

JURNAL INFO KESEHATAN



Published by:
Research and Community Service Unit, Poltekkes Kemenkes Kupang

JURNAL INFO KESEHATAN	VOLUME 22	NUMBER 2	PAGE 214-472	MONTH JUNE	YEAR 2024
--------------------------	--------------	-------------	-----------------	---------------	--------------

JURNAL INFO KESEHATAN

Editorial Team

Editor in Chief

Wanti

Managing Editor

Margareta Teli

Editors

Irfan

Maria Hilaria

Taufik Anwar

Yanuar Fahrizal

Erma Mahmiyah

Slamet Wardoyo

Ni Nyoman Yuliani

Leny Marlina A. Pinat

Muhammad Ifham Hanif

Widyana Lakshmi Puspita

Shelfi Dwi Retnani Putri Santoso

International Editor

Febi Dwirahmadi

Isabella S. Ziyane

Zainab Mohd Shafie

Suzanne Patricia Dardeau

Administrative Staff

M. Ibraar Ayatullah

Technical Support

Semly A. Kase

Editorial Address

Piet A.Tallo Street, Liliba-Kupang, East Nusa Tenggara, Indonesia

Phone/fax: (0380) 8800256

Email: jurnalinfokesehatan@gmail.com

Website: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>

Accredited by:

Ministry of Research, Technology and Higher Education of the Republic of Indonesia
(Kemenristekdikti) Number: 105/E/KPT/2022, April 7, 2022

This journal is indexed on:



Published by:

Research and Community Services Unit, Poltekkes Kemenkes Kupang

Published Every 3 Months

March, June, September and December

JURNAL INFO KESEHATAN

p-ISSN 0216-504X | e-ISSN 2620-536X

Volume 22 Number 2, June 2024

TABLE OF CONTENTS

- Comparison of Severity in Diabetic Ulcer Patients with and Without Sepsis** 214-220
Siti Roisya Aga Maydiana, Evi Nurhayatun, Desy Puspa Putri
- The Relationship of Dietary Pattern and Sarcopenia in Type II Diabetes Mellitus Patients** 221-234
Veronika Merika Kedang, Ahmad Syauqy, Ani Margawati
- Husband Support and Coping Stress on Exclusive Breastfeeding in Primipara** 235-243
Roganda Simanjuntak, Diadjeng Setya Wardani, I Wayan Agung Indrawan, Fariha Nuzulul Hinisa
- The Effects of Black Garlic on Ovarian Malondialdehyde, Oviduct Muscle, and Endometrial Arterioles in Smoke-Exposed Rats** 244-258
Noni Fidya Ayu Anandasari, Novalia Kridayanti, Ni Ketut Devy Kaspirayanthi, Tatit Nurseta, Tri Yudani Mardining Raras, Husnul Khotimah, Subandi Reksohusodo, Kenty Wantri Anita, Hendy Setyo Yudhanto
- The Effect of Sub-Acute Inhalation Exposure to Polyethylene Micro-Nano Plastics on the Histopathological Features of the Mammary Glands in Female Wistar White Rats (*Rattus Norvegicus*)** 259-271
Ihda Dian Kusuma, Laksmitha Janasti, Riana Trinovita Sari, Britania Laila Nanda, Hikmawan Wahyu Sulistomo, Nurdiana
- Analysis of the Effect of Black Garlic (*Allium sativum*) Extract on Ovarian Follicular Atresia, Endometrial VEGF Expression, and Fallopian Tube Epithelial Cell Count in Rats (*Rattus norvegicus*) Exposed to Cigarette Smoke** 272-288
Novalia Kridayanti, Noni Fidya Ayu Anandasari, Ni Ketut Devy Kaspirayanthi, Tatit Nurseta, Tri Yudani Mardining Raras, Husnul Khotimah, Subandi Reksohusodo, Aina Angelica, Kenty Wantri Anita, Hendy Setyo Yudhanto
- Nurses Experience of Prone Position in The Absence of Positioning Tool, and Suggestion of Prone Position Tool: A Phenomenology Study** 289-299
Sriyono, Hakim Zulkarnain, Erna Dwi Wahyuni, Wikan Purwihantoro Sudarmaji, Jujuk Proboningsih, María Pilar Mosteiro-Díaz
- Exploring Key Determinants of Trail Run Athlete's Preparedness to Perform Pre-Hospital First Aid for Ankle Sprain** 300-306
Hakim Zulkarnain, Galih Indhiantoro, Yulis Setiya Dewi, Wahyu Sri Astutik, Filomena Adelaide de Matos

The Effect Flavonoids Phaleria macrocarpa Fruit Extract on Thickness of Trabeculae, Cortex Ratio Femoral Bone and Aortic Intima-Media in Mice Menopause Model	307-316
R. A. Rahmawati Nurul Fadilah, Ani Khoirinda, Sutrisno, Yahya Irwanto, Kenty Wantri Anita, R. A. Rose Khasana Dewi	
The Effect of Flavonoids of Phaleria macrocarpa Fruit Extract on Aortic Diameter Mice Menopause Model	317-325
Ani Khoirinda, R. A. Rahmawati Nurul Fadilah, Sutrisno Sutrisno, Yahya Irwanto, R. A. Rose Khasana Dewi	
The Influence of Providing Information and Educational Media in Efforts to Prevent Stroke	326-334
Cindy Eka Wijaya, Irza Haicha Pratama, Ermi Girsang	
Vibration Stimulation to Increase Milk Production in Puerperal Mothers	335-346
Rhela Panji Raraswati, Krisdiana Wijayanti, Heni Hendriyani	
Evaluation of Hematotoxicity in Female Wistar Rats Following Sub-Acute Inhalation Exposure to Polyethylene Microplastic	347-356
Hikmawan Wahyu Sulistomo, Anisa Setyowati, Melani Chysti Situmorang, Ita Sulistiani, Dewi Azar Nuria Wardani, Kharisma Ciptaning Gusti, Nurdiana, Ihda Dian Kusuma, Bambang Rahardjo, Subandi Reksohusodo	
The Effect of Edamame Jelly on Lowering Blood Pressure in Primary Hypertension Patients	357-368
Yualeny Valensia, Budiyantri Wiboworini, Nur Hafidha Hikmayan	
The Effectiveness of Diabetic Self-Management Education (DSME) on Dietary Habit, Obesity, and Physical Activity Among Patients with DM Type II	369-377
Liana Lidia Agow, Fery Agusman Motuho Mendrofa, Sonhaji	
Effectiveness of Hae-Band in Measuring Hb Levels in Postpartum Hemorrhage Risk Monitoring	378-386
Dinda Dian Meidita, Krisdiana Wijayanti, Heni Hendriyani	
Factors Contributing to Hypertension Self-Care Management Behavior in Elderly Rural Residents	387-394
Irwina Angelia Silvanasari, Achmad Ali Basri, Nurul Maurida, Trisna Vitaliati	
The Role of Hemoglobin in Maintaining Health: A Literature Review	395-408
Nireza Agesti, Damrah Damrah, Willadi Rasyid, Wilda Welis, Dally Rahman, Fiky Zarya	
The Effect of Preeclampsia in Pregnant Women on The Incidence of Maternal Mortality: Literature Review	409-418
Riski Resa Oktaria, Tia Setriana, Sofia Merylista, Yenni Fusfitasari, Nunu Harison	
Quality Evaluation and Flavonoid Content of Honey from Riau Forest, Indonesia	419-428
Eva Yuniritha, Nur Ahmad Habibi, Hasneli, Alsri Windra Doni	

- Partner and Household Factors Associated with Breastfeeding Practice: A Systematic Review** 429-440
Supriatin, Ni Made Dwi Yunica Astriani, Mochamad Heri, Mohamad Sadli
- Moringa oleifera as Anticancer: A Review of Recent Studies** 441-461
Norma Tiku Kambuno, Erni Hernawati Purwaningsih
- The Role of Nutritional Status in Improving Physical Endurance in Athletes: A Literature Review** 462-472
Samrian Sandi, Sayuti Syahara, Kamal Firdaus, Donie Donie, Dally Rahman, Fiky Zarya

GUIDELINES FOR AUTHORS

JURNAL INFO KESEHATAN

MANUSCRIPT PREPARATION AND SUBMISSION

Initial submission

The article is the result of a research study in health. The article should be written in English and Indonesian. The initial submission should be clean and complete but does not have to comply with all Jurnal Info Kesehatan (Journal of Health Info) specifications yet.

Manuscripts of insufficient priority or quality are promptly rejected. Manuscripts retained to undergo a review process for potential publication in the Journal will be submitted to a technical check. Authors will be informed immediately if their manuscripts need reformatting and will be given 14 days to make specific changes.

Visit <http://jurnal.poltekkeskupang.ac.id/index.php/infokes> for online manuscript submission.

Questions? Write jurnalinfokesehatan@gmail.com or call M. Ibraar Ayatullah at (+62) 813-1411-9647).

Manuscript File Formats

All manuscripts should be submitted in Word document format. Jurnal Info Kesehatan submission system is located at <http://jurnal.poltekkeskupang.ac.id/index.php/infokes>

Manuscript Components

Research result article consists of: Title, author's name, abstract in English, keywords, introductions, research methods, results and discussions, conclusions, and References.

Review article consists of: Title, author's name, abstract in English, Keywords, Introductions, research methods, results and discussions, Conclusion, and References.

Author must attach a plagiarism checking document (can use Turnitin, iThenticate or something else). The maximum similarity tolerated is 25%.

WRITING GUIDE

Title

- a) The title is written in English and informative, concise, and not too long or short (10- 25 words).
- b) Consists of the variables under study and describes the content of the manuscript.
- c) A title does not contain abbreviations or formulas.
- d) The title page should include the title of the manuscript only. The names of authors should be deleted to ensure the double-blinding of the paper during the peer review process.

Abstract and Keyword

- a) The abstract is concisely written, about the most important ideas, and contain the problems or research objectives, research method, and research results.
- b) Written in English language with 300-350 words maximum.
- c) Keywords contain main words.

Introduction

An introduction is presented in an integrated manner without subtitles. It is written in the form of paragraphs with a contains:

- a) Background or research rational.
- b) Theoretical basis (literature review in brief).
- c) Research objective.

Research Method

The method is written with a containing:

- a) The study design.
- b) Data collection techniques and data sources.
- c) Method of data analysis.

Results and Discussion

The results represent a major part of scientific articles containing:

- a) Results of data analysis.
- b) Results of hypothesis testing.
- c) It can be presented with a table or graph to clarify results verbally.
- d) Discussion is an important part of the entire scientific article. The purposes of the discussion are: answer the research problem, interpret the findings, integrate the findings of research into the existing knowledge, and formulate a new theory or modify the existing theories.
- e) A serial number that is used is number 1, 2, 3, and so on, do not need to use a composite number. Hyphens should not change the serial number.

Conclusion

- a) Contain conclusions and recommendations.
- b) Conclusions contain answers to the research questions.
- c) Recommendations refer to the results of research and practical form of action, specify to whom and for what recommendation intended.
- d) Written in essay form, not in numerical form.

References

The main references are national journal, international journals and proceeding. All references should be to the most pertinent and up-to-date sources:

- a) Contain literature that is referenced in the content, arranged alphabetically, and written in the APA (*American Psychological Association*) 6th Edition system.
- b) Fully written, appropriate with the references in the content.
- c) Only load literature referenced in the content.
- d) Source of reference are at least 80% from literature published last 10 years
- e) References are least 80% from research article in journal or research reports.
- f) Reference format is APA (*American Psychological Association*) 6th Edition style better to use Reference Manager (Mendeley, Zotero or Endnote, etc).

Example:

Journal:

Author1A, Author2 B. (Year).Title of Manuscript.*Name of Journal or its Abbreviation*.
Vol.(Issue),pages. doi:.....

Casadei, D., Serra, G., Tani, K. (2007). Implementation of a Direct Control Algorithm for Induction Motors Based on Discrete Space Vector Modulation. *IEEE Transactions on Power Electronics*,15(4), 769-77.

Casadei, D., Serra, G., Tani, K. (2007). Implementation of a Direct Control Algorithm for Induction Motors Based on Discrete Space Vector Modulation. *IEEE Transactions on Power Electronics*,15(4), 769-77. doi: <http://doi.org/10.1109/63.849048>

Proceeding:

If the proceedings consists of several volumes

Author1 A, Author2 B.(Year).*Title of Manuscript*. Name of Conference of Seminar. City.Volume: pages. doi:.....

Calero C, Piatini M, Pascual C, Serrano MA. (2009). Towards Data Warehouse Quality Metrics. Proceedings of the 3rd Intl. Workshop on Design and Management of Data Warehouses (DMDW). *Interlaken*. 39, 2-11. doi: <http://doi.org/10.1109/63.849048>

Texbooks:

If the references are refer to specific page range in a book

Author1 A, Author2 B. (Year). *The Title of the Book*. Edition. City: Publisher. pages.

De Vaus, D. A. (2014). *Surveys in social research*. Sydney, Australia: Allen & Unwin.

Book chapter:

If the references are refer to specific page range in a book chapter

Author1 A, Author2 B. (Year). The Title of the Book. Edition. City: Publisher. pages.

McKenzie, H., Boughton, M., Hayes, L., & Forsyth, S. (2008). Explaining the complexities and value of nursing practice and knowledge. In I. Morley & M. Crouch (Eds.), *Knowledge as value: Illumination through critical prisms* (pp.209-224). Amsterdam, Netherlands: Rodopi.

Newspaper article:

Author1 A, Author2 B. (Year, Month date). *The Title of the Newspaper Article*. Publisher. Retrieved from.....

Fellner, C. (2019, April 7). Time bomb: Two new cases as NSW faces worst measles outbreak in years. *The Sydney Morning Herald*. Retrieved from <https://www.smh.com.au>.

The editor would like to give a great acknowledgment to all of the reviewers. Without their contribution and expertise, it would be difficult to maintain the high standards of a peer-reviewed journal.

Dr. Helen Bradely
Dr. Smathi Chong
Dr. Gillian Mashman
Israfil, S. Kep, M. Kes
Setiawan, SKM, M.Kes
Norzawani Jaffar, Ph.D
Mohd Razif Shahril, Ph.D
Azmahani Abdullah, Ph.D
Dr. Kusmiyati, SKM, MPH
Prof. Sakinah Harith, Ph.D
Solikhah, SKM, M.Kes, PhD
Abbe Maleyki Mhd Jalil Ph.D
Dr. Sabina Gero, S.Kp., M. Sc
Dr. R.H. Kristina, SKM, M.Kes
Dr. Drs. Abdul Wahab, M. Kes
Dr. Fathmawati, S.Si.T, M.Kes
Prof. Dr. Khayan, SKM, M.Kes
Priska E. Tenda, SF, Apt., MSc
Dr. drg. Jusuf Kristianto, M.Kes
Dr.drg. Christina Ngadilah, MPH
Marichatul Jannah, S.ST, M.Kes
Dr. drg. Wiworo Haryani, M. Kes
Dr. drg. Christina Ngadilah, MPH
Dr. Linda Suwarni, SKM., M. Kes
Ermi Ndoen, S.KM, MSc.PH, Ph.D
Dr. Drs. Jefrin Sambara, Apt, M.Si
Kholisotul Hikmah, S.S.T., M. Epid
Dr. Indah Budiastutik, SKM, M.Kes
Agus Hendra Al Rahmad, SKM, MPH
Dr. Mareta Bakale Bakoil, S.ST, MPH
Mariana Ngunju Awang, SST, M. Kes
Alfiyana Yuliasari, S.Keb., Bd. M.K.M
Adam Astrada, Ns., MHS, CNS, DHSc
Dr. Yessi Dessy Arna, M. Kep., Sp. Kom
Norma Tiku Kambuno, S.Si., Apt, M. Kes
Dr. Arif Setyo Upoyo, S.Kp, Ners, M. Kep
Kraichat Tantrakarnapa, B.S., M.Sc., Ph.D.
Dr. Yuanita Clara Luhi Rogaleli, S.Si, M.Kes
Dr. Brahma Putra Marjadi, MPH, PhD, SFHEA
Prof. Dr. Ridwan Amiruddin, SKM, M.Kes, M.Sc, PH
Dr. Ns. Ni Luh Putu Inca Buntari Agustini, S.Kep., MNS
Prof. Nelson Martins, B. Med, MD, GCERT PH, MHM, PhD



Jurnal Info Kesehatan

Vol. 22, No. 2, June 2024, pp. 214-220

P-ISSN 0216-504X, E-ISSN 2620-536X

DOI: [10.31965/infokes.Vol22.Iss2.1221](https://doi.org/10.31965/infokes.Vol22.Iss2.1221)

Journal homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>



RESEARCH

Open Access

Comparison of Severity in Diabetic Ulcer Patients with and Without Sepsis

Siti Roisya Aga Maydiana^{1a}, Evi Nurhayatun^{2b*}, Desy Puspa Putri^{2c}

¹ Faculty of Medicine, Sebelas Maret University, Surakarta, Central Java, Indonesia

² Department of Internal Medicine, Faculty of Medicine, Sebelas Maret University, Surakarta, Central Java, Indonesia

^a Email address: sasha.mydn@gmail.com

^b Email address: evi.nurhayatun@staff.uns.ac.id

^c Email address: desypuspa@staff.uns.ac.id

Received: 12 June 2023

Revised: 15 February 2024

Accepted: 2 April 2024

Abstract

Diabetes mellitus patients in Central Java grow yearly. According to data provided by the Central Java Health Department in 2019, Diabetes mellitus ranks second as the largest non-communicable disease (NCD), covering 13,4% of the total NCD patients. The prevalence of diabetic ulcers is 15-25% of all diabetes mellitus patients in Indonesia, which concerns the author regarding the possible increase of the diabetic ulcer population in Central Java. The severity of diabetic ulcers depends on various factors, including infection and complications. The most common complication is sepsis, a systemic infection often arising from local infections that are not appropriately treated, causing progressive damage and increasing mortality rates. This study aims to examine the comparison of ulcer severity in diabetic ulcer patients with sepsis and non-sepsis complications using Meggitt Wagner's criteria. This study utilizes an analytic observational study using a cross-sectional approach. The author sampled 126 patients diagnosed with Diabetic Ulcers in 2022 at Dr. Moewardi General Hospital for this study, using a purposive sampling method. The research was conducted by looking at the patient's medical record data, and data analysis using SPSS software. The test used in this research includes a Parametric T-independent test and a Pearson bivariate relationship test. This study shows that the comparison of ulcer severity in diabetic ulcer patients with sepsis and without sepsis was statistically significant (p -value = 0.000002). The mean of the group with sepsis (3.4127) was greater than the group without sepsis (2.5238). In conclusion, the comparison of ulcer severity in diabetic ulcer patients with sepsis and without sepsis is significant. We recommend future researchers include patients' comorbidities, total hospitalization time, and detailed information on drug use and patient adherence to treatment in their research for more nuanced findings.

Keywords: Diabetes Mellitus, Diabetic Ulcer Severity, Sepsis, Wagner's Criteria.

*Corresponding Author:

Evi Nurhayatun

Department of Internal Medicine, Faculty of Medicine, Sebelas Maret University, Surakarta, Central Java, Indonesia

Email: evi.nurhayatun@staff.uns.ac.id



©The Author(s) 2024. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

1. INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disease characterized by persistent hyperglycemia. It is caused by insulin secretion damage or peripheral insulin resistance (Goyal, Jialal, & Delhi, 2023). In 2019, Indonesia was ranked 7th as the country with the most diabetic patients in the world (Kementerian Kesehatan, 2020). Meanwhile, in Central Java itself, diabetes is the second largest non-communicable disease (NCD) after hypertension with 618,546 patients, covering 10.7% of the total number of patients with NCD (Dinas Kesehatan Provinsi Jawa Tengah, 2019). Therefore, it is vital for the medical institution to pay additional attention in order to reduce the prevalence and complications of Diabetes Mellitus in Central Java.

Common Diabetes Mellitus complications that often emerge are diabetic ulcers (Wang et al., 2021). The term diabetic ulcer is used to describe a wound on the skin of a person with diabetes, which does not heal properly (Monteiro-Soares et al., 2019; Syafril, 2018). Wounds with longer periods of healing increase its risk of infection, and if not treated accordingly, it may cause sepsis (Perez-Favila et al., 2019). Patients with sepsis experience systemic inflammation and immune disorders that involve altering multiple organs in the body, thus increasing the risk for amputation, disability, and death (Huang, Cai, & Su, 2019)

Diabetic ulcer is a potentially fatal complication that can cause mortality and morbidity in patients. Previous studies suggest that 19-34% of diabetic patients are likely to develop diabetic ulcers in their lifetime (Everett & Mathioudakis, 2018). In Indonesia, the probability of developing diabetic ulcer reaches 15% of the total diagnosed DM patients with a prevalence rate of diabetic ulcers occurring in 15-25% of patients (Sukartini, Theresia Dee, Probawati, & Arifin, 2020).

During the course of the disease, diabetic ulcer is so susceptible to infection that it commonly caused septic foot or infected diabetic wound (Lin, Hung, Huang, & Yeh, 2019). Previous study showed that 98.8% of diabetic ulcer patients in Indonesia suffer from infection, which requires patients to be assessed for possible infection during admission (Najihah, 2020). In 2021, it is stated that sepsis is the most common complication for diabetic ulcer patients, which is evident in 68.26% of the total patients (Hariftyani, Novida, & Edward, 2021).

Previous research regarding the profile of diabetic ulcer patients proves that sepsis is the most common complication in DM, and ulcer severity determined with Meggitt-Wagner criteria grade 4 and 5 have manifested in 41,31% of patients (Hariftyani et al., 2021). However, it has not been clarified whether there is a difference in the severity of ulcers in patients with sepsis and without sepsis. Based on this information, this study aims to contribute novelty by analyzing the comparison of ulcer severity in patients with sepsis and without sepsis using Meggitt-Wagner's criteria. The increasing number of diabetic ulcer patients and the lack of knowledge regarding these complications indicates the urgency to research this matter.

2. RESEARCH METHOD

This is analytic observational research utilizing a cross sectional method as its research design, conducted at the Internal Medicine Department of Dr. Moewardi General Hospital Surakarta. The population of this research are type 2 DM patients diagnosed with diabetic ulcers in the year of 2022 at Dr. Moewardi General Hospital Surakarta with inclusion of patients with pneumonia excluding patients with a history of malignancy and autoimmune; patients undergoing immunosuppression therapy; patients with other infection such as UTI and gastrointestinal infection; and patients with insufficient medical records.

The independent variable is the presence or absence of sepsis in patients measured using Sequential Organ Failure Assessment (SOFA) criteria (Cecconi, Evans, Levy, & Rhodes, 2018). The dependent variable is severity of diabetic ulcer measured with Megitt-Wagner's

criteria (Utami, Marselin, & Hartanto, 2021). Confounding variables include age, gender, length of stay, types of pathogens in the ulcer, and patients' mortality. Samples were taken using a purposive sampling method, with 63 patients in each category with and without sepsis, making the total sample of 126 patients. Data analysis was performed using SPSS software. The population was first subjected to normality and homogeneity tests using Kolmogorov-Smirnov test and Levene's test (Conover, Guerrero-serrano, & Gustavo, 2018; Orcan, 2020). Furthermore, data on ulcer severity and the presence or absence of sepsis was analyzed using a parametric comparison test to determine the difference between the severity in both categories (Kim, 2015). A significant difference is obtained if the p-value is <0.05. Ethical approval for this study was obtained in Dr. Moewardi General Hospital Surakarta with ethical number: 364/III/HREC/2023.

3. RESULTS AND DISCUSSION

Table 1. Subject Characteristics (age, gender, comorbidities)

Subject	Characteristic	Frequency (n)	Percentage (%)
Age	15-44 years old	7	6%
	45-59 years old	61	48%
	≥60 years old	58	46%
Gender	Female	67	53%
	Male	59	47%
Comorbidities	Yes	64	51%
	No	62	49%

Table 1 shows that the population of this study consists of 67 female patients and 59 male patients, with the age ranging from 33-83 years old. The data indicates that the most common age group of this study population is between 45-59 years old. The percentage of patients with comorbidities prior to admission is slightly higher than that of patients without comorbidities

Table 2. Ulcer Severity on Patients with and Without Sepsis

Sepsis	Wagner's Criteria Grade					Total	Mean	p-value
	1	2	3	4	5			
Yes	2	7	22	26	6	63	2.5238	0.000002
No	11	23	16	12	1	63	3.4127	

Table 2 shows that this study obtained medical records from 126 diabetic ulcer patients at Dr. Moewardi General Hospital Surakarta in the year 2022. The data was later examined for ulcer severity using Meggitt-wagner's criteria, and the presence or absence of sepsis. The outcome shows that the average Wagner's Criteria grade for ulcer patients with sepsis is 3.4127, with grade 4 being the frequent Wagner's grade. For patients without sepsis, the average Wagner's Criteria grade is 2.5238 with grade 2 contributing to a third of the total patient's severity grade.

Data acquired in this study suggest that diabetic ulcer patients with sepsis had a higher Wagner grade (3.4127) compared to its counterparts without sepsis (25238). Further analyzing the data using T-Independent test resulted in p-value <0.001 (p<0.05), meaning that the comparison between two groups is significantly different.

Ulcer Severity is influenced by many factors, such as infection. Infection in diabetic ulcers varies in type, ranging from uncomplicated cellulitis to systemic inflammation or sepsis (Goh et al., 2020). The presence of infection is one of the bases for assessing the severity of ulcers. Likewise, in Wagner's criteria used in this study, the presence of infection is specified in grade 3 (Stang & Young, 2018). Infection in diabetic ulcers is progressive, and ulcer patients

with infections are 10 times more probable of hospitalization and have an increased amputation risk up to 155 times compared to individuals without infections (Najihah, 2020; Primadina, Basori, & Perdanakusuma, 2019). In a previous study, it is mentioned that 59% of diabetic ulcer patients were the result of sepsis (Matta-guti, Garc, Garc, Á, & Luis, 2021). Moreover, the Department of Orthopedic Surgery in Korea university found a positive and linear correlation between patients diagnosed with sepsis and the severity of diabetic ulcers (Park, 2017).

Table 3. Samples Distributions Based on Age and Mortality

Subject		Wagner's Criteria Grade					Mean	p-value	
		1	2	3	4	5			Total
Pathogens	MDR	2	5	11	17	1	36	3.2778	0.029
	Non-MDR	11	25	27	21	6	90	2.8286	
Age	≤60 years old	9	14	25	17	3	68	2.8678	0.263
	>60 years old	4	16	13	21	4	58	3.0862	

Table 3 shows that the patients with multi-drug resistant (MDR) pathogens experience higher severity grade albeit a lower population count. Based on the information acquired in this research it is stated that 36 out of 126 patients are infected with MDR pathogens, and the average Wagner's Criteria Grade on those patients are 3.2778. Whilst, Wagner's Criteria grade for patients infected with non-MDR pathogens averaged at 2.8286.

Furthermore, it appears that patients over 60 years old had a higher Wagner's Criteria Grade during admission over patients aged 60 years and younger. With the average Wagner Grade for patients over 60 are 3.0862.

In patients infected with MDR pathogens, the Wagner grade averaged higher than patients infected with non-MDR pathogens. This analysis also bears significant results with a p-value of 0.29 ($p < 0.05$). A previous study supported this result by stating that patients infected with MDR pathogens require longer healing periods, resulting in an increase in the Wagner grade due to bacterial reproduction which exacerbates tissue damage and increases the risk of infection (Liu, 2022; Yan, Song, Zhang, & Li, 2022)

Patients over 60 years old are found to have higher Wagner grade over patients age 60 years and younger. Despite this, after analyzing the comparison between both groups using the T-Independent test, the resulting p-value is proven to be insignificant. Meaning the difference between the two groups mean are indistinguishable in comparison. The Previous study in 2021 and 2022 affirms this result in that they found no correlation between age and diabetic ulcer severity (Dörr et al., 2020; Syauta, Hendarto, Mariana, Kusumanegara, & Faruk, 2021). In 2018, a study by Jeffcoate found that older patients had a longer recovery period, which is believed to have contributed to ulcer severity (Jeffcoate, Vileikyte, Boyko, Armstrong, & Boulton, 2018).

Table 4. Sepsis Distribution on Hospital Length of Stay

Sepsis	Length of Stay			Mean	p-value
	0-5 days	6-10 days	>10 days		
Yes	22	26	15	7.6984	0.372
No	22	33	8	7.0635	

Table 4 shows that Patients with and without sepsis are further examined on their hospital length of stay, comparing the time spent for treating both groups. The results are longer periods of hospital stay for patients with sepsis. Patients with sepsis had an average length of stay of 7.7 days, and patients without sepsis spent an average 7 days at the hospital.

In comparing the length of stay of patients with and without sepsis, the results for patients with sepsis appear to be longer in duration. However, after analyzing the comparison between the two groups, the result does not hold significance as the p-value equals to 0.372. In the T-independent test, the p-value needs to be <0.05 for the comparison to have any significant difference. In contrast with previous study, it is said that diabetic ulcer patients with sepsis experience 1.6 times longer hospital stays than patients without sepsis (Gunawan, Pangalila, & Ludong, 2019). This contrasting outcome is likely due to study limitations; however, the author believes that said contrast warrants further study to determine such findings.

Table 5. Sepsis Distribution on Patients Mortality

Sepsis	Mortality (n)		Pearson Correlation	p-value
	Yes	No		
Yes	27	36	0.496	<0.0001
No	1	62		

Table 5 shows Out of 126 medical records from patients that had been acquired, this study found that 28 patients died in total. 27 of them were patients with sepsis, and only one patient died without sepsis. A significant correlation between sepsis patients and mortality is characterized by the p-value result at <0.0001 ($p<0.05$). It is discovered that out of 28 patients in the sample population who died, 27 of them are patients with sepsis. Moreover, after further examining the data, the Pearson Correlation is valued at 0.496 which indicates a moderate correlation. These findings are in accordance with the previous study by Schofield, 2021 claiming that sepsis is the leading cause of death in ulcer patients, and another study by Chen, 2023 affirms this result by mentioning that systemic and local infection was the second leading cause of death after cardiovascular disease in patients with diabetic ulcer (Chen, Sun, Gao, & Ran, 2023; Schofield, Haycocks, Anderson, Heald, & Robinson, 2021).

4. CONCLUSION

In accordance with this research, which utilizes a cross-sectional approach on 126 diabetic ulcer patients' medical records at Dr. Moewardi General Hospital during the year 2022, it is evident that the comparison of ulcer severity in diabetic ulcer patients with and without sepsis is statistically significant. It is also concluded that the severity of ulcer on patients infected with MDR pathogens are more severe than patients who are infected with non-MDR pathogens. Lastly, this study also noted the moderate correlation between the presence of sepsis and patient mortality.

For future researchers, it is advised to classify comorbidities according to organ system and analyze the severity for each group to see if there are significant results, also to consider the total time of hospitalization for patients with multiple admissions in order to extensively comprehend the patient's condition, lastly, future studies should provide a detailed picture of drug use and patients treatment adherence.

REFERENCES

- Cecconi, M., Evans, L., Levy, M., & Rhodes, A. (2018). Sepsis and septic shock. *The Lancet*, 392(10141), 75–87. [https://doi.org/10.1016/S0140-6736\(18\)30696-2](https://doi.org/10.1016/S0140-6736(18)30696-2)
- Chen, L., Sun, S., Gao, Y., & Ran, X. (2023). Global mortality of diabetic foot ulcer: A systematic review and meta-analysis of observational studies. *Diabetes, Obesity and Metabolism*, 25(1), 36–45. <https://doi.org/10.1111/dom.14840>
- Conover, W. J., Guerrero-Serrano, A. J., & Tercero-Gómez, V. G. (2018). An update on 'a

- comparative study of tests for homogeneity of variance'. *Journal of Statistical Computation and Simulation*, 88(8), 1454-1469. <https://doi.org/10.1080/00949655.2018.1438437>
- Dinas Kesehatan Provinsi Jawa Tengah. (2019). *Profil Kesehatan Provinsi Jateng Tahun 2019*. Dinas Kesehatan Provinsi Jawa Tengah.
- Dörr, S., Schlecht, M., Chatzitomaris, A., Weisser, G., Lucke-Paulig, L., Friedl, A., ... & Lobmann, R. (2021). Predictive effect of inflammatory response and foot ulcer localization on outcome in younger and older individuals with infected diabetic foot syndrome. *Experimental and Clinical Endocrinology & Diabetes*, 129(12), 878-886.
- Everett, E., & Mathioudakis, N. (2018). Update on management of diabetic foot ulcers. *Annals of the New York Academy of Sciences*, 1411(1), 153-165. <https://doi.org/10.1111/nyas.13569>
- Goh, T. C., Bajuri, M. Y., Nadarajah, S. C., Rashid, A. H. A., Baharuddin, S., & Zamri, K. S. (2020). Clinical and bacteriological profile of diabetic foot infections in a tertiary care. *Journal of foot and ankle research*, 13(1), 1-8.
- Goyal, R., Jialal, I., & Delhi, N. (2023). *Type 2 Diabetes*. StatPearls Publishing.
- Gunawan, B., Pangalila, F., & Ludong, M. (2019). Hubungan tingkat keparahan sepsis dengan Diabetes Melitus terkontrol dan tidak terkontrol menggunakan parameter HbA1C di Rumah Sakit Royal Taruma Jakarta Barat periode 2015-2017. *Tarumanagara Medical Journal*, 1(2), 277-290.
- Huang, M., Cai, S., & Su, J. (2019). The pathogenesis of sepsis and potential therapeutic targets. *International Journal of Molecular Sciences*, 20(21). <https://doi.org/10.3390/ijms20215376>
- Jeffcoate, W. J., Vileikyte, L., Boyko, E. J., Armstrong, D. G., & Boulton, A. J. M. (2018). *Current Challenges and Opportunities in the Prevention and Management of Diabetic Foot Ulcers*. 41(April), 645-652. <https://doi.org/10.2337/dc17-1836>
- Kementerian Kesehatan, R.I. (2020). *Peraturan Menteri Kesehatan Republik Indonesia Nomor 2 Tahun 2020 Tentang Standar Antropometri Anak*. Jakarta: Kementerian Kesehatan Republik Indonesia.
- Kim T. K. (2015). T test as a parametric statistic. *Korean journal of anesthesiology*, 68(6), 540-546. <https://doi.org/10.4097/kjae.2015.68.6.540>
- Lin, C. W., Hung, S. Y., Huang, C. H., Yeh, J. T., & Huang, Y. Y. (2019). Diabetic foot infection presenting systemic inflammatory response syndrome: a unique disorder of systemic reaction from infection of the most distal body. *Journal of Clinical Medicine*, 8(10), 1538.
- Liu, X., Ren, Q., Zhai, Y., Kong, Y., Chen, D., & Chang, B. (2022). Risk factors for multidrug-resistant organisms infection in diabetic foot ulcer. *Infection and Drug Resistance*, 1627-1635.
- Matta-Gutierrez, G., Garcia-Morales, E., Garcia-Alvarez, Y., Álvaro-Afonso, F. J., Molines-Barroso, R. J., & Lazaro-Martinez, J. L. (2021). The influence of multidrug-resistant bacteria on clinical outcomes of diabetic foot ulcers: a systematic review. *Journal of Clinical Medicine*, 10(9), 1948.
- Monteiro-Soares, M., Russell, D., Boyko, E. J., Jeffcoate, W., Mills, J. L., Morbach, S., & Game, F. (2019). IWGDF Guideline on the classification of diabetic foot ulcers. *International Working Group on the Diabetic Foot*, 1-15.
- Najihah. (2020). Infeksi Luka Kaki Diabetik dan Faktor Resikonya : Literature Review. *Jurnal Ilmiah Kesehatan Pencerah*, 09(2), 179-185.
- Orcan, F. (2020). *Parametric or Non-parametric : Skewness to Test Normality for Mean Comparison*. 7(2), 255-265.

- Park, J. H., Suh, D. H., Kim, H. J., Lee, Y. I., Kwak, I. H., & Choi, G. W. (2017). Role of procalcitonin in infected diabetic foot ulcer. *Diabetes research and clinical practice*, 128, 51-57.
- Perez-Favila, A., Martinez-Fierro, M. L., Rodriguez-Lazalde, J. G., Cid-Baez, M. A., Zamudio-Osuna, M. D. J., Martinez-Blanco, M. D. R., ... Garza-Veloz, I. (2019). Current therapeutic strategies in diabetic foot ulcers. *Medicina (Lithuania)*, 55(11), 1–21. <https://doi.org/10.3390/medicina55110714>
- Primadina, N., Basori, A., & Perdanakusuma, D. S. (2019). Proses penyembuhan luka ditinjau dari aspek mekanisme seluler dan molekuler. *Qanun Medika-Medical Journal Faculty of Medicine Muhammadiyah Surabaya*, 3(1), 31-43.
- Schofield, H., Haycocks, S., Robinson, A., Edmonds, M., Anderson, S. G., & Heald, A. H. (2021). Mortality in 98 type 1 diabetes mellitus and type 2 diabetes mellitus: Foot ulcer location is an independent risk determinant. *Diabetic Medicine*, 38(10), e14568. <https://doi.org/10.1111/dme.14568>
- Stang, D., & Young, M. (2018). ulcer classification system in Scotland : part 2. *The Diabetic Foot Journal*, 21(2), 100–106.
- Sukartini, T., Theresia Dee, T. M., Probowati, R., & Arifin, H. (2020). Behaviour model for diabetic ulcer prevention. *Journal of Diabetes and Metabolic Disorders*, 19(1), 135–143. <https://doi.org/10.1007/s40200-019-00484-1>
- Syafril, S. (2018). Pathophysiology diabetic foot ulcer. *IOP Conference Series: Earth and Environmental Science*, 125(1). <https://doi.org/10.1088/1755-1315/125/1/012161>
- Syauta, D., Hendarto, J., Mariana, N., Kusumanegara, J., & Faruk, M. (2021). Risk factors affecting the degree of diabetic foot ulcers according to Wagner classification in diabetic foot patients &. *Medicina Clínica Práctica*, 4, 100231. <https://doi.org/10.1016/j.mcpsp.2021.100231>
- Utami, M. P. S., Marselin, A., & Hartanto, F. A. D. (2021). *Indonesian Journal of Global Health Research*. 3(3), 407–414.
- Wang, Y., Shao, T., Wang, J., Huang, X., Deng, X., Cao, Y., ... Zhao, C. (2021). An update on potential biomarkers for diagnosing diabetic foot ulcer at early stage. *Biomedicine and Pharmacotherapy*, 133(July 2020), 110991. <https://doi.org/10.1016/j.biopha.2020.110991>
- Yan, X., Song, J., Zhang, L., & Li, X. (2022). Analysis of risk factors for multidrug - resistant organisms in diabetic foot infection. *BMC Endocrine Disorders*, 1–7. <https://doi.org/10.1186/s12902-022-00957-0>

Jurnal Info Kesehatan

Vol. 22, No. 2, June 2024, pp. 221-234

P-ISSN 0216-504X, E-ISSN 2620-536X

DOI: [10.31965/infokes.Vol22.Iss2.1476](https://doi.org/10.31965/infokes.Vol22.Iss2.1476)Journal homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>**RESEARCH****Open Access****The Relationship of Dietary Pattern and Sarcopenia in Type II Diabetes Mellitus Patients****Veronika Merika Kedang^{1a}, Ahmad Syauqy^{2b*}, Ani Margawati^{2c}**¹ Postgraduate Program of Nutrition Sciences, Faculty of Medicine, Diponegoro University, Central Java, Indonesia² Department of Nutrition Sciences, Faculty of Medicine, Diponegoro University, Central Java, Indonesia^a Email address: learaga031213@gmail.com^b Email address: syauqy@fk.undip.ac.id^c Email address: animargawati@gmail.com

Received: 2 May 2024

Revised: 19 May 2024

Accepted: 7 June 2024

Abstract

Diet plays an important role in managing mass, and muscle strength and preventing diseases related to malnutrition. A dietary pattern that contains several nutrients has a role in improving muscle performance and reducing the incidence of sarcopenia. This study aims to determine the relationship between dietary patterns and the incidence of sarcopenia in subjects with type 2 diabetes mellitus. This study was a cross-sectional study conducted on 150 subjects with type 2 diabetes mellitus aged 40 years or older. Multivariate logistic regression tests were conducted to measure the effect of adherence to each dietary pattern on the possibility of sarcopenia. Three dietary patterns were identified through analysis of major components. After adjusting for confounding variables, subjects with healthy dietary patterns on the highest tertile had a lower odds ratio for developing sarcopenia (OR 0.584; 95% CI 0.070-4.865; p=0.000). In contrast, adherence to unhealthy dietary patterns was not associated with sarcopenia (OR 1.508; 95% CI 0.305-7.452; p=0.612). Similarly, adherence to a mixed dietary pattern did not affect the likelihood of sarcopenia (OR 1.297; 95% CI 0.341-4.931; P=0.704). This study shows that adherence to a healthy dietary pattern is associated with a lower chance of developing sarcopenia in people with type II diabetes mellitus.

Keywords: Dietary Patterns, Sarcopenia, Muscle Mass, Muscle Strength, Walking Speed, Type II Diabetes Mellitus.

***Corresponding Author:**

Ahmad Syauqi

Department of Nutrition Sciences, Faculty of Medicine, Diponegoro University, Central Java, Indonesia

Email: syauqy@fk.undip.ac.id

©The Author(s) 2024. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

1. INTRODUCTION

Sarcopenia is a condition where the skeletal muscle is involuntarily resorbed, leading to decreased muscle strength and function (Cruz-Jentoft et al., 2019; Sun et al., 2021). This condition has recently been identified as a new complication in patients with diabetes. The prevalence of sarcopenia varies, depending on age groups studied, ethnicities, associated comorbidities, and diagnostic criteria employed. For community-dwelling elderly individuals, the prevalence ranges from 9.9% to 40% (Mayhew et al., 2019), while among the elderly with type 2 diabetes mellitus (T2DM), it ranges from 15% to 44%, depending on the diagnostic criteria used (Shaikh et al., 2020; Wang et al., 2016).

Sarcopenia has multiple causes, including chronic disorders such as neurological, inflammatory, and autoimmune diseases, malnourishment, hypogonadism, any chronic systemic illness, and multiple drugs, especially glucocorticoids (Borba et al., 2019). Diabetes is one of the most common causes of sarcopenia. Impaired muscle function contributes to a sedentary life. The pathogenesis of sarcopenia is complicated and associated with aging, sedentary lifestyle, nutritional factors, increased production of reactive oxygen species, increased levels of proinflammatory cytokines, hormonal changes, and decreased neuromuscular function (Chen et al., 2021; Tan et al., 2021). Nutritional causes of sarcopenia include inadequate energy and protein intake, low blood levels of vitamin D, and inadequate intake of carotenoids and antioxidants (Yakout et al., 2019).

The link between sarcopenia and diabetes mellitus is due to the presence of similar risk factors between both conditions. Sarcopenia may be caused by inadequate energy and nutritional factors. Nutritional factors are linked to dietary patterns, which are defined as the quantities, proportions, variety, or combination of different foods, drinks, and nutrients in diets, and the frequency with which they are habitually consumed. Dietary pattern (DP) is the general profile of food and nutrient consumption which is characterized based on the usual eating habits (Snetselaar, et al., 2021). The analysis of dietary patterns gives a more comprehensive impression of the food consumption habits within a population (Omage & Omuemu, 2018). It may be better at predicting the risk of diseases than the analysis of isolated nutrients or foods because the joint effect of various nutrients involved would be better identified.

A dietary pattern containing several nutrients may have a role in improving muscle performance and decreasing the incidence of sarcopenia (Göbl & Tura, 2022). In the people of East Nusa Tenggara, especially in Belu Regency, the diet pattern is commonly high in carbohydrate, vegetable, and legume intake but low in animal and fruit food intake (Carolina & Wahyu, 2020). This may increase the risk of sarcopenia. Therefore, the purpose of this study was to determine the relationship of dietary patterns with the incidence of sarcopenia in patients with type II Diabetes Mellitus at Gabriel Manek General Hospital, SVD Atambua.

2. RESEARCH METHOD

This study used a cross-sectional design with purposive sampling the outpatient room of Gabriel Manek General Hospital, SVD Atambua. A total of 183 individuals with age ≥ 40 year met the criteria of type two diabetes mellitus between November 2023 until January 2024. After excluding the individuals ($n = 23$) who had been unable to walk independently or used assistive devices and ($n = 10$) who did not cooperate and withdrew during the research, a total of 150 subjects were finally recruited for analysis in this study. Ethical approval for this research was received from the research ethics committee of the Faculty of Medicine, Diponegoro University, Indonesia (*Ethical Clearance* No. 542/EC/KEPK/FK-UNDIP/XI/2023).

Definition and assessment of sarcopenia. Sarcopenia is defined as low muscle mass, decreased muscle strength, and/or slow walking speed. It is assessed based on the guidelines

of the Asian Working Group of Sarcopenia (AWGS). In men, low muscle mass index is <7.0 kg/m² and muscle strength decreases when <26 kg. In women, low muscle mass index is <5.7 kg/m² and muscle strength decreases when <18 kg. Walking speed decreases when <0.8 (m/s) (Chen et al., 2020).

Dietary pattern assessment. Trained nutritionists collected dietary data using food frequency questionnaires (FFQs). The FFQs contained 25 food items that represent typical East Nusa Tenggara food patterns. Participants were asked how often they consumed each food item. The frequency scores were used to obtain a dietary pattern, which was classified into tertiles (Syauqy et al., 2018b). A healthy diet is characterized by a higher intake of foods with higher benefits in improving health (such as fruits and vegetables, whole grains, fish, lean meats, low-fat dairy, nuts, and olive oil), but lower in energy. Unhealthy diet patterns are characterized by low nutrient food intake (for example, refined grains, sweets, and animal products that are high in saturated fat) while mixed diet patterns are characterized by food intake consisting of a mixture of healthy and unhealthy diet patterns (Dominguez et al. 2022).

Anthropometric measurements are taken by trained health professionals using standard protocols and tools such as ONEMED brand Microtoice for height measurement and bioelectrical impedance analysis instrument (brand Omron HBF-375) for measuring body weight (kg) and body fat (%). Body mass index (BMI) is calculated as body weight divided by height square and classified as normal nutritional status (18.5-25 kg/m³), undernutrition status (< 18.5 kg/m³), and over nutritional status (> 25 kg/m³) (Kementerian Kesehatan RI, 2014). Waist and hip circumference are measured using a tape measure and used to calculate waist-to-hip ratio, which is defined as low (< 0.90 for men and < 0.85 for women) and high (≥ 0.90 for men and ≥ 0.85 for women) (Syauqy et al., 2018a).

Biochemical measurements. We used laboratory test data from patient medical records with the provision of test results that will be used if Fasting blood glucose levels (FGP) ≥ 126 mg/dl. Blood glucose levels 2 hours postprandial > 200 mg/dl, and Hemoglobin A1c (HbA1c) levels are $\geq 6.5\%$.

Demographic and lifestyle characteristics such as gender, age, duration of suffering from type 2 diabetes mellitus, metformin consumption, and physical activity were collected using questionnaires. The duration of suffering from type 2 diabetes mellitus dichotomy is ≤ 5 years and >5 years. Physical activity using the pre-developed PASE (Physical Activities Scale for Elderly) questionnaire was categorized as less (total score < 17), moderate (total score ≥ 17), and good (total score ≥ 22) (VandeBunte et al. 2022).

The study used chi-square and ANOVA tests to determine differences in variables between subjects with sarcopenia and different dietary patterns. Multivariate logistic regression analysis was used to compare the relationship between dietary patterns and sarcopenia. The models were adjusted for various factors, and SPSS 26 software was used for analysis with a significance level of $p \leq 0.05$.

3. RESULTS AND DISCUSSION

Table 1. Study's Subject Characteristics

Characteristics	Sarcopenia (%)		Total (N= 150)	p-value
	Yes (n= 77)	No (n= 73)		
Age (mean \pm SD)	60.91 \pm 9.63	55.04 \pm 9.60	58.25 \pm 10.02	0.000
40-59	57.1	42.9	56.7	0.048
≥ 60	34.2	65.8	43.3	
Sex				
Male	61.0	41.1	51.3	0.023
Female	39.0	58.9	48.7	
Weight (kg)	46.59 \pm 5.35	57.28 \pm 6.02	51.44 \pm 7.77	0.000

Height (cm)	161.65± 4.44	163.82±8.32	162.64±6.56	0.044
BMI (kg/m ²)	17.82±1.86	21.48± 2.97	19.48±3.03	0.000
Illness duration (years)				
≤ 5	1.3	69.9	34.7	0.000
>5	98.7	30.1	65.3	
Waist (cm)	83.42±6.62	98.38±7.19	90.20±10.14	0.000
Hip circumference (cm)	97.85±83	105.30±5.61	101.23±6.38	0.000
Waist-hip ratio	0.85± 0.04	0.93±0.06	0.88± 0.07	0.000
% body fat	26.23± 5.15	27.78± 5.36	26.93± 5.28	0.073
Muscle mass index (kg/m ²)	6.44± 1.14	8.16± 1.56	7.22± 1.59	0.000
Muscle strength (kg)	19.35± 3.51	24.92± 3.39	21.98± 3.93	0.000
Walking speed (m/s)	0.74± 0.23	1.08± 0.25	0.90± 0.29	0.000
Physical activity (%)				
Less	76.6	4.1	40.7	0.000
Moderate	15.6	11.0	14.0	
Good	7.8	84.9	45.3	
HbA1c Levels (%)	7.30±0.73	6.76±0.93	7.06±0.87	0.000
Random blood glucose (mg/dl)	158.90±15.66	151.09±21.59	154.56±19.09	0.011
Blood glucose 2 hours postprandial (mg/dl)	265.88±45.60	242.74±62.32	255.39±54.86	0.010
Metformin consumption (%)				
Consumed	13.0	94.5	52.7	0.000
Not consumed	87.0	5.5	47.3	
Total intake				
Energy (kcal)	1083.66± 30.26	1363.91± 37.97	1210.71± 26.39	0.000
Protein (gr)	43.46±1.30	56.66± 1.42	49.45± 1.09	0.000
Fat (gr)	31.59±1.28	35.03± 1.54	33.15± 0.99	0.086
Carbohydrate (gr)	204.70±4.25	236.19±5.40	218.97± 3.60	0.000

Table 1 shows that the research analyzed participants' sarcopenia conditions and found that the majority of those affected were older than 60 years. The study revealed a significant relationship between body weight, height, and nutritional status with the incidence of sarcopenia. Patients with type II diabetes mellitus who have had pain for over five years had a higher incidence of sarcopenia. The research found a significant relationship between muscle mass index, muscle strength, and walking speed with the incidence of sarcopenia. HbA1C levels were also associated with sarcopenia. In addition, the study found a significant relationship between low physical activity and sarcopenia.

Table 2. Loading of Dietary Factors and Patterns Resulting from Principal Component Analysis for 25 Food Groups

Food Group	Factor I (Unhealthy dietary pattern)	Factor II (Mix dietary pattern)	Factor III (Healthy dietary pattern)
Rice/flour/corn products	0.154	0.488	0.098
Root crops	0.162	-0.287	-0.036
Whole grains	-0.097	-0.166	0.613

Staple food ingredients cooked in oil	0.353	0.076	0.063
Nuts	-0.160	-0.274	0.380
Soya bean	-0.242	-0.119	0.367
Dairy products and dairy products	0.046	0.099	-0.250
Light colored vegetables	0.094	0.531	-0.069
Dark colored vegetables	0.050	-0.248	0.516
Egg	0.244	0.049	0.296
Fish and seafood	-0.007	0.190	0.339
Meat	0.194	-0.523	0.057
Poultry	0.106	0.516	0.193
Fast food	0.648	0.123	0.094
Processed foods	0.517	-0.349	-0.306
Fried food	0.374	0.313	-0.204
Animal organs	0.011	0.250	-0.020
Fruits	0.096	0.137	0.501
Processed fruit	-0.023	-0.298	-0.261
Traditional snacks	0.360	0.204	0.008
Jam/honey	0.734	-0.074	-0.058
Sweet drinks	0.621	-0.061	0.044
Sweet dessert	0.629	0.037	-0.061
Tea and coffee	0.597	0.202	-0.193
Alcoholic beverages	0.342	-0.129	0.189

Table 2 shows that three dietary patterns have emerged. The first factor is referred to as unhealthy dietary patterns, which consists of ten highly loaded food groups such as staple foods cooked in oil, fast food, processed foods, fried foods, traditional snacks, jams/honey, sugary drinks, sugary desserts, tea and coffee, and alcoholic beverages. The second factor, labeled mixed diet pattern, includes seven types of food comprising high consumption of rice/flour/corn products, root crops, light-colored vegetables, meat, poultry, animal organs, and processed fruits. The third factor called a healthy dietary pattern, includes eight food groups consisting of high consumption of whole grains, nuts, soybeans, dairy and processed products, dark-colored vegetables, eggs, fish and seafood, and fruits. Factor 1 explains 12.13% of intake variance, factor 2 explains 7.56% of the variance, and factor 3 explains 7.62% of the variance. The total variance of the three factors was 27.31%, and the eigenvalue of the three factors was greater than 1.5.

Table 3. Test the characteristics of research subjects with diet

Variables	Unhealthy Dietary Pattern				Mixed Dietary Pattern				Healthy Dietary Pattern			
	T1	T2	T3	p	T1	T2	T3	p	T1	T2	T3	p
Age (mean±sd)	50.54± 9.87	60.42± 10.18	58.80± 9.59	0.045	56.10± 9.21	60.48± 9.94	58.18± 10.58	0.091	59.40± 10,13	59.34± 9,7	56.02± 9.98	0.156
Age category												
40-59	66.0	38.0	58.0	0.015	64.0	44.0	54.0	0.134	54.0	50.0	58.0	0.725
≥60	34.0	62.0	42.0		36.0	56.0	46.0		46.0	50.0	42.0	
Sex												
Male	64.0	52.0	38.0	0.034	48.0	60.0	46.0	0.317	42.0	54.0	58.0	0.250
Female	36.0	48.0	62.0		52.0	40.0	54.0		58.0	46.0	42.0	
Weight (kg)	44.14± 1.17	49.93± 4.14	60.24± 5.43	0,000	49.85± 6.32	52.21± 9.40	52.25± 7.17	0.211	51.02± 7.38	52.27± 7.81	51.44± 7.77	0.657
Height (cm)	159.86± 3.57	164.48± 4.02	163.58± 9.47	0.001	163.36 ± 7.05	160.90 ± 6.65	163.66± 5.56	0.069	163.26± 6,85	162.48± 7.06	162.1± 5.78	0.700
BMI (kg/m2)	17.29± 0.68	18.47± 1.57	22.69± 2.93	0.000	18.67± 1.81	20.24± 3.87	19.53± 2.72	0.034	19.15± 2.63	19.87± 3.33	19.42± 3.10	0.484
Illness duration												
≤ 5 years	40.0	28.0	36.0	0.439	44.0	28.0	32.0	0.216	36.0	22.0	46.0	0.040
>5 years	60.0	72.0	64.0		56.0	72.0	68.0		64.0	78.0	54.0	
Waist circumference (cm)	79.26±0.86	81.75± 1.04	89.96± 4.51	0.000	82.84± 4.92	82.01± 5.79	84.11± 5.32	0.419	83.44± 5.17	83.96± 5.58	83.56± 5.29	0.877
Hip circumference(c m)	93.96±2.57	98.13± 0.56	102.32± 3.74	0.000	97.64± 4.55	98.9± 4.32	98.70± 4.06	0.470	98.21± 3.82	98.11± 4.18	98.11± 4.94	0.992
Waist Hip Ratio	0.84± 0.03	0.83± 0.03	0.87± 0.05	0.000	0.84± 0.03	0.86± 0.05	0.85± 0.04	0.464	0.84± 0.03	0.86± 0.04	0.85± 0.04	0.483

% Body fat	22.80±1.60	29.19± 1.85	35.18± 3.17	0.000	27.70± 5.02	29.51± 6.18	29.95± 5.28	0.101	28.88± 5.76	29.46± 5.92	28.83± 5.06	0.824
Muscle Mass Index(kg/m ²)	7.50± 1.78	7.17± 1.29	6.98± 1.66	0.254	7.53± 1.74	7.14± 1.54	6.98± 1.48	0.217	6.92± 1.30	7.05± 1.71	7.68± 1.66	0.037
Muscle strength (kg)	23.15± 3.37	22.43± 4.15	20.51± 3.83	0.512	22.17± 4.03	22.07± 3.47	21.53± 4.17	0.537	20.71± 3.62	21.72± 3.74	23.56± 3.95	0.001
Walking speed (m/s)	0.87± 0.29	0.89± 0.34	0.93± 0.24	0.570	0.93± 0.27	0.87± 0.28	0.89± 0.32	0.537	1.22± 0.21	0.83± 0.13	0.63± 0.14	0.000
Physical activity												
Less	46.0	34.0	42.0		40.0	42.0	40.0		34.0	58.0	30.0	
Moderate	10.0	20.0	12.0	0.567	10.0	14.0	18.0	0.816	18.0	4.0	20.0	
Good	44.0	46.0	46.0		50.0	44.0	42.0		48.0	38.0	50.0	0.018
HbA1c Levels (%)	7.30± 0.78	7.35±0.87	7.42± 0.80	0.790	7.53± 0.84	7.20± 0.73	7.33± 0.85	0.131	8.16± 0.58	7.27± 0.43	6.63± 0.55	0.000
Fasting blood glucose(FPG) (mg/dl)	148.96± 20.59	151.94± 23.15	152.30± 21.56	0.703	152.34 ±21.04	151.12 ± 22.73	149.74± 21.65	0.837	174.26± 10.83	150.94± 9.42	128.00± 11.56	0.000
Blood sugar 2 hours post- prandial(mg/dl)	247.58± 59.08	248.96± 54.83	265.24± 61.97	0.250	258.82 ± 58.21	250.72 ± 56.81	252.24± 62.29	0.768	264.58± 35.57	261.40± 32.24	195.80± 41.55	0.000

Metformin consumption (%)												
Consumed	56.0	58.0	44.0	0.317	56.0	52.0	50.0	0.829	50.0	42.0	66.0	0.050
Not consumed	44.0	42.0	56.0		44.0	48.0	50.0		50.0	58.0	34.0	
Total intake												
Energy (kcal)	869.50± 26.93	1136.59± 35.47	1749.95 ± 43.06	0.000	1173.08± 61.53	1295.2 7± 65.69	1287.69± 62.15	0.312	1276.68± 65.40	1251,28 ± 62.85	1228.08 ± 62.47	0.864
Protein (gr)	50.51± 1.95	49.76± 2.03	48.08± 1.73	0.656	50.53± 1.81	47.60± 1.91	50.21± 1.98	0.494	37.66± 0.98	46.73± 1.04	63.95± 1.30	0.000
Fat (gr)	21.44± 0.95	36.18± 0.56	46.70± 1.47	0.000	32.36± 1.79	35.22± 1.78	36.74±1.84	0.023	35.88± 1.73	34.60± 1.84	33.84± 1.86	0.726
Carbohydrate (gr)	179.82± 3.85	212.60± 2.27	264.54± 4.89	0.000	207.54 ± 6.02	221.57 ± 6.42	227.84± 6.03	0.042	225.19± 5.78	216.97± 7.12	214.80± 5.74	0.466

The value is presented as % for category variables or mean ± SD for continuous variables; *The p value is obtained from the chi-square test for category variables and the anova test for continuous variable.

Table 3 shows the characteristics of study subjects across categories of dietary tertile. The highest tertile (T3) of the three dietary patterns was dominated by study subjects who had suffered from type II diabetes mellitus > 5 years. Significant differences were found between tertiles related to weight, height, and nutritional status in dietary pattern I (unhealthy dietary pattern). In contrast to this dietary pattern, there were no significant differences between tertiles related to weight, height, and nutritional status in dietary pattern 2 (mixed dietary pattern) and dietary pattern 3 (healthy dietary pattern). Table 3 also reveals differences in average energy, protein, fat, and carbohydrate intake between tertile categories of each dietary pattern.

Table 4. Risk Estimation For Sarcopenia and Its Components Across Tertile Dietary Patterns

Variables	Unhealthy Dietary Pattern		Mixed Dietary Pattern		Healthy Dietary Pattern		
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	
Low Muscle Mass Index	T1	1	1	1	1	1	
	T2	1.085 (0.491-2.395)	1.146 (0.244-5.382)	1.273 (0.579-2.795)	1.290 (0.303-5.502)	0.444 (0.199-0.989)	0.542 (0.162-1.807)
	T3	1.496 (0.679-3.294)	1.969 (0.468-8.282)	1.000 (0.454-2.203)	1.342 (0.329-5.474)	0.345 (0.153-0.777)	0.674 (0.192-2.367)
	p	0.569	0.442	0.786	0.641	0.024	0.000
	T1	1	1	1	1	1	1
Low muscle strengths	T2	1.084 (0.493-2.384)	1.180 (0.265-5.252)	1.287 (0.575-2.881)	1.040 (0.290-3.727)	0.663 (0.302-1.472)	0.880 (0.241-3.209)
	T3	1.379 (0.628-3.029)	1.412 (0.357-5.585)	1.090 (0.484-2.455)	1.157 (0.303-4.421)	0.351 (0.152-0.814)	0.510 (0.132-1.972)
	p	0.706	0.519	0.822	0.732	0.044	0.000
	T1	1	1	1	1	1	1
	Low-speed walking	T2	1.278 (0.578-2.825)	1.383 (0.236-8.083)	1.381 (0.627-3.040)	1.120 (0.273-4.590)	0.568 (0.257-1.256)
T3		1.385 (0.627-3.058)	1.953 (0.356-10.712)	1.000 (0.452-2.213)	1.417 (0.349-5.762)	0.343 (0.152-0.775)	0.589 (0.120-2.897)
p		0.703	0.489	0.650	0.571	0.032	0.000
T1		1	1	1	1	1	1
Sarcopenia		T2	1.282 (0.577-2.849)	1.307 (0.239-7.146)	1.278 (0.578-2.852)	1.194 (0.311-4.589)	0.615 (0.279-1.359)
	T3	1.390 (0.626-3.084)	1.508 (0.305-7.452)	1.086 (0.489-2.411)	1.297 (0.341-4.931)	0.375 (0.167-0.842)	0.584 (0.070-4.865)
	p	0.700	0.612	0.827	0.704	0.032	0.000

Table 4 shows the Odds Ratio (OR) for sarcopenia and its components across tertile dietary patterns. The highest tertile of an unhealthy dietary pattern has a greater chance of sarcopenia incidence than other tertiles. There was no significant association with sarcopenia in mixed dietary patterns. A healthy dietary pattern has a lower chance of developing sarcopenia and its components.

In this study, three main dietary patterns were identified using factor analysis techniques: dietary pattern 1 (unhealthy dietary pattern), dietary pattern 2 (mixed diet pattern), and dietary pattern 3 (healthy dietary pattern). The study found no significant association between dietary

pattern 1 (unhealthy dietary pattern) and the incidence of sarcopenia, even after controlling for potential confounders such as gender, age, nutritional status, physical activity, duration/length of suffering from type II diabetes mellitus, HbA1C levels, and metformin consumption habits. This dietary pattern is similar to the Western dietary pattern and is known to have a high content of fast food, sweet foods, oily foods, and alcohol consumption, which can reduce muscle mass, and strength, and increase inflammatory mediators (Bagheri et al., 2021).

Dietary pattern 2 (mixed dietary pattern) is characterized by high levels of consumption of rice/flour/corn products, root crops, light-colored vegetables, meat, poultry, animal organs, and processed fruits. However, adherence to this pattern was not associated with a decrease in the likelihood of sarcopenia even after adjusting for confounding variables (Ganapathy & Nieves, 2020). Several previous studies have shown that animal protein as a source of essential amino acids did not significantly affect the increase in muscle mass (Granic et al., 2020) and that diets high in saturated fat can influence the occurrence of sarcopenia due to increased catabolism such as inflammation and oxidative stress as well as fat accumulation leading to muscle aging (Enos et al., 2013).

In contrast to dietary pattern 1 and dietary pattern 2, adherence to dietary pattern 3 (healthy dietary pattern) was found to significantly reduce the likelihood of sarcopenia, even after adjusting for other possible confounding variables. This healthy diet pattern is similar to the Mediterranean diet, and several studies have reported positive effects on reducing the risk of chronic diseases such as diabetes mellitus (Bagheri et al., 2021). Results from this study also showed that participants who consumed fruits, nuts, and dairy daily had a lower risk of sarcopenia. Fruits and nuts provide abundant antioxidants, which can contribute to reducing oxidative stress and preventing or reducing muscle breakdown (Nishikawa et al., 2021). Legumes are also rich in vegetable proteins, unsaturated fatty acids, phytochemicals, vitamins, and minerals, which may act synergistically to prevent and manage sarcopenia in older adults (Del Río-Celestino & Font, 2020). Therefore, plant-derived protein can be the most appropriate source to meet protein requirements in older adults with or at risk of type II diabetes mellitus (Göbl & Tura, 2022).

The study also examined the relationship between diet and the three components of sarcopenia by comparing the average levels of those components across the tertiles of each diet. In this study found no significant relationship between unhealthy dietary pattern and mixed dietary pattern with low muscle mass index, muscle strength and walking speed even after adjusting for confounding variables. However, in the case of healthy dietary pattern the direction of the relationship is significant as expected. Muscle mass index, muscle strength, and walking speed increase with more adherence to the diet. A longitudinal study found that greater adherence to a "westernized" dietary pattern was associated with an increased risk of muscle mass, lower muscle strength and slow walking speed, after a three-and-a-half-year follow-up period (Talegawkar et al., 2012). In another study, men with a diet high in red meat had poorer physical performance than those with a low meat pattern (Oh et al., 2014).

The study also found that animal protein intake was not significantly associated with sarcopenia, and in fact, a diet high in animal protein was found to have an insignificant association with sarcopenia. However, animal protein intake was associated with an increased risk of overall type 2 diabetes mellitus compared to vegetable protein intake. Foods high in saturated fats, such as red meat and butter, were found to increase the prevalence of sarcopenia compared to a relatively healthy diet of unsaturated fats, olive oil, and vegetable fats.

Dietary pattern 3 (healthy dietary pattern) as a whole is better in maintaining muscle mass, muscle strength, and walking speed than diet pattern 1 (unhealthy dietary pattern) and dietary pattern 2 (mixed dietary pattern). A cross-sectional study found that in women, higher fruit and vegetable variation scores were associated with higher mid-arm muscle areas (Kojima

et al., 2015). Another cross-sectional study found that adherence to healthy dietary patterns such as the Mediterranean dietary was associated with better muscle mass outcomes in women, but not in men (Shahar et al., 2012). The intake of alkaline-producing foods such as fruits and vegetables contained in this dietary pattern is also known to prevent acidosis and as a consequence maintain muscle mass and strength during aging and prevent the possibility of sarcopenia (Papadopoulou et al., 2023). One cross-sectional study found a healthier diet was significantly associated with higher hand grip strength in women but not men. A healthier variety of foods is associated with a lower risk of decreased grip strength in the future (Bollwein et al., 2013). High adherence to healthy dietary patterns such as the Mediterranean has also been linked to better walking speed, better physical function, and a slower decline in mobility over time (Bagheri et al., 2021). Two cross-sectional studies found a link between adherence to healthy dietary patterns such as the Mediterranean diet and faster walking speed (better physical performance) (León-Muñoz et al., 2015) (Xu et al., 2012). A healthier variety of foods was also associated with a lower risk for a decrease in walking speed in the future (Bollwein et al., 2013).

Strengths and limitations. This study supports further information about the relationship between dietary patterns and the incidence of sarcopenia in people with type 2 diabetes mellitus in adult and elderly populations. The limitations in this study are first, the nature of the cross-sectional study limits the ability to generalize the results of this study. Second, due to budget constraints, researchers used BIA for muscle mass estimation, while computerized tomography (CT scan), magnetic resonance imaging (MRI) and dual-energy X-ray absorptiometry (DEXA) were more accurate than BIA. Nevertheless, this method is considered a valid and reliable method and is generally applied in previous studies. Third, it should also be remembered that loss of muscle mass and muscle strength is different in men and women, so an accurate gender stratification analysis is needed to determine the relationship between dietary patterns with muscle mass and muscle strength. However, due to the small sample size and given that separating the sexes in the analysis resulted in a small number of people in each category, researchers did not conduct such an analysis.

4. CONCLUSION

It has been found that dietary patterns can play a crucial role in managing sarcopenia in patients with type II diabetes mellitus. Studies have shown that a healthy diet has a protective relationship with sarcopenia and its components. However, there is no relationship between an unhealthy dietary pattern and a mixed dietary pattern with sarcopenia and its components. There is a higher chance of sarcopenia incidence with these dietary patterns.

This study suggests that a healthy dietary pattern, similar to the Mediterranean diet, can help prevent or reduce the risk of sarcopenia in older adults. Plant-derived protein may be the most appropriate source to ascertain protein requirements in older adults with or at risk of type II diabetes mellitus. Fruits, nuts, and legumes provide abundant antioxidants and nutrients that can contribute to reducing oxidative stress and preventing or reducing muscle breakdown.

ACKNOWLEDGMENTS

The authors express their gratitude to the Ministry of Health of the Republic of Indonesia, specifically the directorate general of health workers, as well as the Belu district government in NTT for their support in the author's achievement of the M.Gizi degree. In addition, the author was also given scholarships by the Ministry of Health of the Republic of Indonesia through the Directorate General of Health Workers.

REFERENCES

- Bagheri, A., Hashemi, R., Heshmat, R., Motlagh, A. D., & Esmailzadeh, A. (2021). Patterns of nutrient intake in relation to sarcopenia and its components. *Frontiers in Nutrition*, 8, 645072. <https://doi.org/10.3389/fnut.2021.645072>
- Bollwein, J., Diekmann, R., Kaiser, M. J., Bauer, J. M., Uter, W., Sieber, C. C., & Volkert, D. (2013). Dietary quality is related to frailty in community-dwelling older adults. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*, 68(4), 483–489. <https://doi.org/10.1093/gerona/gls204>
- Borba, V. Z. C., Costa, T. L., Moreira, C. A., & Boguszewski, C. L. (2019). Mechanisms of Endocrine Disease: Sarcopenia in endocrine and non-endocrine disorders. *European journal of endocrinology*, 180(5), R185-R199. <https://doi.org/10.1530/EJE-18-0937>
- Carolina, & Wahju, E. H. (2020). Kajian Agroekologi terhadap Strategi Pemenuhan Kebutuhan Pangan Masyarakat di Kabupaten Belu Nusa Tenggara Timur. *Pangan*, 25(2), 83–94.
- Chen, L. K., Woo, J., Assantachai, P., Auyeung, T. W., Chou, M. Y., Iijima, K., Jang, H. C., Kang, L., Kim, M., Kim, S., Kojima, T., Kuzuya, M., Lee, J. S. W., Lee, S. Y., Lee, W. J., Lee, Y., Liang, C. K., Lim, J. Y., Lim, W. S., ... Arai, H. (2020). Asian Working Group for Sarcopenia: 2019 Consensus Update on Sarcopenia Diagnosis and Treatment. *Journal of the American Medical Directors Association*, 21(3), 300-307.e2. <https://doi.org/10.1016/j.jamda.2019.12.012>
- Chen, X., Hou, L., Zhang, Y., & Dong, B. (2021). Analysis of the Prevalence of Sarcopenia and Its Risk Factors in the Elderly in the Chengdu Community. *Journal of Nutrition, Health and Aging*, 25(5), 600–605. <https://doi.org/10.1007/s12603-020-1559-1>
- Cruz-Jentoft, A. J., Bahat, G., Bauer, J., Boirie, Y., Bruyère, O., Cederholm, T., Cooper, C., Landi, F., Rolland, Y., Sayer, A. A., Schneider, S. M., Sieber, C. C., Topinkova, E., Vandewoude, M., Visser, M., Zamboni, M., Bautmans, I., Baeyens, J. P., Cesari, M., ... Schols, J. (2019). Sarcopenia: Revised European consensus on definition and diagnosis. In *Age and Ageing* (Vol. 48, Issue 1, pp. 16–31). Oxford University Press. <https://doi.org/10.1093/ageing/afy169>
- Del Río-Celestino, M., & Font, R. (2020). The health benefits of fruits and vegetables. *Foods*, 9(3), 1–4. <https://doi.org/10.3390/foods9030369>
- Dominguez, L. J., Veronese, N., Baiamonte, E., Guarrera, M., Parisi, A., Ruffolo, C., ... & Barbagallo, M. (2022). Healthy aging and dietary patterns. *Nutrients*, 14(4), 889. <https://doi.org/10.3390/nu14040889>
- Enos, R. T., Davis, J. M., Velázquez, K. T., McClellan, J. L., Day, S. D., Carnevale, K. A., & Murphy, E. A. (2013). Influence of dietary saturated fat content on adiposity, macrophage behavior, inflammation, and metabolism: Composition matters. *Journal of Lipid Research*, 54(1), 152–163. <https://doi.org/10.1194/jlr.M030700>
- Ganapathy, A., & Nieves, J. W. (2020). *Nutrition and Sarcopenia—What Do We Know?* 12(6), 1755. <https://doi.org/10.3390/nu12061755>
- Göbl, C., & Tura, A. (2022). Focus on Nutritional Aspects of Sarcopenia in Diabetes: Current Evidence and Remarks for Future Research. *Nutrients*, 14(2), 1–7. <https://doi.org/10.3390/nu14020312>
- Granic, A., Jagger, C., Davies, K., Adamson, A., Kirkwood, T., Hill, T. R., Siervo, M., Mathers, J. C., & Sayer, A. A. (2016). Effect of dietary patterns on muscle strength and physical performance in the very old: Findings from the newcastle 85+ study. *PLoS ONE*, 11(3), 1–17. <https://doi.org/10.1371/journal.pone.0149699>
- Granic, A., Mendonça, N., Sayer, A. A., Hill, T. R., Davies, K., Siervo, M., Mathers, J. C., & Jagger, C. (2020). Effects of dietary patterns and low protein intake on sarcopenia risk in

- the very old: The Newcastle 85+ study. *Clinical Nutrition*, 39(1), 166–173. <https://doi.org/10.1016/j.clnu.2019.01.009>
- Kementerian Kesehatan RI. (2014). *Peraturan Menteri Kesehatan Republik Indonesia Nomor 41 Tentang Pedoman Gizi Seimbang*. Jakarta: Kementerian Kesehatan Republik Indonesia
- Kojima, N., Kim, M., Saito, K., Yoshida, H., Yoshida, Y., Hirano, H., Obuchi, S., Shimada, H., Suzuki, T., & Kim, H. (2015). Lifestyle-related factors contributing to decline in knee extension strength among elderly women: A cross-sectional and longitudinal cohort study. *PLoS ONE*, 10(7), 1–13. <https://doi.org/10.1371/journal.pone.0132523>
- León-Muñoz, L. M., García-Esquinas, E., López-García, E., Banegas, J. R., & Rodríguez-Artalejo, F. (2015). Major dietary patterns and risk of frailty in older adults: A prospective cohort study. *BMC Medicine*, 13(1), 1–9. <https://doi.org/10.1186/s12916-014-0255-6>
- Mayhew, A. J., Amog, K., Phillips, S., Parise, G., McNicholas, P. D., De Souza, R. J., ... & Raina, P. (2019). The prevalence of sarcopenia in community-dwelling older adults, an exploration of differences between studies and within definitions: a systematic review and meta-analyses. *Age and ageing*, 48(1), 48-56. <https://doi.org/10.1093/ageing/afy106>
- Nishikawa, H., Fukunishi, S., Asai, A., Yokohama, K., Ohama, H., Nishiguchi, S., & Higuchi, K. (2021). Sarcopenia, frailty and type 2 diabetes mellitus (Review). *Molecular Medicine Reports*, 24(6), 1–8. <https://doi.org/10.3892/mmr.2021.12494>
- Nugraha, G. (2022). *Teknik Pengambilan dan Penanganan Spesimen Darah Vena Manusia untuk Penelitian*. LIPI Press.
- Oh, C., No, J. K., & Kim, H. S. (2014). Dietary pattern classifications with nutrient intake and body composition changes in Korean elderly. *Nutrition Research and Practice*, 8(2), 192–197. <https://doi.org/10.4162/nrp.2014.8.2.192>
- Omage, K., & Omuemu, V. O. (2018). Assessment of dietary pattern and nutritional status of undergraduate students in a private university in southern Nigeria. *Food Science and Nutrition*, 6(7), 1890–1897. <https://doi.org/10.1002/fsn3.759>
- Papadopoulou, S. K., Detopoulou, P., Voulgaridou, G., Tsoumana, D., Spanoudaki, M., Sadikou, F., Papadopoulou, V. G., Zidrou, C., Chatziprodromidou, I. P., Giaginis, C., & Nikolaidis, P. (2023). Mediterranean Diet and Sarcopenia Features in Apparently Healthy Adults over 65 Years: A Systematic Review. *Nutrients*, 15(5), 1–17. <https://doi.org/10.3390/nu15051104>
- Shahar, D. R., Houston, D. K., Hue, T. F., Lee, J. S., Sahyoun, N. R., Tyllavsky, F. A., Geva, D., Vardi, H., & Harris, T. B. (2012). Adherence to mediterranean diet and decline in walking speed over 8 years in community-dwelling older adults. *Journal of the American Geriatrics Society*, 60(10), 1881–1888. <https://doi.org/10.1111/j.1532-5415.2012.04167.x>
- Shaikh, N., Harshitha, R., & Bhargava, M. (2020). Prevalence of sarcopenia in an elderly population in rural South India: a cross-sectional study. *F1000Research*, 9, 175. <https://doi.org/10.12688/f1000research.22580.1>
- Shang, X., Scott, D., Hodge, A. M., English, D. R., Giles, G. G., Ebeling, P. R., & Sanders, K. M. (2016). Dietary protein intake and risk of type 2 diabetes: Results from the Melbourne Collaborative Cohort Study and a meta-analysis of prospective studies. *American Journal of Clinical Nutrition*, 104(5), 1352–1365. <https://doi.org/10.3945/ajcn.116.140954>
- Snetselaar, L. G., de Jesus, J. M., DeSilva, D. M., & Stoody, E. E. (2021). Dietary Guidelines for Americans, 2020-2025: Understanding the Scientific Process, Guidelines, and Key Recommendations. *Nutrition today*, 56(6), 287–295. <https://doi.org/10.1097/NT.0000000000000512>

- Sun, T., Ma, Z., Gao, L., Wang, Y., & Xie, H. (2021). Correlation between Sarcopenia and Arteriosclerosis in Elderly Community Dwellers: A Multicenter Study. *Journal of Nutrition, Health and Aging*, 25(5), 692–697. <https://doi.org/10.1007/s12603-021-1624-4>
- Syaury, A., Hsu, C. Y., Rau, H. H., & Chao, J. C. J. (2018a). Association of dietary patterns, anthropometric measurements, and metabolic parameters with C-reactive protein and neutrophil-to-lymphocyte ratio in middle-aged and older adults with metabolic syndrome in Taiwan: A cross-sectional study. *Nutrition Journal*, 17(1), 1–12. <https://doi.org/10.1186/s12937-018-0417-z>
- Syaury, A., Hsu, C. Y., Rau, H. H., & Chao, J. C. J. (2018b). Association of dietary patterns with components of metabolic syndrome and inflammation among middle-aged and older adults with metabolic syndrome in Taiwan. *Nutrients*, 10(2), 1–12. <https://doi.org/10.3390/nu10020143>
- Talegawkar, S. A., Bandinelli, S., Bandeen-Roche, K., Chen, P., Milaneschi, Y., Tanaka, T., Semba, R. D., Guralnik, J. M., & Ferrucci, L. (2012). A higher adherence to a mediterranean-style diet is inversely associated with the development of frailty in community-dwelling elderly men and women. *Journal of Nutrition*, 142(12), 2161–2166. <https://doi.org/10.3945/jn.112.165498>
- Tan, V. M. H., Pang, B. W. J., Lau, L. K., Jabbar, K. A., Seah, W. T., Chen, K. K., Ng, T. P., & Wee, S. L. (2021). Malnutrition and Sarcopenia in Community-Dwelling Adults in Singapore: Yishun Health Study. *Journal of Nutrition, Health and Aging*, 25(3), 374–381. <https://doi.org/10.1007/s12603-020-1542-x>
- VandeBunte, A., Gontrum, E., Goldberger, L., Fonseca, C., Djukic, N., You, M., ... & Casaletto, K. B. (2022). Physical activity measurement in older adults: Wearables versus self-report. *Frontiers in Digital Health*, 4, 869790. <https://doi.org/10.3389/fdgth.2022.869790>
- Wang, T., Feng, X., Zhou, J., Gong, H., Xia, S., Wei, Q., Hu, X., Tao, R., Li, L., Qian, F., & Yu, L. (2016). Type 2 diabetes mellitus is associated with increased risks of sarcopenia and pre-sarcopenia in Chinese elderly. *Scientific Reports*, 6. <https://doi.org/10.1038/srep38937>
- Xu, B., Houston, D. K., Locher, J. L., Ellison, K. J., Gropper, S., Buys, D. R., & Zizza, C. A. (2012). Higher healthy eating index-2005 scores are associated with better physical performance. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*, 67 A(1), 93–99. <https://doi.org/10.1093/gerona/qlr159>
- Yakout, S. M., Alkahtani, S. A., Al-Disi, D., Aljaloud, K. S., Khattak, M. N. K., Alokail, M. S., Reginster, J. Y., Sabico, S., & Al-Daghri, N. M. (2019). Coexistence of Pre-sarcopenia and Metabolic Syndrome in Arab Men. *Calcified Tissue International*, 104(2), 130–136. <https://doi.org/10.1007/s00223-018-0477-2>

Jurnal Info Kesehatan

Vol. 22, No. 2, June 2024, pp. 235-243

P-ISSN 0216-504X, E-ISSN 2620-536X

DOI: [10.31965/infokes.Vol22.Iss2.1505](https://doi.org/10.31965/infokes.Vol22.Iss2.1505)Journal homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>**RESEARCH****Open Access****Husband Support and Coping Stress on Exclusive Breastfeeding in Primipara****Roganda Simanjuntak^{1a*}, Diadjeng Setya Wardani^{1b}, I Wayan Agung Indrawan^{1c}, Fariha Nuzulul Hinisa^{1d}**¹ Master Program of Midwifery, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia^a Email address: rogandasimanjuntak773@gmail.com^b Email address: diadjeng_wardani@ub.ac.id^c Email address: dr_iwa.fl@ub.ac.id^d Email address: farihanh@student.ub.ac.id

Received: 16 May 2024

Revised: 2 June 2024

Accepted: 7 June 2024

Abstract

Breastfeeding is the best strategy to ensure optimal growth and development for infants. Social support, fatigue, and stress are the main factors that hinder breastfeeding for primiparous mothers. This cross-sectional study analyzes husband support and coping stress of exclusive breastfeeding for primiparous mothers with a quantitative approach. The samples were 70 respondents taken with a total sampling technique. The research instruments were the husband support questionnaire and coping strategies inventory. The researchers analyzed the data with chi-square and multiple logistic regression. The results found 48 (68.5%) mothers with excellent husband support and exclusive breastfeeding. Meanwhile, mothers with excellent husband support but without exclusive breastfeeding were 18 respondents (25.71%). The results of statistical tests with a significance level of 5% obtained a sig value (p-value) of husband support (p=0.002) and coping stress p=0.000. The result indicates a correlation between husband support and coping stress on exclusive breastfeeding with OR value = (3.667). On the other hand, mothers with excellent husband support could provide exclusive breastfeeding 4 times higher than those with poor husband support. Husband support and coping stress affect exclusive breastfeeding of primiparous mothers in the employing area of Puskesmas Dinoyo Malang City. Coping stress is the dominant factor influencing exclusive breastfeeding in primiparous mothers in the employing area of Puskesmas Dinoyo Malang City. The research recommends public health centers improve programs that support maternal care in helping to improve skills in managing stress, on exclusive breastfeeding both at the public health center, the integrated public health service, and the auxiliary health center.

Keywords: Exclusif Breastfeeding, Coping Stress, Husband Support, Primipara.***Corresponding Author:**

Roganda Simanjuntak

Master Program of Midwifery, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia

Email: rogandasimanjuntak773@gmail.com

©The Author(s) 2024. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

1. INTRODUCTION

Breast milk is an essential and dominant nutrient for infants in early life. Breastfeeding is the best strategy to provide necessities for infant growth and development (Nurek et al., 2021). Breastfeeding for six months to two years to reduce morbidity and mortality (Sukatin et al., 2022). Exclusively breastfed infants only receive breast milk without other meals or fluids (Nurek et al., 2021). Exclusive breastfeeding has an impact on the emotional relationship between infants and mothers (Kementerian Kesehatan Republik Indonesia, 2022).

Primiparous mothers experience barriers that can prevent them from exclusively breastfeeding, including lack of knowledge, difficulty with breastfeeding techniques, milk production, social support, and fatigue (Fukui, et al., 2021). Primiparous mothers also feel anxious and uncertain about their capability to breastfeed and correct breastfeeding techniques (Hörnell et al., 1999). These situations may influence mothers' confidence including interfering the breastfeeding. Primiparous mothers are often worried that the baby lacks nutritional intake from breast milk. They are also worried and may feel unable to provide adequate care for the babies, such as suffering from underweight (Goger et al., 2020). Primiparous mothers feel uncomfortable when breastfeeding in public because they will be visible from the crowd. The mothers may also find this situation could lead to public judgment toward their breastfeeding capability. Some mother may find it inconvenient to expose their body parts which are different from their young ages. This situation may influence their confidence in breastfeeding (Hauck et al., 2021). Breastfeeding may burden the thoughts of mothers; make them feel fatigued and worried about their physical and emotional fatigue management and interrupt their sleeping quality (Ahmad et al., 2022).

WHO explains the achievement of breastfeeding coverage in the world was 40% in 2022 and is targeting 70% by 2030 (World Health Organization, 2021). Exclusive breastfeeding coverage in Indonesia, based on the Ministry of Health in 2021, reached a percentage of 56.9%. The data also shows that West Nusa Tenggara Province reaches the highest rank with a coverage percentage of 82.4% while the lowest is Maluku at 13% (Kementerian Kesehatan Republik Indonesia, 2022). In Eastern Java, breastfeeding coverage in 2020 reached 79%. The other finding found the breastfeeding coverage percentage in Malang, 2022, was 76.96%. Of this percentage, Dinoyo Health Center had the lowest breastfeeding coverage percentage, 51.18% (Dinas Kesehatan Kota Malang, 2022).

The low coverage may influence both babies and mothers because babies without low breastfeeding coverage may suffer from unstable physical and mental conditions (childbirth trauma), and prevention of ovarian and breast cancer. The trauma of childbirth tends to be greater in primiparous mothers than in other mothers due to swollen and painful breasts, sticky-sensation nipples, and decreased milk production. In addition, primiparous mothers also tend to experience fatigue due to a lack of rest and sleep (Oktafia & Deviana, 2021).

The affected mental state in mothers requires emotional support from the closest person (husband), both emotional support and informational support (Darwiche et al., 2019). This support can relieve the stress level of primiparous mothers so they can be more motivated and feel confident to provide exclusive breastfeeding. However, the high stress level of these mothers may decrease exclusive breastfeeding (Choiriyah & Yudi, 2022). Therefore, primiparous mothers must receive effective coping stress strategies such as getting support from partners, and family, maintaining rest patterns, and attending educational programs of breastfeeding to obtain information (Corby, Kane, & Dayus, 2021). On the other hand, mothers' stress management capability also influences their success and so does the support of the husband. This involvement could improve the quality of breastfeeding and the success of exclusive breastfeeding (Durmazoğlu et al., 2021).

Based on this exposure, the basis for conducting the following research is on the impact of husband support and coping stress on exclusive breastfeeding in primiparous mothers. The purpose of this study was to analyze the relationship between husband support and coping stress on exclusive breastfeeding for primiparous mothers.

2. RESEARCH METHOD

The cross-sectional research took 70 primiparous mothers as the samples with a total sampling technique. The research site was in the performance area of the Dinoyo Health Center in Malang City and was conducted from January 2024 to March 2024. The inclusion criteria are postpartum mothers with exclusive breastfeeding for their babies aged between 6 and 24 months; and mothers with excellent physical and mental health. The applied research instruments were: the PSQI (the Pittsburgh Sleep Quality Score) consisting of 10 items with a Guttman scale (Shahid et al., 2012; Tobbback et al., 2017; Burta, 2018), the husband-support questionnaire consisting of 10 items with a Guttman scale (Wahyuningsih & Machmudah, 2013; Wulandari & Winarsih, 2023; Hani, 2020), the coping strategy inventory consists of 10 items with a Guttman scale, and the exclusive breastfeeding questionnaire consists of one question with a Guttman scale (Bella, Wardhani & Indrawan, 2023). The Cronbach alpha scores of each instrument are consecutively 0.63, 0.60, and 0.9.

The researchers analyzed the univariate, bivariate, and multivariate data. The univariate analysis deals with the frequency distribution; bivariate analysis with Chi-square correlation analysis, and multivariate with multiple logistic regression to examine the influence. This research granted the ethical feasibility of the health research ethics commission of the Faculty of Medicine, Brawijaya University No. 7923/UN.10.F08.14.21/PP/2023.

3. RESULTS AND DISCUSSION

Table 1. The Analysis results of respondent characteristics at the Dinoyo Public Health Center

Variable	Frequency (f)	Percentage (%)
Age		
At risk (<20 and >35 y.o)	1	1,4%
No risk (20-35 y.o)	69	98,6 %
Education		
High	59	82,3%
Low	11	17,7%
Job		
Unemployed	54	77,1%
Employed	16	22,9%
Husband Support		
Poor	4	5,7%
Excellent	66	94,3%
Coping stress		
Poor	19	27,1%
Excellent	51	72,9%
Exclusive Breastfeeding		
Not Exclusive	22	31,4%
Exclusive	48	68,6%

Table 1 confirms that most respondents are not at risky ages, 69 respondents (98.6%). On the other hand, the table shows only 1 respondent at risky age level, between 25 and 35 years old, 1.4%. The highest maternal education was observable on 59 respondents (82.3%) while

the lowest maternal education was observable on 11 respondents (11.7%). The table shows 54 unemployed mothers (77.1%) while 16 mothers are employed, 22.9%. The table also shows excellent husband support for breastfeeding in 66 respondents, (94.3%) while only 4 mothers received poor husband support, (5.7%). 51 respondents or 72.9% have excellent maternal coping stress against the exclusive breastfeeding problem. 19 respondents of 27.1% have poor maternal coping stress against exclusive breastfeeding problems. A total of 48 respondents (68.6%) provided exclusive breastfeeding and only 22 respondents (31.4%) did not.

Table 2. Bivariate analysis of husband support and primipara exclusive breastfeeding at the Dinoyo Primary Health Center

Husband Support	Not Exclusive		Exclusive		Total	p-value*	OR	95% CI Lower-upper
	n	%	n	%				
Poor	4	18,2	0	0	4	0,002	3,667	2,473-5,437
Excellent	18	81,8	48	100	66			
Total	22	100	48	100	70			

The table shows 48 mothers (100%) with excellent husband support and exclusive breastfeeding provision. 18 mothers, 81.8%, have excellent husband support but without exclusive breastfeeding. The significant statistic test, at the 5% level, obtains a p-value of 0.002, lower than 0.05. The result indicates an effect of the husband's support on exclusive breastfeeding with the OR value = (3.667). The interpretation of the table is mothers with optimal husband support tend to provide exclusive breastfeeding 4 times greater than those with poor husband support.

Table 3. Bivariate analysis coping stress and primipara exclusive breastfeeding at the Dinoyo Primary Health Center

Coping stress	Not Exclusive		Exclusive		Total	p-value*	OR	95% CI Lower-upper
	n	%	n	%				
Poor	19	86,4	0	0	19	0,000	17,000	5,671-50,959
Excellent	3	13,6	48	100	51			
Total	22	100	100	100	70			

Table 3 shows 48 mothers, 100%, with excellent coping stress and exclusive breastfeeding. On the other hand, 3 mothers, 13.6%, and mothers with excellent coping stress but not exclusive breastfeeding were 3 respondents (13.6%). The finding of statistical testing with a 5% significance scale obtains a p-value of 0.000 lower than 0.05. The result shows the correlation between coping with stress and exclusive breastfeeding. The OR value = (17.000) indicates mothers with excellent coping stress provide exclusive breastfeeding 17 times higher than those with poor coping stress.

Table 4. The multivariate analysis logistic regression

Variable	p-value*	B-value	OR	(95% CI)	
				Lower	Upper
Husband Support	0,004	4,975	13,532	-3,6976	3,6632
Coping stress	0,000	6,446	17,522	1,5654	8,3853
Constanta		-8,037			

Table 4 shows the variables of husband support and coping stress have a significant effect on exclusive breastfeeding in primiparous mothers at the Dinoyo Health Center area. This is confirmed by the p-value of husband support (0.004), and coping stress (0.000) < α (0.05). The

table shows the p-value of the coping stress is 0.000 lower than 0.05. The result indicates coping stress dominantly influences exclusive breastfeeding at the Dinoyo Public Health Center in Malang. The evidence is the sig-value of 0.000 and OR-value of 17.522.

DISCUSSION

Influence of Husband Support with Exclusive Breastfeeding in Primipara

Husband support is defined as a caring attitude that is intended to foster excellent cooperation. Husband support is a clause of mutual help and has a special value for the wife as a sign of a close bond. Husband support can be in the form of psychological help in the form of motivation, attention, and material support, supporting the wife by helping directly or providing several facilities to facilitate the wife's activities and reduce maternal stress because lack of husband support is a risk factor for postpartum anxiety and depression (Darwichea et al., 2019).

The test results show that the research hypothesis is accepted or there is a relationship between husband support and exclusive breastfeeding in primiparous mothers. Optimal husband support is associated with increased exclusive breastfeeding, the higher the level of husband support, the higher the likelihood of primiparous mothers providing exclusive breastfeeding. This study is in line with previous research which confirms that the husband's positive attitude towards breastfeeding greatly influences the mother's intention for exclusive breastfeeding (Gebremariam et al., 2021). Mothers who feel that their partners support breastfeeding tend to stop breastfeeding less when compared to mothers who feel that their partners are hesitant to support breastfeeding. In addition, the husband's physical and emotional support for his partner has a positive impact on exclusive breastfeeding, therefore involving the husband in breastfeeding is very supportive of the success of exclusive breastfeeding. A significant relationship was also suggested in a previous study that the influence of attitudes and support as well as the involvement of husbands/partners in maternal behavior in exclusive breastfeeding is very necessary to make decisions to continue exclusive breastfeeding after childbirth (Han et al., 2023).

Research on family support found that husband support has an important role in exclusive breastfeeding in the form of knowledge, positive attitudes, involvement in decision-making, practical support, and emotional support for exclusive breastfeeding. Husband support can escalate the mother's motivation and confidence in exclusive breastfeeding for infants and the success of exclusive breastfeeding practices. Another study found that family support and husband support simultaneously influenced exclusive breastfeeding (Marks et al., 2018)

In Minangkabau families, mothers have an important role in deciding on exclusive breastfeeding, and husband support is a crucial aspect of the procession of supporting exclusive breastfeeding. The results of the study are also related to those revealed in the study that when pregnant women and husbands agree on exclusive breastfeeding until the age of 6 months, the baby will likely receive breast milk longer, this shows that the support of the spouse in supporting the mother's intention to provide exclusive breastfeeding, so that the agreement of the husband and wife regarding the intention to provide breast milk can increase the initiation and duration of exclusive breastfeeding (Marks et al., 2018).

In the research area at the Dinoyo Health Center in Malang City, it can be concluded that the presence of a husband's support, especially support in the form of assessment or appreciation, can cause breastfeeding mothers to feel more valued, listened to and given more attention at home setting. This situation positively influences the practice of exclusive breastfeeding on infants.

The Effect of Coping Stress with Exclusive Breastfeeding in Primipara

Coping stress is a strategy used by breastfeeding mothers to overcome stress during exclusive breastfeeding because the breastfeeding process can be stressful and challenging for primiparous mothers, especially those with first-time breastfeeding process experience. An individual with stress management requires some steps to relieve the negative consequences of the breastfeeding process (Shiraishi et al., 2020).

The partial test results confirmed that the stress-coping variable had a significant impact on the exclusive breastfeeding variable of primiparous mothers. Swastiningsih, (2014) also found the mother's capability to cope with stress during breastfeeding influenced exclusive breastfeeding. Thus, mothers must manage their stresses effectively to provide excellent exclusive breastfeeding for babies.

Another study also found the influence of maternal stress toward the stopped exclusive breastfeeding in primiparous mothers. Therefore, effective techniques are important to provide exclusive breastfeeding. Examples of the strategies include mental and emotional preparations by organizing a breastfeeding schedule, seeking information to overcome problems for employed and unemployed mothers, seeking emotional support from spouses, family, and friends by sharing the encountered problems, and maintaining a balance between profession and private life to realize mental health and exclusive breastfeeding provisions (Islami et al., 2021).

Research also found that high levels of stress in the postpartum period could increase the risk of early termination of exclusive breastfeeding. Stress can also affect the level of confidence in breastfeeding by decreasing oxytocin release, interfering with the milk ejection reflex, and lowering confidence in breastfeeding. In this study coping stress management was carried out by providing education to pregnant women and postpartum women regarding exclusive breastfeeding, informing mothers about physical and mental changes after childbirth, helping mothers plan for childbirth properly, providing education related to the essence of exclusive breastfeeding, breastfeeding techniques, and the benefits of breast milk for mothers and babies, overcoming problems while breastfeeding, relieving the stress and anxiety of mothers to breastfeed, providing emotional support to help mothers feel heard, understood and supported, and providing positive encouragement for the continuity of exclusive breastfeeding in primiparous mothers (Azizi et al., 2020).

In the research area at Puskesmas Dinoyo Malang City, excellent maternal coping stress has a positive impact on breastfeeding mothers. Mothers with effective coping stress strategies tend to excellently cope with stress in breastfeeding. Effective coping can help mothers stay calm, focused, and motivated to continue to provide exclusive breastfeeding. Adjusting to a new role as a mother and finding ways to cope with stress could facilitate the adaptation of the mothers to the breastfeeding demand.

The most influential coping stress and emotional support on exclusive breastfeeding among primipara

The results of the last multivariate test show the most influential variable toward exclusive breastfeeding at the Dinoyo Health Center in Malang City, namely coping stress. The findings of partial testing confirmed that coping stress had a significant impact on exclusive breastfeeding in primiparous mothers. Then, the coping stress variable has an odds ratio value of 17.522, so the chances of mothers with excellent coping stress tend to provide exclusive breastfeeding to primiparous mothers in the working area at the Dinoyo health center are 17.522 times greater than those with poor coping stress.

The following study found the influence of psychological stress and anxiety during breastfeeding on mental well-being and exclusive breastfeeding administration. Thus, mothers

must manage problems in breastfeeding effectively (Nagel et al., 2023). Therefore, primiparous mothers must have effective coping strategies in managing stress such as seeking social support, namely many experiences and feelings of the closest people or maternal support groups that provide channels to reduce stress and improve psychological well-being, relaxation techniques such as meditation, deep breathing or yoga can relieve the stress of breastfeeding mothers, maintaining a balanced life and paying attention to other aspects such as adequate sleep, healthy diet and regular exercise, and managing time so that mothers could focus on providing exclusive breastfeeding. By applying effective coping stress strategies, mothers can increase their ability to provide exclusive breastfeeding. The evidence is the OR result for emotional support, 17.522.

Emotional support, namely emotional support from husbands can relieve stress in mothers. The evidence is the OR value, 13.532. This situation could increase milk production, positive mood, and psychological support; and assist mothers to feel relaxed and comfortable in breastfeeding. Mothers also need instrumental support, namely helping with household tasks, caring for babies, or giving mothers time to rest can help relieve the mother's workload so that the mothers can focus on breastfeeding. The other support could be informational from the husband to provide knowledge of the benefits of breast milk, correct breastfeeding techniques, and breastmilk management solutions. These matters influence breast milk production. Excellent knowledge can help mothers understand exclusive breastfeeding effectively. Excellent knowledge could also help primiparous mothers feel more confident, comfortable, and motivated to continue providing exclusive breastfeeding to their babies (Pratiwi et al., 2022).

Counseling and psychological support are important to assist mothers in overcoming stress and anxiety in breastfeeding. Providing resources for relaxation techniques such as deep breathing can help mothers overcome stress at the Public Health Center so that primiparous mothers can reach out and obtain optimal results. Supporting mothers' ability to manage stress effectively can influence primiparous mothers' success in exclusively breastfeeding their infants, which can improve the health and well-being of both mothers and their children.

4. CONCLUSION

Husband support and coping stress affect exclusive breastfeeding of primiparous mothers in the Employed area of Puskesmas Dinoyo Malang City. Coping stress is the dominant factor influencing exclusive breastfeeding in primiparous mothers in the Employed area of Puskesmas Dinoyo Malang City. The research recommendations are that it is hoped that the public health center will improve programs that support maternal care in helping to improve skills in managing stress, on exclusive breastfeeding both at the public health center, the integrated public health service, and auxiliary health center so that it can run according to the ministry of health program evenly. Public health centers must improve the husband support for primiparous mothers to share experiences and prenatal counseling related to exclusive breastfeeding especially the emotional support. Thus, they can help mothers understand and anticipate sources of stress and strategies to manage them. A need for postpartum counseling that focuses on stress management, sleep quality, and husband support is important to support the mother's mental health. These efforts are useful to improve the breastfeeding process smoothly. Support from health workers and the surrounding environment is important in facilitating primiparous mothers in providing exclusive breastfeeding.

REFERENCES

- Ahmad, M., Beheshti, Z., Alimoradi, Z., Bahrami, N., Allen, K., & Lissack, K. (2022). Predictors of breastfeeding self-efficacy during the covid-19 pandemic. *Journal of Neonatal Nursing*, 28(5), 349–355. <https://doi.org/10.1016/j.jnn.2021.08.012>
- Azizi, E., Maleki, A., Mazloomzadeh, S., & Pirzeh, R. (2020). Effect of Stress Management Counseling on Self-Efficacy and Continuity of Exclusive Breastfeeding. *Breastfeeding Medicine*, 15(8), 501–508. <https://doi.org/10.1089/bfm.2019.0251>
- Bella, Wardhani, D.S., & Indrawan, I.W.A. (2023). Analisis faktor yang berhubungan dengan pernikahan dini terhadap pemberian asi eksklusif di kabupaten tulungagung. *Magister thesis. Universitas Brawijaya*.
- Choiriyah, F. N., & Yudi, T. H. (2022). Hubungan Dukungan Sosial dan Stres Pada Ibu yang Memberikan Asi Eksklusif. *Seminar Nasional Psikologi UM, Senapih*, 178–190.
- Corby, K., Kane, D., & Dayus, D. (2021). Investigating predictors of prenatal breastfeeding self-efficacy. *Canadian Journal of Nursing Research*, 53(1), 56-63. <https://doi.org/10.1177/0844562119888363>
- Darwiche, J., Milek, A., Antonietti, J. P., & Vial, Y. (2019). Partner support during the prenatal testing period after assisted conception. *Women and Birth*, 32(2), e264-e271. <https://doi.org/10.1016/j.wombi.2018.07.006>
- Dinas Kesehatan Kota Malang. (2022). *Profil Kesehatan Kota Malang Tahun 2022*. Malang: Dinas Kesehatan Kota Malang Kota Malang.
- Durmazoğlu, G., Çiçek, Ö., & Okumuş, H. (2021). The effect of spousal support perceived by mothers on breastfeeding in the postpartum period. *Turkish Archives of Pediatrics*, 56(1), 57–61. <https://doi.org/10.14744/TurkPediatriArs.2020.09076>
- Fukui, N., Motegi, T., Watanabe, Y., Hashijiri, K., Tsuboya, R., Ogawa, M., ... & Someya, T. (2021). Exclusive breastfeeding is not associated with maternal–infant bonding in early postpartum, considering depression, anxiety, and parity. *Nutrients*, 13(4), 1184. <https://doi.org/10.3390/nu13041184>
- Gebremariam, K. T., Zelenko, O., Mulugeta, A., & Gallegos, D. (2021). A cross-sectional comparison of breastfeeding knowledge, attitudes, and perceived partners' support among expectant couples in Mekelle, Ethiopia. *International Breastfeeding Journal*, 16(1), 1–8. <https://doi.org/10.1186/s13006-020-00355-z>
- Goger, P., Rozenman, M., & Gonzalez, A. (2020). Journal of Behavior Therapy and Experimental Psychiatry The association between current maternal psychological control, anxiety symptoms, and emotional regulatory processes in emerging adults. *Journal of Behavior Therapy and Experimental Psychiatry*, 68(February), 101563. <https://doi.org/10.1016/j.jbtep.2020.101563>
- Han, F. L., Ho, Y. J., & McGrath, J. M. (2023). The influence of breastfeeding attitudes on breastfeeding behavior of postpartum women and their spouses. *Heliyon*, 9(3), e13987. <https://doi.org/10.1016/j.heliyon.2023.e13987>
- Hani, R. U. (2020). Hubungan Dukungan Suami Terhadap Keberhasilan Pemberian ASI Eksklusif Pada Ibu Primipara di Wilayah Kerja Puskesmas Pisangan. *Skripsi*. UIN Syarif Hidayatullah Jakarta
- Hauck, Y. L., Bradfield, Z., & Kuliukas, L. (2021). Women's experiences with breastfeeding in public: An integrative review. *Women and Birth*, 34(3), e217-e227. <https://doi.org/10.1016/j.wombi.2020.04.008>
- Hörnell, A., Aarts, C., Kylberg, E., Hofvander, Y., & Gebre-Medhin, M. (1999). Breastfeeding patterns in exclusively breastfed infants: a longitudinal prospective study in Uppsala, Sweden. *Acta paediatrica*, 88(2), 203-211. <https://doi.org/10.1111/j.1651-2227.1999.tb01083.x>

- Islami, M. J., Broidy, L., Baird, K., Rahman, M., & Zobair, K. M. (2021). Early exclusive breastfeeding cessation and postpartum depression: Assessing the mediating and moderating role of maternal stress and social support. *PLoS ONE*, *16*(5 May), 1–19. <https://doi.org/10.1371/journal.pone.0251419>
- Kementerian Kesehatan Republik Indonesia. (2022). *Profil Kesehatan Indonesia 2021*. Jakarta: Kementerian Kesehatan Republik Indonesia.
- Leyn, S. P. W. C. B. (2019). Evaluasi Penerapan Keselamatan dan Kesehatan Kerja (K3)(Studi Kasus di PT. Indokon Raya). Skripsi. Universitas 17 Agustus 1945 Surabaya.
- Marks, E. J., Grant, C. C., De Castro, T. G., Bandara, D. K., Wall, C., & Morton, S. M. B. (2018). Agreement between future parents on infant feeding intentions and its association with breastfeeding duration: Results from the Growing Up in New Zealand cohort study. *International Journal of Environmental Research and Public Health*, *15*(6), 1–16. <https://doi.org/10.3390/ijerph15061230>
- Nagel, E. M., Howland, M. A., Pando, C., Stang, J., Mason, S. M., Fields, D. A., & Demerath, E. W. (2023). *Outcomes: A narrative review*. *44*(2), 215–227. <https://doi.org/10.1016/j.clinthera.2021.11.007> Maternal
- Nurek, J., Buraczyńska-Andrzejewska, B., Kolasa, I., Czyżniewski, B., & Gibas-Dorna, M. (2021). Fatigue, sleepiness, perceived stress, and physical activity in postpartum mothers according to the infants' feeding method. A pilot study. *Pediatrics i Medycyna Rodzinna*, *17*(4), 339–345. <https://doi.org/10.15557/PIMR.2021.0054>
- Oktafia, R., & Deviana, R. (2021). Hubungan Kelelahan Postpartum Dengan Motivasi Pemberian Asi. *Jurnal Kesehatan Mercusuar*, *4*(2), 66–73. <https://doi.org/10.36984/jkm.v4i2.221>
- Pratiwi, B. A., Lesmi, A., Husin, H., Angraiani, W., & Suryani, D. (2022). Does Husband Support Associated with the Duration of Breastfeeding? *Journal of Maternal and Child Health*, *7*(3), 326–333. <https://doi.org/10.26911/thejmch.2022.07.03.09>
- Shahid, A., Wilkinson, K., Marcu, S., & Shapiro, C. M. (Eds.). (2012). *STOP, THAT and one hundred other sleep scales*. Springer Science & Business Media. <https://doi.org/10.1007/978-1-4419-9893-4>
- Shiraishi, M., Matsuzaki, M., Kurihara, S., Iwamoto, M., & Shimada, M. (2020). Post-breastfeeding stress response and breastfeeding self-efficacy as modifiable predictors of exclusive breastfeeding at 3 months postpartum: a prospective cohort study. *BMC Pregnancy and Childbirth*, *20*(1), 1–11. <https://doi.org/10.1186/s12884-020-03431-8>
- Sukatin, S., Nurkhalipah, N., Kurnia, A., Ramadani, D., & Fatimah, F. (2022). Bimbingan Dan Konseling Belajar. *Humantech: Jurnal Ilmiah Multidisiplin Indonesia*, *1*(9), 1278-1285.
- Swastiningsih, N. (2014). Coping strategies on breastfeeding-Employed mother. *International Seminar on Wellbeing Among Vulnerable Groups, January 2014*, 101–107.
- Tobback, E., Behaeghel, K., Hanouille, I., Delesie, L., & Mariman, A. (2017). *Comparison of subjective sleep and fatigue in breast- and bottle-feeding mothers*. *47*(September 2016), 22–27. <https://doi.org/10.1016/j.midw.2017.01.009>
- Wahyuningsih, D., & Machmudah. (2013). Dukungan suami dalam pemberian asi eksklusif. *Jurnal Keperawatan Maternitas*, *1*(2), 93–101.
- Wulandari, S. R., & Winarsih, W. (2023). Hubungan Dukungan Suami Dengan Pemberian ASI Eksklusif. *Jurnal Kesehatan Samodra Ilmu*, *14*(01), 8–12. <https://doi.org/10.55426/jksi.v14i01.245>
- World Health Organization. (2021). *Global Breastfeeding Scorecard, 2021: protecting breastfeeding through bold national actions during the COVID-19 pandemic and beyond*. World Health Organization.

Jurnal Info Kesehatan

Vol. 22, No. 2, June 2024, pp. 244-258

P-ISSN 0216-504X, E-ISSN 2620-536X

DOI: [10.31965/infokes.Vol22.Iss2.1533](https://doi.org/10.31965/infokes.Vol22.Iss2.1533)

Journal homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>



RESEARCH

Open Access

The Effects of Black Garlic on Ovarian Malondialdehyde, Oviduct Muscle, and Endometrial Arterioles in Smoke-Exposed Rats

Noni Fidya Ayu Anandasari^{1a*}, Novalia Kridayanti^{1b}, Ni Ketut Devy Kaspirayanti^{1c}, Tatit Nurseta^{2d}, Tri Yudani Mardining Raras^{3e}, Husnul Khotimah^{4f}, Subandi Reksohusodo^{1,2g}, Kenty Wantri Anita^{5h}, Hendy Setyo Yudhanto⁵ⁱ

¹ Master Program of Midwifery, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia

² Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia

³ Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia

⁴ Faculty of Medical, Universitas Brawijaya, Malang, East Java, Indonesia

⁵ Department of Pathological Anatomy, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia

^a Email address: nonifidyaayu@gmail.com

^b Email address: novalia.kri@gmail.com

^c Email address: devykaspira@student.ub.ac.id

^d Email address: tns_obg.fk@ub.ac.id

^e Email address: daniraras@ub.ac.id

^f Email address: husnul_farmako.fk@ub.ac.id

^g Email address: desobg@gmail.com

^h Email address: kenty_wa@ub.ac.id

ⁱ Email address: hendy.setyo@ub.ac.id

Received: 22 May 2024

Revised: 11 June 2024

Accepted: 19 June 2024

Abstract

The effect of cigarette smoke exposure on reproductive health has been widely studied, showing various physiological disorders induced by free radicals and oxidative stress. This study aimed to assess the protective effect of ethanol extract of black garlic (*Allium sativum*) on some reproductive parameters of female rats *Rattus norvegicus* exposed to subacute cigarette smoke. A proper experimental method with a post-test-only control group design was used, involving 25 rats divided into five groups with three different doses of the extract. The rats were exposed to cigarette smoke and then treated for 28 days. Results showed a significant decrease in ovarian Malondialdehyde levels and increased fallopian tube smooth muscle thickness in the extract-treated group. In addition, black garlic extract successfully increased the number of endometrial arterioles in the group exposed to cigarette smoke and extract, in contrast to the group exposed to cigarette smoke only. This study concludes that black garlic extract has the potential to be a protective agent against oxidative damage in the reproductive system of rats exposed to cigarette smoke, with effectiveness that depends on the dose of extract given. Further studies should investigate the long-term effects and optimal dosing of black garlic extract in humans. Additionally, exploring the molecular mechanisms behind its protective properties could enhance its application in reproductive health management.

Keywords: Black Garlic Extract, Cigarette Smoke, Oviduct Muscle, Malondialdehyde Levels, Endometrial Arterioles.

*Corresponding Author:

Noni Fidya Ayu Anandasari

Master Program of Midwifery, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia

Email: nonifidyaayu@gmail.com



©The Author(s) 2024. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

1. INTRODUCTION

Cigarette smoke has been globally recognized as one of the leading causes of many severe diseases and health complications. According to a study conducted (Widyanti *et al.*, 2020);(Rahma *et al.*, 2019), cigarette smoke contributes to an increased risk of cardiovascular disease, chronic lung disease, as well as various neurological disorders such as Alzheimer's and Parkinson's. In Indonesia, the prevalence of smokers has shown a significant increase from 2011 to 2021, with more than 70 million residents actively smoking, resulting in a large population of secondhand smoke exposure in homes and public spaces (GATS, 2021).

Secondhand smoke is known to be a major risk factor for numerous chronic diseases globally, such as heart disease, reproductive issues, and lung cancer (Widyanti *et al.*, 2020). Cigarette smoke, which contains more than 4800 chemicals, including free radicals, causes significant oxidative stress in the body, which can affect reproductive health, especially in women (Rahma *et al.*, 2019).

Exposure to cigarette smoke has been linked to reduced fertility and a higher likelihood of miscarriage and various pregnancy complications (Trofor *et al.*, 2018). This exposure has severe implications for reproductive health, including decreased pregnancy potential and a high risk of disorders of reproductive organs such as the ovaries and endometrium.

Studies (Ardiana, 2021) show that cigarette smoke can reduce the chances of pregnancy by 30%, disrupt the normal function of reproductive hormones, and induce oxidative stress that damages reproductive cells. Harmful substances in cigarette smoke, including nicotine and carbon monoxide, have been shown to interfere with the expression of hormone receptors such as estrogen and progesterone and growth factors such as VEGF, which are critical for endometrial and ovarian function (Park *et al.*, 2022).

These substances have been linked to various health issues, including cancer. Studies have shown that cigarette smoking is associated with an increased risk of ovarian cancer (Faber *et al.*, 2013), and endometrial carcinoma (Felix *et al.*, 2014). Additionally, cigarette smoke exposure has been found to induce autophagy, dysregulate mitochondrial dynamics, and generate reactive oxygen species, leading to apoptosis and cell damage (Gannon *et al.*, 2013).

Furthermore, research has demonstrated that smoking cigarettes prevents bone-marrow-derived stem cells from being recruited to the uterus (Budani *et al.*, 2021), affecting uterine receptivity and potentially leading to fertility issues. It can also impact the expression of genes involved in the inflammatory response in the uterus, affecting processes essential for uterine growth and remodeling. Moreover, cigarette smoke has been implicated in causing damage to the ovary, inhibiting ovarian follicle growth, and affecting ovarian function (Aningsih *et al.*, 2020).

Recent studies have revealed that the prevalence of smoking in Indonesia is very high, with exposure to cigarette smoke occurring not only in the public environment but also at home, which increases the health risks of secondhand smoke, including women and children. The prevalence of smoking in Indonesia is very high. According to recent data, there are approximately 3,461,279 active female smokers in Indonesia. Additionally, the exposure to secondhand smoke is a significant issue, impacting both women and children in various environments, including homes and public places (Sala & Gotti, 2023; Tobacco Atlas, 2024; World Health Organization, 2024). The damage that secondhand smoke causes to the female reproductive system is a severe public health issue that requires effective intervention strategies.

Research in Indonesia shows an urgent need to address the health problems caused by cigarette smoke, especially among women who are at risk of infertility and other pregnancy complications due to oxidative stress and hormonal disruption induced by nicotine and other toxic substances in cigarette smoke (Kida *et al.*, 2021).

Faced with this problem, black garlic (*Allium sativum*) in ethanol extract may offer a potential solution. Black garlic, known for its high antioxidant content, "has shown potential in reducing oxidative damage induced by free radicals found in cigarette smoke" (Lu *et al.*, 2017). Previous studies have demonstrated that black garlic can improve antioxidant status and reduce biomarkers of oxidative damage in animal models and humans (Agustina *et al.*, 2020).

Therefore, this study aimed to evaluate the protective effect of ethanol extract of black garlic (*Allium sativum*) against the negative impact of cigarette smoke on the reproductive system of rats. Black garlic, a fermented form of garlic, is known to have a high concentration of antioxidants that can fight free radicals and potentially reduce oxidative damage to reproductive tissues (Lu *et al.*, 2017).

This study is expected to provide new insights into the potential use of black garlic as a mitigation strategy against the damaging effects of cigarette smoke, especially on Malondialdehyde levels, oviduct smooth muscle thickness, and the number of endometrial arterioles in animal models exposed to subacute cigarette smoke.

The results of this study are expected to provide new insights into the application of herbal medicine as a supporting therapy to protect reproductive organs from the adverse effects of cigarette smoke, offering a safer and more sustainable alternative therapy to reduce the health impacts caused by smoking habits that are still high in Indonesia.

2. RESEARCH METHOD

This research presents a unique laboratory experimental study that utilizes a 'post-test only control group' approach. The novelty lies in its focus on the direct effect of ethanol extract of black garlic on rats exposed to subacute cigarette smoke. The study was conducted at the Pharmacology Laboratory, Anatomical Pathology Laboratory Faculty of Medicine Brawijaya University, and Laboratorium Herbal Medika Batu City, from December 2024 to March 2024. The entire experimental procedure was approved by the Ethics Committee of the Faculty of Medicine, Brawijaya University, with ethical number 367/EC/KEPK-S2/11/2023.

The study population included 25 female Wistar strain white rats (*Rattus norvegicus*), aged 8-10 weeks with body weights between 150-200 grams, randomly selected into five groups of five animals each. The groups included one negative control group that was not exposed to cigarette smoke or extract, one positive control group that was only exposed to cigarette smoke, and three experimental groups that were exposed to cigarette smoke and given black garlic extract at doses of 50 mg/kgBW, 100 mg/kgBW, and 150 mg/kgBW. Each group was exposed to cigarette smoke for five minutes per session, two sessions per day for 28 days. The following is the division of the groups and the duration of exposure:

- 1) CN (Negative Control): This group was not exposed to cigarette smoke and was not given black garlic extract.
- 2) CP (Positive Control): Exposed to cigarette smoke twice a day for five minutes per session for 28 days without black garlic extract.
- 3) P1 (Treatment 1): Exposed to cigarette smoke twice a day for five minutes per session and given a dose of 50 mg/kgBW black garlic extract for 28 days.
- 4) P2 (Treatment 2): Exposed to cigarette smoke twice a day for five minutes per session and given a dose of 100 mg/kgBW black garlic extract for 28 days.
- 5) P3 (Treatment 3): Exposed to cigarette smoke twice a day for five minutes per session and given a 200 mg/kgBW dose of black garlic extract for 28 days.

The data were meticulously measured through analysis of Malondialdehyde levels with the Thiobarbituric Acid Reactive Substances (TBARS) method, ovarian smooth muscle thickness through histopathological analysis using Hematoxylin and Eosin (H&E) staining, and

the number of endometrial arterioles observed using light microscopy. To ensure the reliability of the findings, statistical analysis was performed using ANOVA to compare between groups, followed by Tukey's post hoc test if significant differences were found, with $p < 0.05$ as the significance limit.

Data presentation will include tables and graphs for straightforward interpretation of results and experimental flow charts and histological images to support the analysis. The tools used in this study include standard laboratory rat cages, a cigarette smoke machine that can control the dose and duration of exposure, ethanol extract of black garlic produced through the extraction of fermented black garlic with 70% ethanol, as well as a Thiobarbituric Acid Reactive Substances (TBARS) kit, microtome, Hematoxylin and Eosin (H&E) staining material, and microscope for histopathology analysis. Data were analyzed using statistical SPSS software to test for significant differences between groups.

3. RESULTS AND DISCUSSION

This study involved 25 female Wistar white rats divided into five study groups, each with five rats. The rats were exposed to two cigarette smoke cigarettes daily for 28 days and given different doses of black garlic extract.

Table 1. Normality and Homogeneousity Test of Malondialdehyde Level Data

Test	p-value	Description
Normality	0.207	Normal
Homogeneousity	0.159	Homogeneous

Table 1 shows that data normal and homogeneous distribution fulfills the prerequisites for further statistical analysis.

Table 2. Comparison of Test Results of Malondialdehyde Levels

Group	n	Mean \pm SD (μ M)	p-value
CN	5	1.157 \pm 0.60 ^a	0.000
CP	5	4.787 \pm 0.70 ^{cd}	
P1	5	2.674 \pm 1.70 ^a	
P2	5	3.980 \pm 0.27 ^{bc}	
P3	5	6.200 \pm 1.54 ^d	

Notes: CN: Negative control group, not exposed to cigarette smoke. CP: Positive control group, only exposed to cigarette smoke. P1, P2, P3: Groups exposed to cigarette smoke were given 50, 100, and 200 mg/kgBW of black garlic extract, respectively. p-value < 0.05 indicates a significant difference.

Table 2, One-way ANOVA analysis showed significant differences in Malondialdehyde levels between the control and treatment groups, with the highest levels in the group receiving the most significant black garlic dose (P3). Figure 1 shows the difference in mean Malondialdehyde levels between the unexposed, cigarette smoke-exposed, and cigarette smoke-exposed groups treated with black garlic extract.

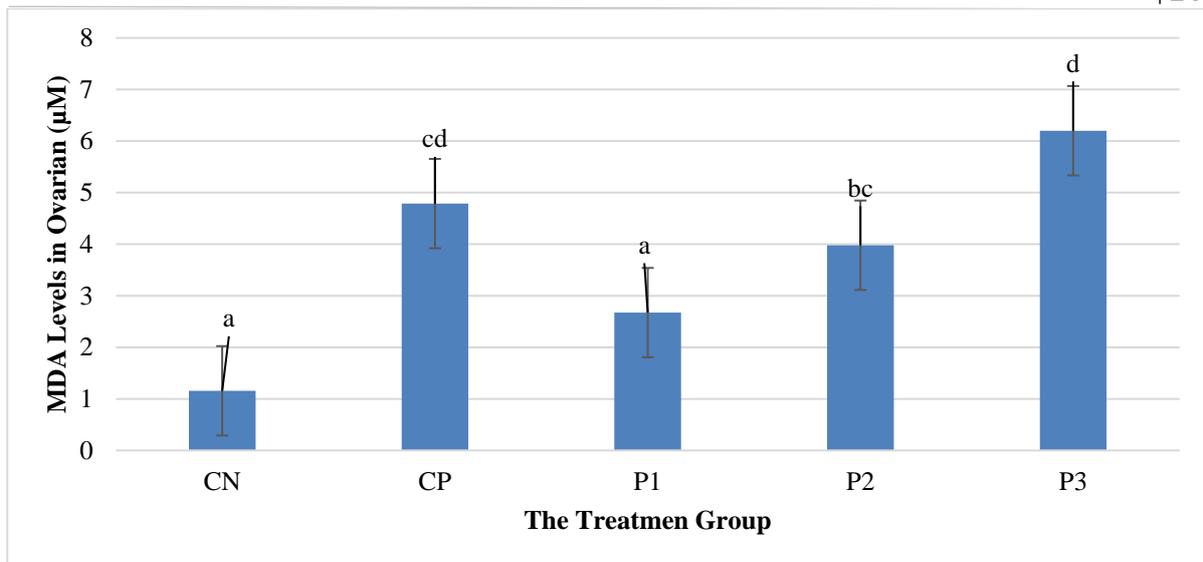


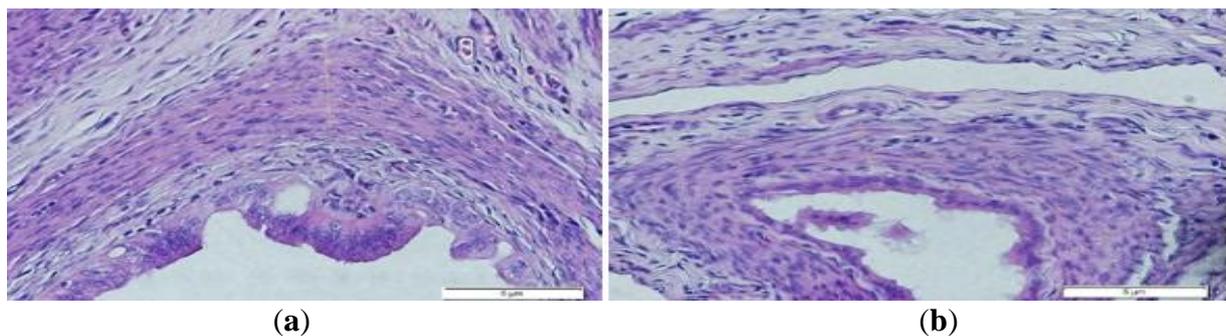
Figure 1. Malondialdehyde Levels

We divided the female *Rattus norvegicus* rats into several groups for our study. The first group (CN) was not exposed to any external factors. The second group (CP) was exposed to cigarette smoke. The third, fourth, and fifth groups (P1, P2, and P3) were also exposed to cigarette smoke, but in addition, they were given black garlic extract at doses of 50, 100, and 200 mg/kgBW, respectively. We then measured the mean ovarian Malondialdehyde levels in each group.

The results showed that black garlic extract could reduce Malondialdehyde levels at low doses (50 mg/kgBW). However, increased Malondialdehyde levels occurred at higher doses, suggesting a potential prooxidative effect at higher doses. These measurements suggest that black garlic extract at a specific dose can reduce cigarette smoke-induced oxidative damage in rat ovaries; however, increasing the dose above a certain threshold can increase Malondialdehyde levels, indicating the importance of proper dosing in the therapeutic application of this extract.

Effects of Black Garlic (*Allium sativum*) Ethanol Extract on Oviduct Smooth Muscle Thickness *Rattus norvegicus* Exposed to Subacute Cigarette Smoke

This study involved 25 female Wistar white rats divided into five research groups, each with five rats. The study lasted for 28 days in the pharmacology laboratory to measure the effect of black garlic extract on the thickness of fallopian tube smooth muscle in rats exposed to subacute cigarette smoke. Observations were made using an Olympus BX53 microscope with 400x magnification and counted in 5 fields of view.



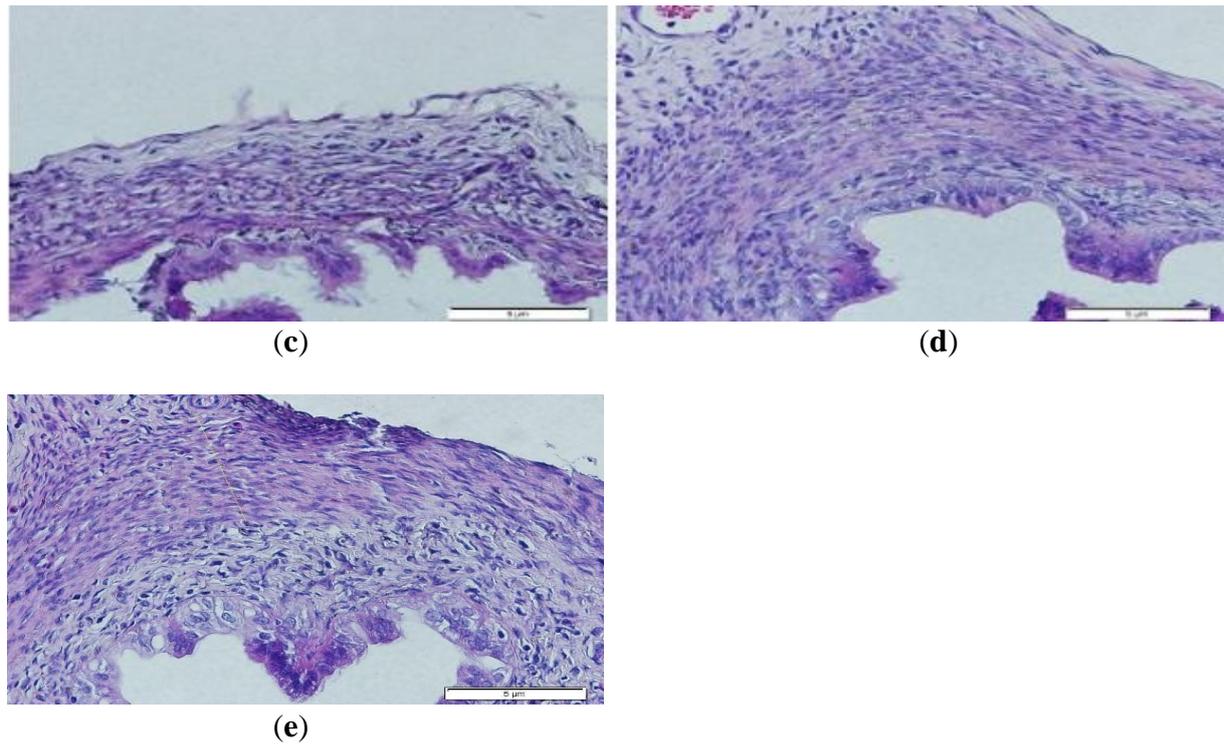


Figure 2. (a) Negative Control Group CN (-); (b) Positive Control Group CP (+); (c) Treatment Group 1 (Cigarette smoke exposure and dose of 50 mg/Kg/BW); (d) Treatment Group 2 (Cigarette smoke exposure and dose of 100mg/kg/BW; (e) Treatment Group 3 (Cigarette smoke exposure and dose of 200mg/kg/BW).

Table 3. Normality and Homogeneity Test of Fallopian Tube Smooth Muscle Thickness Data

Test	p-value	Description
Shapiro-Wilk Normality Test	0.715	Normal
Levene Homogeneity Test	0.087	Homogeneous

Table 3 shows that fallopian tube smooth muscle thickness data were normally distributed and homogeneous, fulfilling the prerequisites for further statistical analysis.

Table 4. One-Way Anova Test of Fallopian Tube Smooth Muscle Layer Thickness

Group	n	Mean \pm SD	p-value
CN	5	6.74160 \pm 1.635602 b	0.002
CP	5	4.08940 \pm 0.256373 a	
P1	5	5.78380 \pm 0.261336 b	
P2	5	5.90960 \pm 0.893802 b	
P3	5	6.17360 \pm 0.511684 b	

Notes: CN: Negative control group, not exposed to cigarette smoke. CP: Positive control group, only exposed to cigarette smoke. P1, P2, P3: Groups exposed to cigarette smoke and given black garlic extract, respectively, 50, 100, and 200 mg/kgBW. p-value <0.05 indicates a significant difference.

The administration of black garlic extract significantly increased the thickness of the smooth muscle layer, with a significant difference at a dose of 50 mg/kg/BW compared to the positive control.

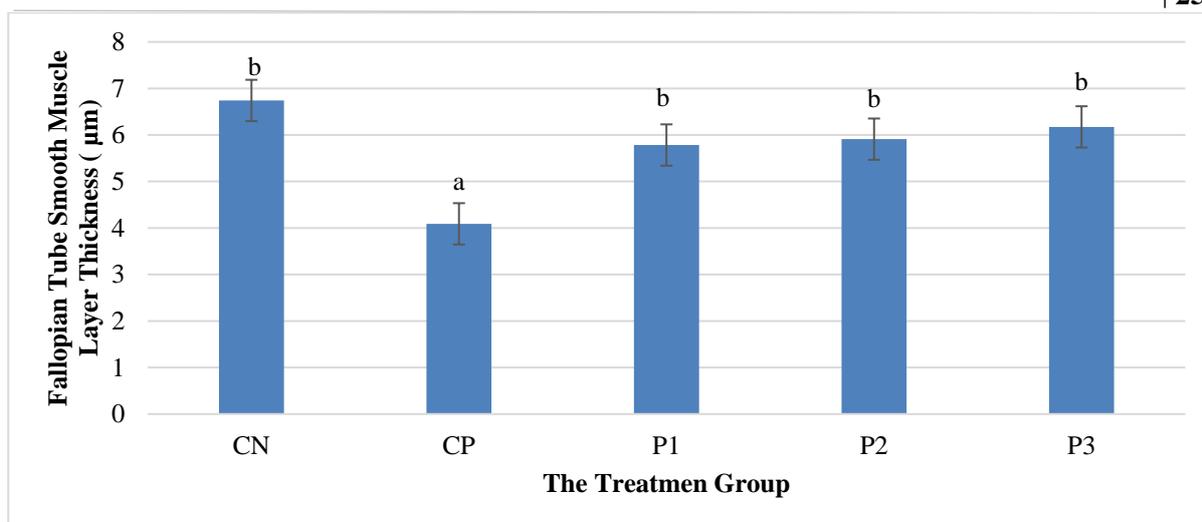


Figure 3. Effect of Black Garlic Extract on Increasing the Thickness of the Smooth Muscle Layer of Fallopian Tubes of White Rats Exposed to Cigarette Smoke.

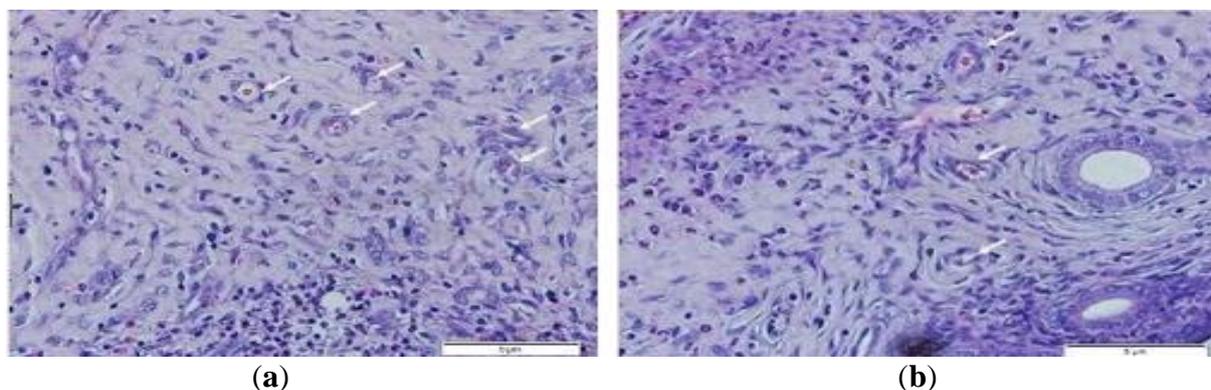
CN: control negative without exposure to cigarette smoke and administration of black garlic extract; CP: control positif exposed to cigarette smoke and without administration of black garlic extract; P1: exposed to cigarette smoke and given 50 mg/kgBW black garlic extract; P2: exposed to cigarette smoke and given 100 mg/kgBW black garlic extract; P3: exposed to cigarette smoke mg/gBW and given 200 mg/kgBW black garlic extract.

Figure 3 shows the fallopian tube smooth muscle thickness in groups exposed to cigarette smoke and those given black garlic extract. The increase in the thickness of the smooth muscle layer is most significant at low doses of extract.

This analysis indicates that black garlic extract can reduce the adverse effects of cigarette smoke exposure on the thickness of the fallopian tube smooth muscle, especially at a dose of 50 mg/kg/BW. These results confirm the potential of black garlic extract as a protective agent against tissue damage due to cigarette smoke exposure.

Effects of Black Garlic (*Allium sativum*) Ethanol Extract on Endometrial Arterioles Count in *Rattus Norvegicus* Exposed to Subacute Cigarette Smoke

This study used female Wistar rats divided into control and treatment groups with exposure to cigarette smoke and different doses of black garlic extract (50 mg/kg, 100 mg/kg, and 200 mg/kg). Observations were made using an Olympus BX53 light microscope, with 400x magnification, to measure the effect on the number of endometrial arterioles.



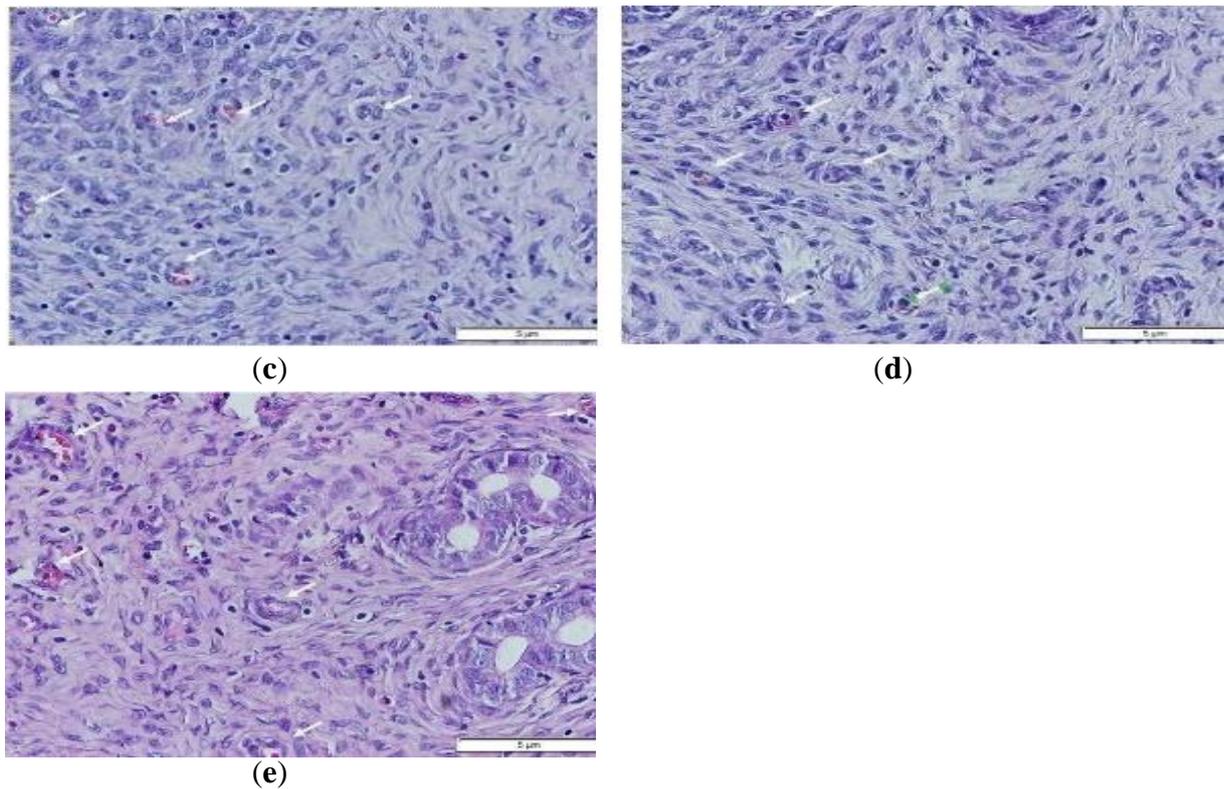


Figure 4. Arrows indicate spiral arterioles in rat endometrium with round tissue shape, with tunica intima, tunica media, and adventitia. (a) negative control group/no treatment; (b) positive control group/exposed to cigarette smoke; (c) treatment group 1 with cigarette smoke and black garlic 50 mg/kgBW; (d) treatment group with cigarette smoke and black garlic 100 mg/kgBW; (e) treatment group 3 with cigarette smoke and black garlic 200 mg/kgBW).

Table 5. Normality and Homogeneity Test of the Number of Endometrial Arterioles

Test	p-value	Description
Normality	0.972	Normal
Homogeneousitas	0.149	Homogeneous

Table 5 shows that the test results show that the data is usually distributed and Homogeneous, which allows further statistical analysis using the One Way ANOVA test.

Table 6. Comparison Test Results of The Number of Endometrial

Group	n	Mean \pm SD	p-value
CN	5	5.24 \pm 1.10 ^b	0.000
CP	5	2.20 \pm 0.38 ^a	
P1	5	5.12 \pm 1.06 ^b	
P2	5	4.24 \pm 0.49 ^b	
P3	5	3.76 \pm 0.72 ^b	

Different notations (a,b) give significant meaning to the mean \pm sd obtained (p-value $<$ α = 0.05), which means that there is a significant difference, and if the notation makes different letters (p-value $>$ α = 0.05), there is no significant difference in the HS post hoc test. Statistical analysis showed significant differences in the number of arterioles between the control and treatment groups, with a significant increase in the group treated with black garlic extract, especially at the lowest dose (50 mg/kg).

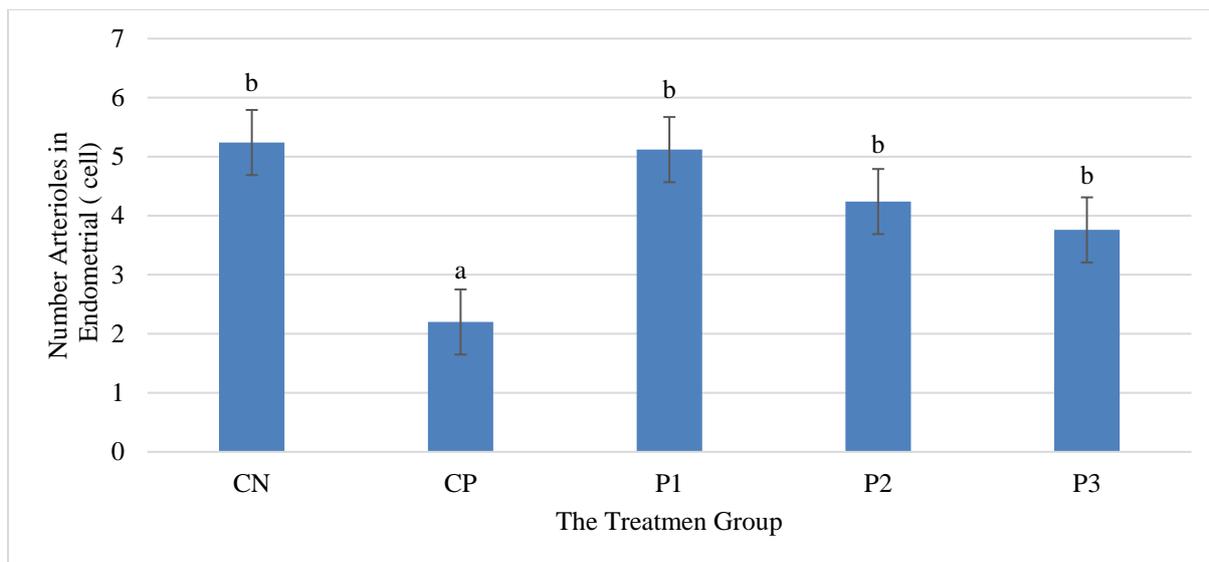


Figure 5. Number of Endometrial Arterioles

Different notations (a,b) give significant meaning to the mean \pm sd obtained (p -value $< \alpha = 0.05$), which means that there is a significant difference, and if the notation makes different letters (p -value $> \alpha = 0.05$), there is no significant difference in the HS post hoc test. Statistical analysis showed significant differences in the number of arterioles between the control and treatment groups, with a significant increase in the group treated with black garlic extract, especially at the lowest dose (50 mg/kg).

Figure 5 shows the mean number of endometrial arterioles in the negative control group, positive control group (exposed to cigarette smoke two cigarettes/day), P1 (exposed to cigarette smoke + BG 50 mg/kg/BW), P2 (exposed to cigarette smoke + BG 100 mg/kg/BW), P3 (exposed to cigarette smoke + BG 200 mg/kg/BW). The mean \pm sd obtained (p -value $< \alpha = 0.05$) can mean that there is a significant difference, and if (p -value $> \alpha = 0.05$), then there is no significant difference. This shows an increase in the number of arterioles in the group given black garlic extract, showing a protective effect against damage due to exposure to cigarette smoke.

Microscopic observations revealed that the arterioles in the group given black garlic extract showed a more preserved structure than those exposed to cigarette smoke in the positive control group. The group with a dose of 50 mg/kgBW of black garlic showed the highest number of arterioles, indicating the strongest positive effect against cigarette smoke damage.

These observations suggest that black garlic extract is potentially a protective agent against endometrial arteriolar damage caused by cigarette smoke exposure, with the most significant effect seen at a dose of 50 mg/kgBW.

DISCUSSION

Effects of Black Garlic (*Allium sativum*) Ethanol Extract on Malondialdehyde Levels in *Rattus Norvegicus* Exposed to Subacute Cigarette Smoke

Cigarette smoke exposure was shown to increase Malondialdehyde levels in *Rattus norvegicus*, indicating increased oxidative stress due to free radical activity derived from toxic substances in cigarette smoke (Aningsih *et al.*, 2020). Higher Malondialdehyde levels in groups exposed to cigarette smoke confirm the role of cigarette smoke in inducing lipid peroxidation

that has the potential to cause damage to cell membranes (Kole *et al.*, 2020);(Ningrum *et al.*, 2021).

Administering black garlic extract at a dose of 50 mg/kgBW led to a notable reduction in Malondialdehyde levels, highlighting black garlic's protective properties against oxidative stress caused by cigarette smoke exposure (Tsai *et al.*, 2019). These results are consistent with the study (Tsai *et al.*, 2019) , which found that black garlic extract was able to reduce Malondialdehyde and inflammatory responses in an acute liver injury model in rats.

Interestingly, increasing the black garlic extract given to the P3 group (200 mg/kgBW) did not provide a better protective effect; instead, it tended to show higher Malondialdehyde levels than the group with lower doses. This may indicate the existence of an optimal dose limit of black garlic extract in reducing oxidative stress, where higher doses can potentially induce pro-oxidative effects or toxicity by the thoughts expressed (Dutta *et al.*, 2021);(Gorrini *et al.*, 2013);(Penke *et al.*, 2017) regarding the potential toxicity of active components at high doses (Marengo *et al.*, 2016).

Furthermore, the decrease in Malondialdehyde levels at low doses (50 mg/kgBW) reflects the potential of black garlic in increasing the body's intrinsic antioxidant capacity through various mechanisms, including increased antioxidant enzyme activity and modulation of oxidative stress, as demonstrated (Oktari *et al.*, 2020);(Djedjibegovic *et al.*, 2020). Active components of black garlic such as S-Allylcysteine (SAC) play an important role in this process, given that SAC is known to have strong antioxidant effects. The differences in results between studies regarding the effectiveness of black garlic doses in reducing Malondialdehyde levels could be due to variations in extraction methods, experimental conditions, and the type and biological condition of the study subjects. This underscores the importance of standardizing research conditions and carefully considering the dose selection.

Effects of Black Garlic (*Allium sativum*) Ethanol Extract on Oviduct Smooth Muscle Thickness *Rattus norvegicus* Exposed to Subacute Cigarette Smoke

This study showed that exposure to cigarette smoke caused a decrease in fallopian tube smooth muscle thickness, which the administration of black garlic extract significantly increased. This decrease can be explained by the impact of cigarette smoke that induces oxidative stress and damages the structural integrity of smooth muscle tissue, as shown by the decrease in muscle thickness in the control group exposed to cigarette smoke (Lee *et al.*, 2017);(Kimura *et al.*, 2017).

The increase in smooth muscle thickness in the group given black garlic indicates the protective effect of bioactive compounds in black garlic against cigarette smoke-induced damage (Lu *et al.*, 2017). Black garlic, which undergoes a fermentation process, contains high concentrations of antioxidant compounds such as S-allyl cysteine (SAC), which are effective in neutralizing free radicals generated from cigarette smoke (Felix *et al.*, 2014).

These results are consistent with a study (Lu *et al.*, 2017), which showed that black garlic extract can reduce oxidative stress and increase antioxidant enzyme levels, reducing inflammation associated with oxidative damage to tissues (Lee *et al.*, 2017). By inhibiting inflammatory pathways and strengthening the body's antioxidant capacity, black garlic extract promotes the maintenance of smooth muscle tissue structure and avoids degradation caused by free radicals (Tsai *et al.*, 2019).

In the context of molecular mechanisms (Dampati & Veronica, 2020) explained that cigarette smoke can interfere with hormone signals necessary for the normal function of the fallopian tubes through disruptive endocrine mechanisms (Recinella *et al.*, 2021). Black garlic extract may mitigate some of these effects by affecting the expression of hormone receptors

and proteins involved in muscle contractility, which might help maintain fallopian tube smooth muscle function in the face of oxidative stress (Szumilas *et al.*, 2020).

Black garlic has metabolic and anti-inflammatory effects in chronic disease models, indicating that black garlic administration not only affects tissue structure but also has the potential to improve physiological functions in the reproductive system affected by exposure to environmental toxins such as cigarette smoke (Amor *et al.*, 2019).

This study underscores the potential of black garlic extract as a therapeutic agent in preventing tissue damage induced by cigarette smoke. These findings may enrich our understanding of the interactions between nutrition and oxidative stress and support the development of nature-based therapeutic approaches in dealing with oxidative damage in the reproductive system.

The absence of articles from Indonesian researchers in this discussion does not imply that black garlic is unavailable in Indonesia or that it lacks research interest there. Black garlic, known for its numerous health benefits, is indeed available and used in Indonesia. The use of international references, such as those cited from Lee, Kimura, and Lu, highlights studies that may have broader recognition or specific findings pertinent to this research.

Black garlic is gaining popularity in Indonesia, both as a culinary ingredient and for its medicinal properties. Indonesian researchers and consumers are increasingly aware of its benefits, and local studies are emerging that focus on its health effects. The fermentation process to produce black garlic, which enhances its antioxidant properties, is well understood and practiced in Indonesia.

However, the inclusion of international articles in this discussion aims to leverage widely recognized studies to support the findings and ensure the study's credibility. Future research could benefit from incorporating more local studies to reflect regional usage and research advancements in Indonesia, thereby providing a comprehensive view of black garlic's impact based on both global and local perspectives.

Effects of Black Garlic (*Allium sativum*) Ethanol Extract on Endometrial Arterioles Count in *Rattus Norvegicus* Exposed to Subacute Cigarette Smoke

This study highlights the significant impact of black garlic extract in increasing the number of endometrial arterioles in rats exposed to subacute cigarette smoke. Cigarette smoke contains compounds that can cause oxidative reactions and inflammation in various body tissues, including the endometrium, which in this study led to a decrease in the number of endometrial arterioles (Oktari *et al.*, 2020).

In the context of this study, black garlic extract, which is rich in antioxidants, plays a vital role in protecting endometrial tissue from cigarette smoke-induced oxidative damage. The increase in the number of arterioles observed in the treated groups, especially at the dose of 50 mg/kgBW, suggests that black garlic has a protective effect on the microvascular structure of the endometrium.

The mechanism behind this effect could be explained through the role of black garlic extract in modulating the VEGF and FGF pathways, which are critical factors in angiogenesis, as revealed (Guo *et al.*, 2021). These two growth factors are responsible for endothelial cell proliferation and migration, which are the initial steps in angiogenesis. Research by Delbandi *et al.* (2020) also showed that black garlic extract supports the formation of new blood vessels by facilitating the migration of these cells, which might explain the increased number of arterioles found in this study.

Administration of black garlic extract is also associated with increased antioxidant activity and reduced oxidative stress in endometrial tissue, as shown (Hoyt *et al.*, 2003), who

found that the extract increased enzymes such as SOD, catalase, and GPx. This is relevant as high oxidative stress is associated with vascular damage and angiogenesis dysfunction.

In addition, studies (Wei *et al.*, 2017) highlighted the potential of black garlic as an anti-inflammatory and anti-cancer agent, showing that it can inhibit angiogenesis in the context of cancer cells, yet in the context of inflammation and damage by cigarette smoke, this extract supports healthy angiogenesis processes (Holbrook, 2016).

This discovery significantly contributes to the development of science, particularly in understanding the interaction between functional food components such as black garlic and biological processes such as angiogenesis under pathological conditions. It also paves the way for the development of therapeutic strategies that can reduce the risk of vascular diseases induced by environmental factors such as cigarette smoke.

In the context of enrichment and technological development for society, these results support using black garlic in the diet as a preventive and therapeutic strategy to ameliorate vascular damage and support reproductive health, particularly in communities exposed to cigarette smoke or other pollutants.

4. CONCLUSION

This study concludes that black garlic extract has the potential to be a protective agent against oxidative damage in the reproductive system of rats exposed to cigarette smoke, with effectiveness that depends on the dose of extract given. Further studies should investigate the long-term effects and optimal dosing of black garlic extract in humans. Additionally, exploring the molecular mechanisms behind its protective properties could enhance its application in reproductive health management.

REFERENCES

- Agustina, E., Andiarna, F., & Hidayati, I. (2020). Uji Aktivitas Antioksidan Ekstrak Bawang Hitam (Black Garlic) Dengan Variasi Lama Pemanasan. *Al-Kauniah: Jurnal Biologi*, *13*(1), 39–50. <https://doi.org/10.15408/kauniah.v13i1.12114>
- Amor, S., González-Hedström, D., Martín-Carro, B., Inarejos-García, A. M., Almodóvar, P., Prodanov, M., García-Villalón, A. L., & García, M. G. (2019). Beneficial effects of an aged black garlic extract in the metabolic and vascular alterations induced by a high fat/sucrose diet in male rats. *Nutrients*, *11*(1). <https://doi.org/10.3390/nu11010153>
- Aningsih, B. S. D., Sujuti, H., Mustofa, E., & Ratnawati, R. (2020). Anthocyanins from *Ipomoea batatas* L. Effect on ovarian malondialdehyde and 17 β -estradiol in rats exposed cigarette smoke. *Russian Open Medical Journal*, *9*(3), 1–5. <https://doi.org/10.15275/rusomj.2020.0308>
- Ardiana, M. (2021). *Telaah Ilmiah Dan Patologi Paparan Asap Rokok Terhadap Penyakit Jantung*. Airlangga University Press.
- Budani, M. C., Carletti, E., & Tiboni, G. M. (2021). In Vivo Cigarette Smoke Exposure to Examine the Expression of Genes Involved in the Inflammatory Response in the Mouse Uterus. *Current Protocols*, *1*(6), 1–9. <https://doi.org/10.1002/cpz1.172>
- Dampati, P. S., & Veronica, E. (2020). Potensi Ekstrak Bawang Hitam sebagai Tabir Surya terhadap Paparan Sinar Ultraviolet. *KELUWIH: Jurnal Kesehatan Dan Kedokteran*, *2*(1), 23–31. <https://doi.org/10.24123/kesdok.v2i1.3020>
- Delbandi, A. A., Mahmoudi, M., Shervin, A., Heidari, S., Kolahdouz-Mohammadi, R., & Zarnani, A. H. (2020). Evaluation of apoptosis and angiogenesis in ectopic and eutopic stromal cells of patients with endometriosis compared to non-endometriotic controls. *BMC Women's Health*, *20*(1), 1–9. <https://doi.org/10.1186/s12905-019-0865-4>
- Djedjibegovic, J., Marjanovic, A., Panieri, E., & Saso, L. (2020). Ellagic Acid-Derived

- Urolithins as Modulators of Oxidative Stress. *Oxidative Medicine and Cellular Longevity*, 2020(Figure 1). <https://doi.org/10.1155/2020/5194508>
- Dutta, A., Dahiya, A., Prakash, A., & Agrawala, P. K. (2021). Acute toxicity of diallyl sulfide derived from *Allium sativum* (garlic) in mice and its possible mechanisms. *Phytomedicine Plus*, 1(3), 100084. <https://doi.org/10.1016/j.phyplu.2021.100084>
- Faber, M. T., Kjær, S. K., Dehlendorff, C., Chang-Claude, J., Andersen, K. K., Høgdall, E., Webb, P. M., Jordan, S. J., Rossing, M. A., Doherty, J. A., Lurie, G., Thompson, P. J., Carney, M. E., Goodman, M. T., Ness, R. B., Modugno, F., Edwards, R. P., Bunker, C. H., Goode, E. L., ... Jensen, A. (2013). Cigarette smoking and risk of ovarian cancer: A pooled analysis of 21 case-control studies. *Cancer Causes and Control*, 24(5), 989–1004. <https://doi.org/10.1007/s10552-013-0174-4>
- Felix, A. S., Yang, H. P., Gierach, G. L., Park, Y., & Brinton, L. A. (2014). Cigarette smoking and endometrial carcinoma risk: The role of effect modification and tumor heterogeneity. *Cancer Causes and Control*, 25(4), 479–489. <https://doi.org/10.1007/s10552-014-0350-1>
- Gannon, A. M., Stämpfli, M. R., & Foster, W. G. (2013). Cigarette smoke exposure elicits increased autophagy and dysregulation of mitochondrial dynamics in murine granulosa cells. *Biology of Reproduction*, 88(3), 1–11. <https://doi.org/10.1095/biolreprod.112.106617>
- GATS. (2021). Gats|Global Adult Tobacco Survey Fact Sheet Indonesia 2021 Gats Objectives. *Fact Sheet Indonesia*, 1–2.
- Gorrini, C., Harris, I. S., & Mak, T. W. (2013). Modulation of oxidative stress as an anticancer strategy. *Nature Reviews Drug Discovery*, 12(12), 931–947. <https://doi.org/10.1038/nrd4002>
- Guo, X., Yi, H., Li, T. C., Wang, Y., Wang, H., & Chen, X. (2021). Role of vascular endothelial growth factor (Vegf) in human embryo implantation: Clinical implications. *Biomolecules*, 11(2), 1–16. <https://doi.org/10.3390/biom11020253>
- Holbrook, B. D. (2016). The effects of nicotine on human fetal development. *Birth Defects Research Part C - Embryo Today: Reviews*, 108(2), 181–192. <https://doi.org/10.1002/bdrc.21128>
- Hoyt, J. C., Robbins, R. A., Habib, M., Springall, D. R., Buttery, L. D. K., Polak, J. M., & Barnes, P. J. (2003). Cigarette smoke decreases inducible nitric oxide synthase in lung epithelial cells. *Experimental Lung Research*, 29(1), 17–28. <https://doi.org/10.1080/01902140303759>
- Kida, N., Nishigaki, A., Kakita-Kobayashi, M., Tsubokura, H., Hashimoto, Y., Yoshida, A., Hisamatsu, Y., Tsuzuki-Nakao, T., Murata, H., & Okada, H. (2021). Exposure to cigarette smoke affects endometrial maturation including angiogenesis and decidualization. *Reproductive Medicine and Biology*, 20(1), 108–118. <https://doi.org/10.1002/rmb2.12360>
- Kimura, S., Tung, Y. C., Pan, M. H., Su, N. W., Lai, Y. J., & Cheng, K. C. (2017). Black garlic: A critical review of its production, bioactivity, and application. *Journal of Food and Drug Analysis*, 25(1), 62–70. <https://doi.org/10.1016/j.jfda.2016.11.003>
- Kole, E., Ozkan, S. O., Eraldemir, C., Akar, F. Y., Ozbek, S. K., Kole, M. C., Kum, T., & Filiz, P. C. (2020). Effects of melatonin on ovarian reserve in cigarette smoking: An experimental study. *Archives of Medical Science*, 16(6), 1376–1386. <https://doi.org/10.5114/AOMS.2019.89409>
- Lee, H. M., Kim, C. W., Hwang, K. A., Sung, J. H., Lee, J. K., & Choi, K. C. (2017). Cigarette smoke impaired maturation of ovarian follicles and normal growth of uterus inner wall

- of female wild-type and hypertensive rats. *Reproductive Toxicology*, 73, 232–240. <https://doi.org/10.1016/j.reprotox.2017.06.187>
- Lu, X., Li, N., Qiao, X., Qiu, Z., & Liu, P. (2017). Composition analysis and antioxidant properties of black garlic extract. *Journal of Food and Drug Analysis*, 25(2), 340–349. <https://doi.org/10.1016/j.jfda.2016.05.011>
- Marengo, B., Nitti, M., Furfaro, A. L., Colla, R., Ciucis, C. De, Marinari, U. M., Pronzato, M. A., Traverso, N., & Domenicotti, C. (2016). Redox homeostasis and cellular antioxidant systems: Crucial players in cancer growth and therapy. *Oxidative Medicine and Cellular Longevity*, 2016. <https://doi.org/10.1155/2016/6235641>
- Ningrum, A. G., Frety, E. E., Diah, I., Shabran, Z. H., Setiani, R. E., & Dewi, E. R. (2021). Antioxidant activity of purslane (*Portulaca oleracea* L.) leaf extract on the levels of ovarian oxidative stress and reproductive hormone in *rattus norvegicus* exposed to cigarette smoke. *Open Access Macedonian Journal of Medical Sciences*, 9(B), 1535–1540. <https://doi.org/10.3889/oamjms.2021.7081>
- Oktari, Kwartika, Azizah, Z., Chandra, B., & Asra, R. (2020). A Review: Antioxidant and Immunomodulator Effects of Black Garlic. *EAS Journal of Pharmacy and Pharmacology*, 2(6), 193–198. <https://doi.org/10.36349/easjpp.2020.v02i06.001>
- Park, S. R., Kim, S. K., Kim, S. R., Yu, W. J., Lee, S. J., & Lee, H. Y. (2022). Effects of smoking on the tissue regeneration-associated functions of human endometrial stem cells via a novel target gene SERPINB2. *Stem Cell Research and Therapy*, 13(1), 1–20. <https://doi.org/10.1186/s13287-022-03061-1>
- Penke, B., Fulop, L., Szucs, M., & Frecska, E. (2017). The Role of Sigma-1 Receptor, an Intracellular Chaperone in Neurodegenerative Diseases. *Current Neuropharmacology*, 16(1), 97–116. <https://doi.org/10.2174/1570159x15666170529104323>
- Rahma, F., Ardiaria, M., & Panunggal, B. (2019). Pengaruh Pemberian Ubi Jalar Ungu (*Ipomoea batatas* L. Poir) Terhadap Kadar Leukosit Total Tikus Wistar Jantan (*Rattus norvegicus*) Yang Dipapar Asap Rokok. *Journal of Nutrition College*, 8(2), 65. <https://doi.org/10.14710/jnc.v8i2.23815>
- Recinella, L., Chiavaroli, A., Masciulli, F., Frascchetti, C., Filippi, A., Cesa, S., Cairone, F., Gorica, E., De Leo, M., Braca, A., Martelli, A., Calderone, V., Orlando, G., Ferrante, C., Menghini, L., Di Simone, S. C., Veschi, S., Cama, A., Brunetti, L., & Leone, S. (2021). Protective effects induced by a hydroalcoholic *allium sativum* extract in isolated mouse heart. *Nutrients*, 13(7). <https://doi.org/10.3390/nu13072332>
- Sala, M., & Gotti, C. (2023). Electronic nicotine delivery systems (ENDS): A convenient means of smoking? *Pharmacological Research*, 195(June). <https://doi.org/10.1016/j.phrs.2023.106885>
- Szumilas, K., Szumilas, P., Grzywacz, A., & Wilk, A. (2020). The effects of e-cigarette vapor components on the morphology and function of the male and female reproductive systems: A systematic review. *International Journal of Environmental Research and Public Health*, 17(17), 1–13. <https://doi.org/10.3390/ijerph17176152>
- Tobacco Atlas. (2024). *Findings from the Third Tobacconomics Cigarette Tax Scorecard*. <https://tobaccoatlas.org/inadequate-tobacco-tax-policies-threaten-global-tobacco-control-progress/>
- Trofor, A. C., Papadakis, S., Lotrean, L. M., Radu-Loghin, C., Eremia, M., Mihaltan, F., Driezen, P., Kyriakos, C. N., Mons, U., Demjén, T., Nogueira, S. O., Fernández, E., Tountas, Y., Przewoźniak, K., McNeill, A., Fong, G. T., & Vardavas, C. I. (2018). Knowledge of the health risks of smoking and impact of cigarette warning labels among tobacco users in six European countries: Findings from the EUREST-PLUS ITC Europe Surveys. *Tobacco Induced Diseases*, 16(October), 1–13.

<https://doi.org/10.18332/tid/99542>

- Tsai, J. C., Chen, Y. A., Wu, J. T., Cheng, K. C., Lai, P. S., Liu, K. F., Lin, Y. K., Huang, Y. T., & Hsieh, C. W. (2019). Extracts from fermented black garlic exhibit a hepatoprotective effect on acute hepatic injury. *Molecules*, 24(6), 1–13. <https://doi.org/10.3390/molecules24061112>
- Wei, Z., Shan, Y., Tao, L., Liu, Y., Zhu, Z., Liu, Z., Wu, Y., Chen, W., Wang, A., & Lu, Y. (2017). Diallyl trisulfides, a natural histone deacetylase inhibitor, attenuate HIF-1 α synthesis, and decreases breast cancer metastasis. In *Molecular Carcinogenesis* (Vol. 56, Issue 10). <https://doi.org/10.1002/mc.22686>
- Widyanti, A. S., Ardiaria, M., & Widyastuti, N. (2020). Pengaruh pemberian ubi jalar ungu (*Ipomoea batatas* L.Poir) terhadap kadar superoksida dismutase (SOD) tikus wistar jantan (*Rattus Norvegicus*) yang dipapar asap rokok. *Jurnal Gizi Indonesia*, 8(1), 45. <https://doi.org/10.14710/jgi.8.1.45-50>
- World Health Organization. (2024). *Tobacco: E-cigarettes*. World Health Organization. <https://www.who.int/news-room/questions-and-answers/item/tobacco-e-cigarettes#:~:text=Are ENDS addictive%3F,can reach dangerously high levels>

Jurnal Info Kesehatan

Vol. 22, No. 2, June 2024, pp. 259-271

P-ISSN 0216-504X, E-ISSN 2620-536X

DOI: [10.31965/infokes.Vol22.Iss2.1534](https://doi.org/10.31965/infokes.Vol22.Iss2.1534)Journal homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>**RESEARCH****Open Access****The Effect of Sub-Acute Inhalation Exposure to Polyethylene Micro-Nano Plastics on the Histopathological Features of the Mammary Glands in Female Wistar White Rats (*Rattus Norvegicus*)****Ihda Dian Kusuma^{1a}, Laksmitha Janasti^{2b}, Riana Trinovita Sari^{2,3c}, Britania Laila Nanda^{4d}, Hikmawan Wahyu Sulistomo^{5e*}, Nurdiana^{5f}**¹ Department of Anatomical Pathology, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia² Master Program of Midwifery, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia³ Department of Midwifery, Kemenkes Poltekkes Kalimantan Timur, Samarinda, East Kalimantan, Indonesia⁴ Bachelor Program of Midwifery, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia⁵ Department of Pharmacology, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia^a Email address: ihdadk.pa.fkub@ub.ac.id^b Email address: laksmithaaj234@student.ub.ac.id^c Email address: rianats@student.ub.ac.id^d Email address: britanialnanda@student.ub.ac.id^e Email address: hikmawan_ws@ub.ac.id^f Email address: nurdianafarmako.fk@ub.ac.id

Received: 23 May 2024

Revised: 17 June 2024

Accepted: 19 June 2024

Abstract

The majority of household appliances are made of plastic derived from synthetic petroleum and the result of polymerization processes. One type of plastic is Polyethylene (PE). Polyethylene (PE) contains antimony trioxide compounds that are carcinogenic in the body if ingested in excessive amounts, triggering cancer and oxidative stress, which can be observed by measuring the levels of malondialdehyde (MDA) in the body. This study aims to determine the proliferation of abnormal cells in the lactiferous ducts and mammary gland acini as well as the increase in MDA levels. The research method used is a true experimental design with a Randomized Post Test Only Group Design. This study used the mammary organs and blood of female white rats that had been exposed to PE for 28 days. The number of samples used in this study was 12 female white rats. The results showed that there were significant differences in the histopathological features and MDA levels in the mammary glands. Based on the results of the Independent T-Test on the number of cell layers in the lactiferous ducts, acini, and the number of acini ($p < 0.05$) and the Mann-Whitney test on MDA levels ($p < 0.05$). There were significant changes in the histopathological features and MDA levels in the mammary glands exposed to Polyethylene (PE) plastic. Exposure to micro-nano plastics of PE type at a dose of 15 mg/m³ for 28 days differed significantly in the MDA levels of mammary glands, histopathological picture of mammary glands at cell proliferation events, both in the lactiferous ducts and acini cells. In addition, exposure to PE plastic has the potential to cause health problems in the breast organs if exposed for a long time and in excess doses.

Keywords: Polyethylene, Cell Mamae Proliferation, Breast Cancer.***Corresponding Author:**

Hikmawan Wahyu Sulistomo

Department of Pharmacology, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia

Email: hikmawan_ws@ub.ac.id

©The Author(s) 2024. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

1. INTRODUCTION

Currently, the majority of household appliances are made from plastic, which is synthesized from petroleum and formed through polymerization processes (Anom & Lombok, 2020). Until now, plastic waste is still an unresolved problem, both in Indonesia and the world. Based on data from the Indonesian Ministry of Environment and Forestry in 2022, obtained from 171 districts/cities throughout Indonesia, the results of waste generation reached 20 million tons per year (Kementerian Lingkungan Hidup dan Kehutanan Republik Indonesia, 2022). Of the various types of waste generated, the largest type of waste comes from food waste as much as 40.6% and the second largest type of waste is plastic waste as much as 18.5% which is included in the category of non-renewable waste. Based on data obtained from Rudend, and Hermana, (2021), Indonesia is the second largest producer of plastic waste in the world with the amount of plastic waste reaching 85,000 tons per year. To deal with this, waste recycling is carried out, either used for crafts or used repeatedly, such as the use of plastic bottles.

Plastic comes in various types used as raw materials in the manufacture of household tools, one of which is Polyethylene (PE) (Sen & Raut, 2015). Polyethylene is a type of plastic composed of extended hydrocarbon chains originating from thermoplastic polymers and is divided into several types, commonly used ones being low-density polyethylene (LDPE) and high-density polyethylene (HDPE) (Sen & Raut, 2015). PE plastic is utilized in the production of plastic bags, disposable containers, bottles, packaging containers, and more (Ghatge et al. 2020). Polyethylene contains antimony trioxide, used as a catalyst in plastic production. Antimony trioxide can enter the body and is carcinogenic, potentially triggering cancer growth (Baharuddin, Asran & Ikhtiar, 2023; Tapiory et al., 2019).

Plastic particles can be categorized by diameter into microplastics (MP) and nano plastics (NP), with MPs measuring 1µm-5mm in diameter and NPs measuring less than 1µm (Shim & Thomposon, 2015). Airborne micro-nanoplastics are produced due to the degradation of larger plastics through UV rays. Once degraded, these particles can easily be blown into the atmosphere by air currents due to their small size and low density (Enyoh et al., 2019). There are three exposure routes for micro-nano plastics to enter the body, oral, respiratory (inhalation), and dermal exposure through cosmetic products (Chang et al., 2020; Vianello et al., 2019). Inhaled micro-nano plastics enter the respiratory system, are then ingested by macrophages, and transferred to the circulatory and lymphatic systems (Karlsson et al., 2017). Micro-nano plastics entering the blood induce free radicals (Campanale et al., 2020).

Cancer cells grow due to a failure to cease the proliferation process, which can be caused by exposure to chemicals that lead to an increase in Reactive Oxidative Species (ROS) (Ilmiawati et al., 2022). Elevated ROS can alter the expression of the p53 tumor suppressor gene, which is crucial for apoptosis. Therefore, oxidative stress can cause changes in gene expression, cell proliferation, and apoptosis, playing a significant role in the initiation, growth, and development of tumors (Jelic et al. 2021). The commonly used parameter to measure ROS in the body is Malondialdehyde (MDA) (Tirani & Haghjou, 2019). Increased ROS in the body will lead to genetic mutations in the nucleus, which can trigger breast cancer.

The entry of PE particles in the body can cause various kinds of adverse effects on the body, even direct exposure causes breast cancer in humans. Breast cancer remains the leading cause of cancer-related deaths in women worldwide. The Indonesian Ministry of Health (2022) reports that, based on data from the Global Burden of Cancer (GLOBOCAN) in 2020, the number of breast cancer cases reached 68,858 (16.6%) out of a total of 396,914 cancer cases in Indonesia, with more than 22,000 deaths (Kementerian Kesehatan Republik Indonesia, 2022). The exact cause of breast cancer is still not fully known. About 60% of all breast cancers are not due to family heredity, suggesting a possibility of breast cancer occurring due to exposure

to chemicals and drugs in the body (Helm & Rudel, 2020). Research on the relationship between polyethylene microplastic exposure and breast cancer is still limited. However, some studies have found that exposure to microplastics can be at risk of triggering breast cancer. The purpose of this study is to determine the increase in MDA levels in the mammary organs and the occurrence of abnormal cell proliferation in the lactiferous ducts and mammary gland acini.

2. RESEARCH METHOD

This study is a true experimental research design with random sample selection using a Randomized Post Test Only Group Design. It involves comparing and analyzing the results obtained at the end of the study after the treatment (post-test) with a control group that did not receive the treatment. The samples used in this study were female Wistar rats (*Rattus norvegicus*) aged 12-15 weeks and weighing 150-200 grams, which had been exposed to Polyethylene (PE) micro-nano plastics at a concentration of 15 mg/m³ by inhalation for 28 days (Cary et al., 2023). In this study, micro-nano plastics were exposed per inhalation with an exposure dose based on OSHA (Occupational Safety and Health Administration) which is 15 mg/m³. The exposure method refers to the research of Cary et al., (2023), namely the whole-body inhalation method because the exposure is similar to the reality of exposure to microplastics in humans today.

The study used 2 groups: 1 control group and 1 Polyethylene (PE) exposure group, each consisting of 6 rats, randomly selected, with a total of 12 rats required. The number of samples in this study refers to the Frommlet & Heinze, (2020) study, which explains that research using experimental animals with a sample size that is too small will not produce significant conclusions, but using a sample that is too large will cause more animals to suffer. This will be contrary to research ethics, namely the 3R (Replacement, Reduction, Refinement). The study showed that the number of repetitions for each treatment group between (3,4,6,8) had almost the same effectiveness and was not much different. The research samples observed were mammary organ preparations and MDA levels in the mammary organs.

The study was conducted from December 2023 to January 2024. The research was carried out at the Faculty of Medicine, Universitas Brawijaya, specifically in the Pharmacology Laboratory, Biochemistry-Biomedicine Laboratory, and Anatomical Pathology Laboratory of FK UB. The data were statistically analyzed with the help of SPSS 27.0 with a level of significance 0,05 ($p < 0,05$). Data analysis employed the parametric Unpaired Independent T-Test and the Mann-Whitney test. This study received ethical approval from the ethics committee of Universitas Brawijaya with the number: 254/EC/KEPK/08/2023.

3. RESULTS AND DISCUSSION

MDA Levels in Mammary Glands

Measurement of MDA levels in the mammary glands of female white rats was conducted to determine the concentration of MDA (nmol/mL) in each group. In the control group, the average MDA level was 57.6 ± 12 (nmol/mL). Meanwhile, in the treatment group, the average MDA level in the mammary organs was 92 ± 43 (nmol/mL). From these results, it can be concluded that the PE (plastic exposure) group has MDA levels 1.6 times higher than the control group, as presented in the graph in Figure 1.

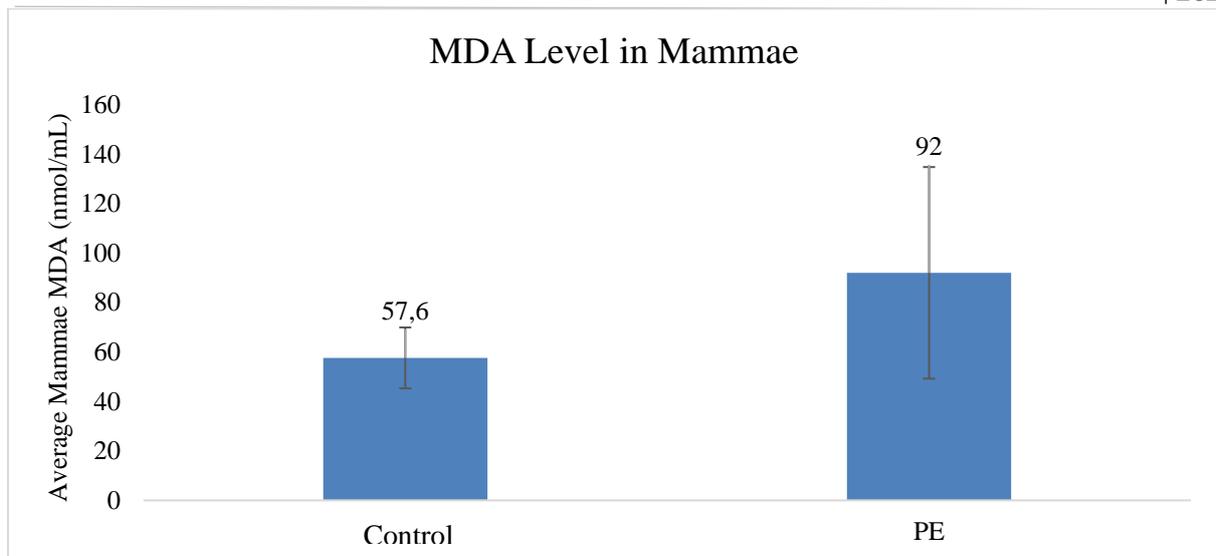
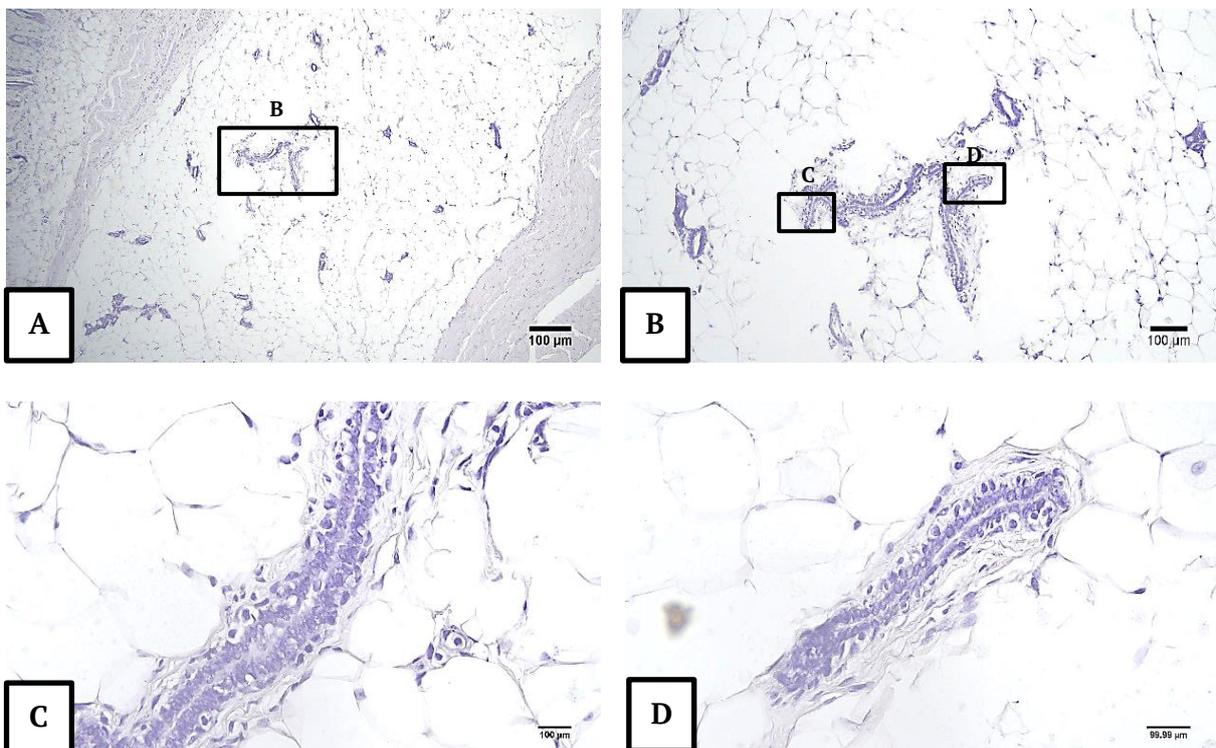


Figure 1. Graph of Average MDA Levels in Mammary Glands of Female White Rats

Observation of Lactiferous Ducts

Observation of epithelial cell proliferation in lactiferous ducts in histopathological images was conducted using an Olympus CX23 microscope at magnifications of 40x, 100x, and 400x. In each field of view, the number of layers of epithelial cells surrounding the lactiferous ducts was counted. The counted epithelial cells were the number of cuboidal epithelial cells from the inner surface to the edge of the lactiferous ducts. Histopathological images were taken in the lactiferous duct area in 10 different fields of view for each group, as presented in Figure 2.

Control Group



PE Group

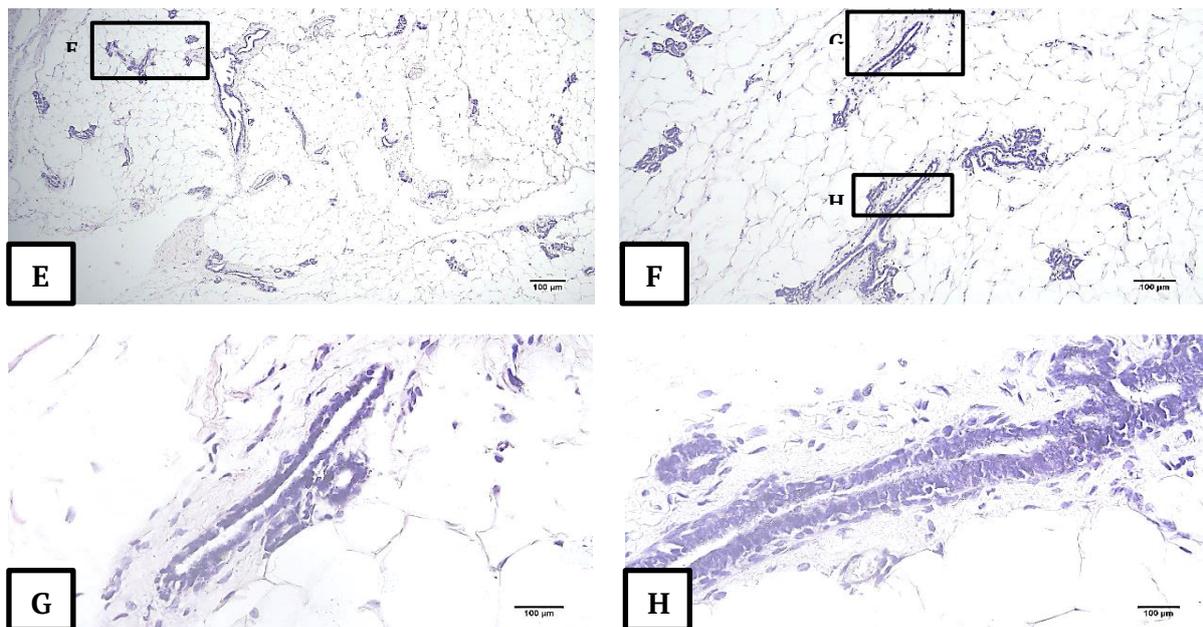


Figure 2. Histopathological Images of Lactiferous Ducts in Mammary Glands with HE Staining in the Control Group (A-D) at magnifications of 40x (A), 100x (B), 400x (C and D), and Treatment Group PE (E-H) at magnifications of 40x (E), 100x (F), 400x (G and H), scale bar: 100 μm.

Average Number of Lactiferous Ducts

Microscopic examination of the mammary glands of female white rats reveals several parts, namely lactiferous ducts and acinar cells. Each sample of mammary tissue from female white rats has a variable number of ducts. The observation of duct numbers in each sample of mammary tissue from female white rats was conducted in 10 fields of view for each sample, and the total number, as well as the average number of ducts for each group, were counted and presented in graphical form in Figure 3 below.

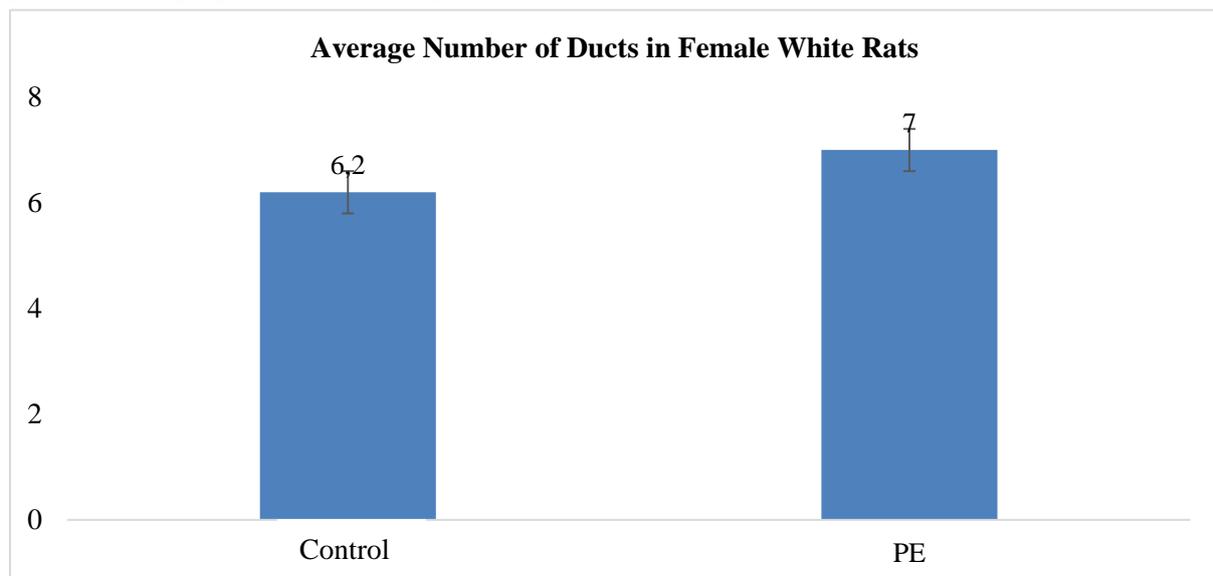


Figure 3. Graph of Average Number of Ducts in Female White Rats

Upon calculation, it was found that in the control group, the average number of ducts was 6 ± 3.42 , while in the PE (plastic exposure) group, the average number of ducts was 7 ± 2.09 . It can be concluded that the total average duct count in the control group and the treatment group did not differ significantly. The normality test results for the number of ducts showed a result of 0.464, indicating a normal data distribution. The Independent T-test resulted in a significance value of 0.644 ($t > 0.05$), hence it can be concluded that exposure to micro-nano plastics did not differ significantly between the two groups.

Number of Layers of Cuboidal Epithelial Cells in Lactiferous Ducts

Microscopic examination of the mammary glands of female white rats in the control group showed normal lactiferous ducts, which were surrounded by an average of 1 layer of slightly elongated square-shaped cuboidal epithelial cells, surrounded by normal connective tissue and fat tissue, with fine chromatin nuclei, and no inflammatory cells were found. In the treatment group, the lactiferous ducts were surrounded by an average of 2 layers of cuboidal epithelial cells that had changed shape to become round, appeared thicker, with coarse chromatin nuclei, and no inflammatory cells were found. Histopathological images of lactiferous ducts in mammary glands for each group are presented in Figure 4.

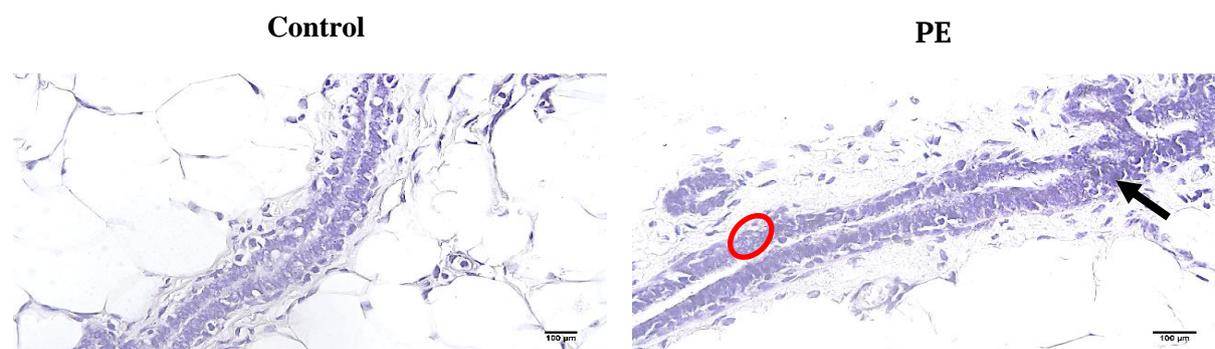


Figure 4. Comparison of Histopathological Images of Lactiferous Ducts in the Control Group and PE Group, red circles indicate the number of layers of cuboidal epithelial cells, which are 1 layer and round in shape, arrows indicate coarse chromatin nuclei, scale bar: 100 µm.

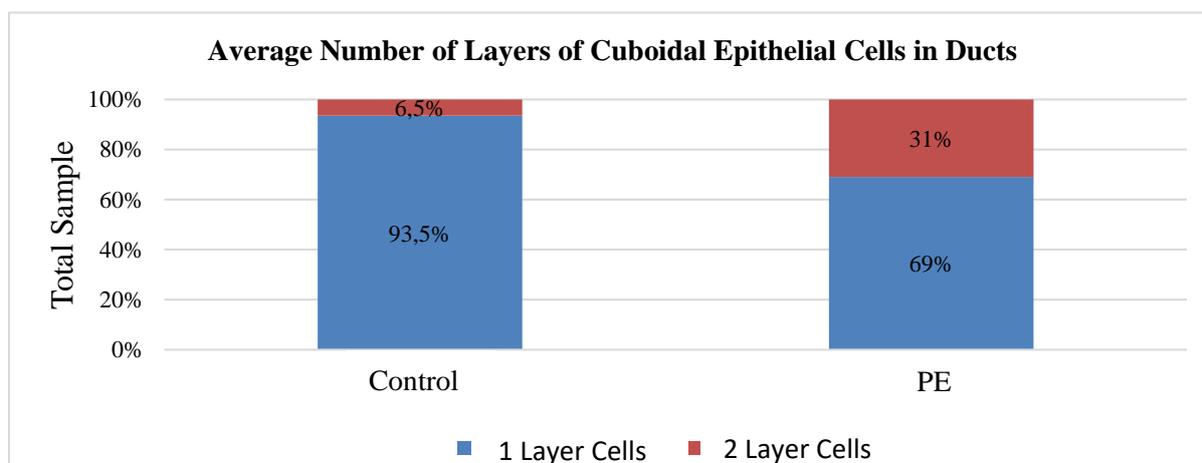


Figure 5. Graph of Average Number of Layers of Cuboidal Epithelial Cells in Ducts

Figure 5 shows the total number of layers of ductal epithelial cells counted per sample with 10 fields of view. It can be observed that in the 2-layer sample, the treatment group has a

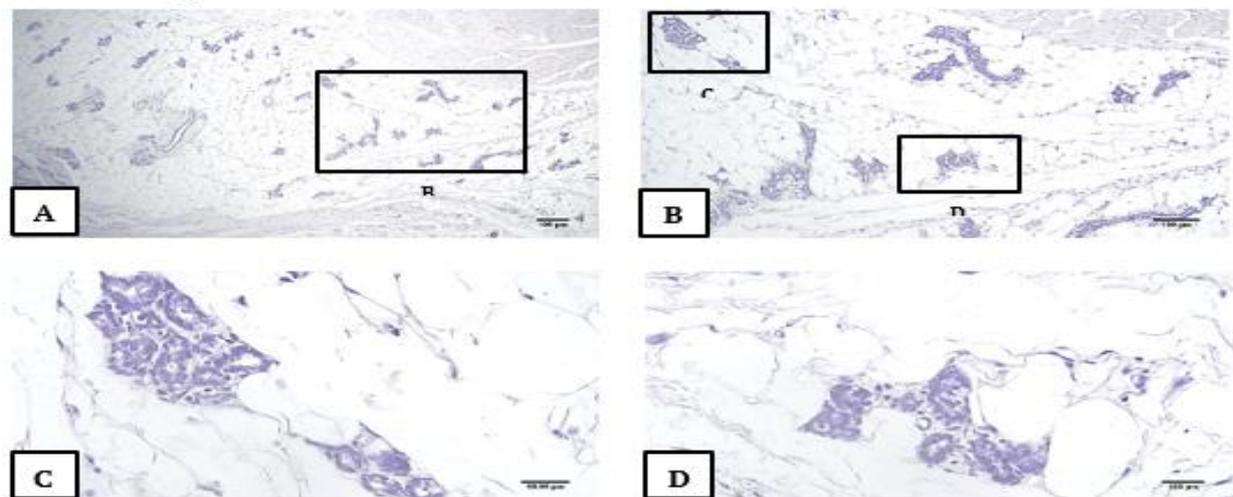
higher percentage (31%) compared to the control group (6.5%), indicating that the PE group experienced a 4.7-fold increase in proliferation compared to the control group.

Data analysis was performed on the 2-layer ductal epithelial cell samples in the control group and PE group. The normality test results showed a result of 0.02, indicating that the data distribution was not normal. The Mann Whitney test resulted in a significance value of 0.013 ($t < 0.05$), concluding that exposure to micro-nano plastics significantly differed between the two groups.

Observation of Acinar Cells

Observation of epithelial cell proliferation surrounding the acini in histopathological images was conducted using an Olympus CX23 microscope at magnifications of 40x, 100x, and 400x. Histopathological images were taken in the acini area in 10 different fields of view, as presented in Figure 6. In each field of view, the number of layers of secretory epithelial cells surrounding the mammary acini was counted. The counted epithelial cells were the number of cuboidal epithelial cells from the inner surface to the outer surface of the mammary acini.

Control Group



PE Group

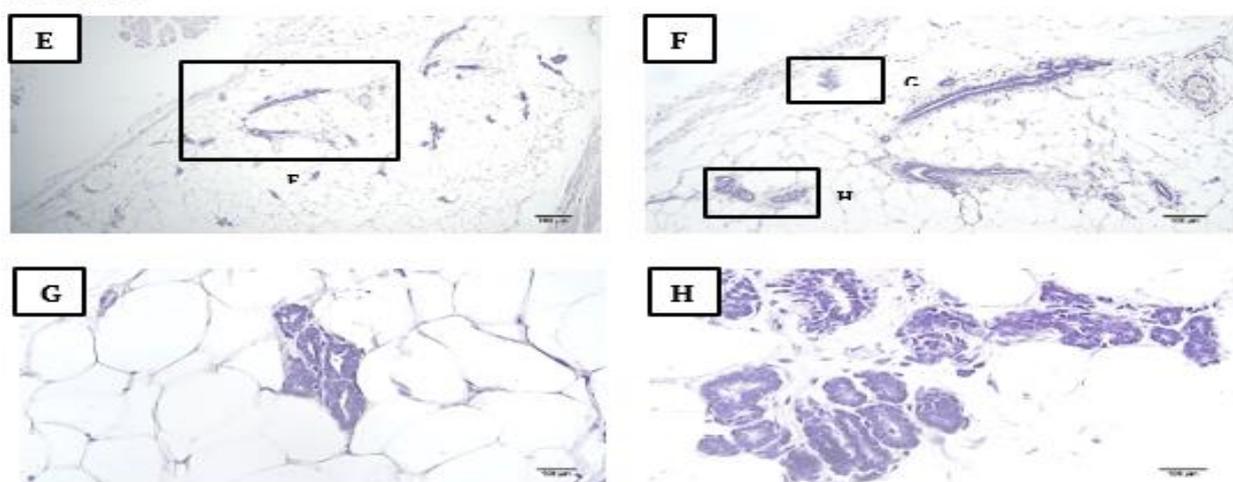


Figure 6. Histopathological Images of Mammary Gland Acini with HE Staining in the Control Group (K) at magnifications of 40x (A), 100x (B), 400x (C and D), and Treatment Group PE (P) at magnifications of 40x (E), 100x (F), 400x (G and H), scale bar: 100 μ m

Average Number of Acinar Cells

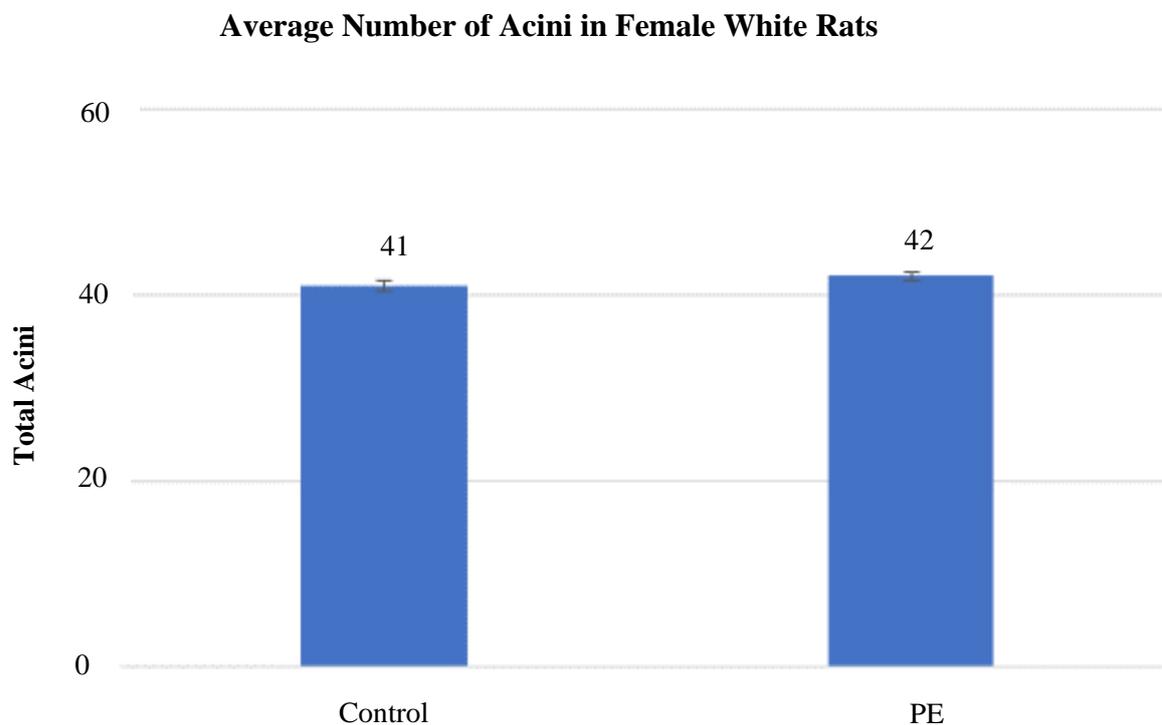


Figure 7. Graph of Average Number of Acini in Female White Rats

Microscopic examination of the mammary glands of female white rats reveals several parts, namely lactiferous ducts and acinar cells. Each sample of mammary tissue from female white rats has a variable number of acinar cells. Observation of acinar cell counts in each sample of mammary tissue from female white rats was conducted in 10 fields of view, and the total number as well as the average number of acinar cells for each group were counted and presented in graphical form, as shown in Figure 7.

Upon calculation, it was found that in the control group, the average number of acinar cells was 41 ± 24.37 , whereas in the PE (plastic exposure) group, the average number of acinar cells was 42 ± 16.46 . These results indicate a significant difference in the total average acini between the control and treatment groups.

The normality test results for the number of mammary acini showed a result of 0.951, indicating a normal data distribution. The Independent T-test resulted in a significance value of 0.960 ($t > 0.05$), hence it can be concluded that exposure to micro-nano plastics did not significantly differ between the two groups.

Number of Layers of Cuboidal Epithelial Cells in Acini

Microscopic examination of the mammary glands of female white rats in the control group revealed acini surrounded by an average of 1 layer of normal cuboidal epithelial cells, slightly elongated in a square shape, surrounded by normal connective tissue and fat tissue, with fine chromatin nuclei.

Meanwhile, in the treatment group, the mammary acini were surrounded by an average of 2 layers of round-shaped cuboidal epithelial cells, thickened, with chromatin nuclei appearing coarse, and no inflammatory cells were found.

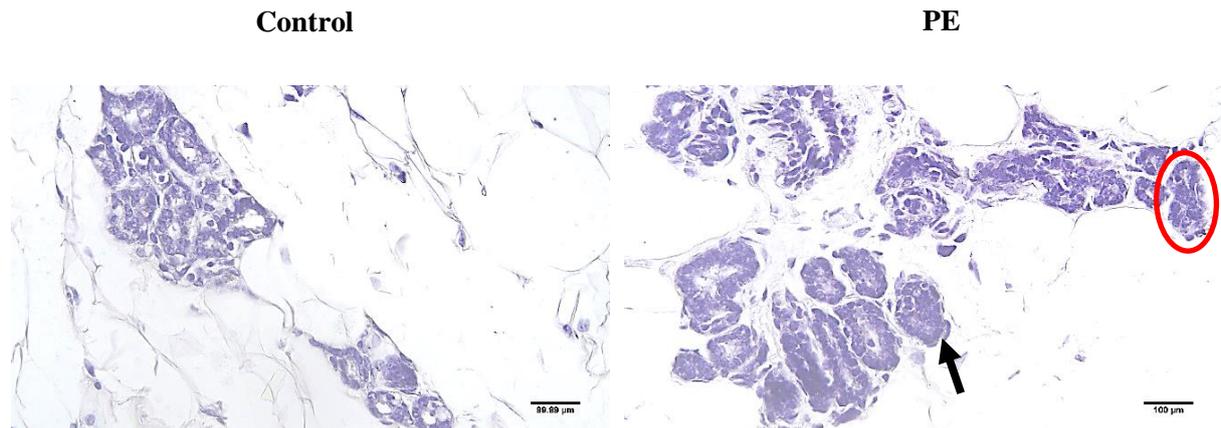


Figure 8. Comparison of Histopathological Images of Acini in Control Group and PE Group, red circles indicate the number of layers of cuboidal epithelial cells which are 1 layer in round shape, arrows indicate coarse chromatin nucleus, scale bar: 100 μ m.

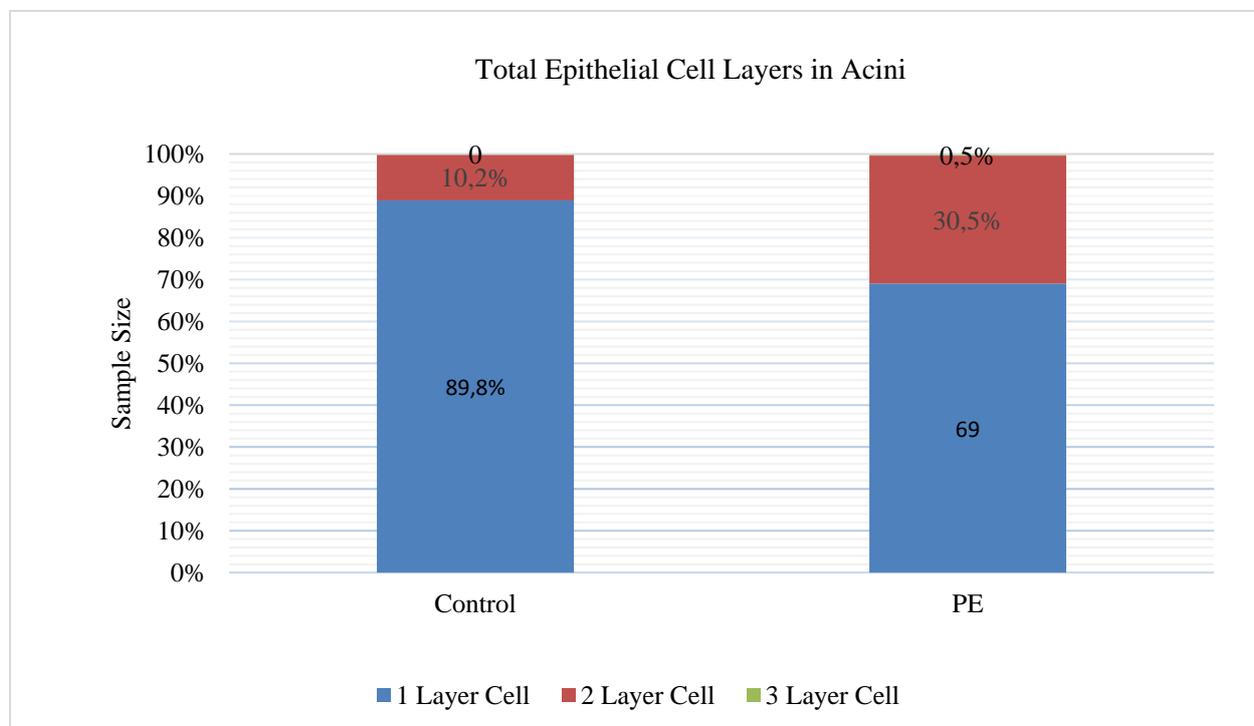


Figure 9. Total Epithelial Cell Layers in Acini

Figure 9 shows the total number of epithelial cell layers in the acini, counted per sample with 10 fields of view. In the control group, acini with a single layer of epithelial cells accounted for 89.8%, two layers for 10.2%, and three layers for 0%. In the PE group, acini with a single layer of epithelial cells accounted for 69%, two layers for 30.5%, and three layers for 0.5%. It can be seen that the percentage of two-layer epithelial cells in the treatment group is higher than in the control group, indicating that the PE group experienced a threefold increase in proliferation compared to the control group.

Data analysis was conducted on samples with two layers of epithelial cells in the ducts from both the control and PE groups. The normality test results for the number of epithelial cell layers surrounding the mammary acini showed a result of 0.928 (p -value > 0.05), indicating

that the data distribution is normal and suitable for parametric testing with the Independent T-test. The Independent T-test results showed a significance of 0.007 ($t < 0.05$), indicating that the exposure to micro-nano plastics resulted in a significant difference between the two groups.

DISCUSSION

Effect on MDA Levels in Mammary Glands

Malondialdehyde (MDA) is the most commonly used biomarker for measuring oxidative stress in the human body in various health issues, especially cancer (Maurya et al., 2021). Oxidative stress is caused by an increase in the production of ROS or free radicals in the body (Nakai & Tsuruta, 2021). Oxidative stress can be caused by various factors, one of which is exposure to chemicals that enter the body which can cause genetic mutations and hormonal dysfunction that can initiate the development of cancer cells in the breast organ (Miao et al., 2021).

In this study, MDA levels in the breast were measured to determine the increase in free radicals in the body after female white rats were exposed to PE. The results showed that MDA levels in the PE group were 1.6 times higher than in the control group. This indicates the presence of excessive free radicals in the body in the PE group, potentially leading to genetic mutations and abnormal cell proliferation (Jelic et al., 2021).

These results are consistent with the study by Ijaz et al., (2022), which investigated the effects of PE exposure on reproductive toxicity in male rats. One of the findings of this study was a significant increase in MDA levels in the treatment rats compared to the control rats. This can occur due to the accumulation of free radicals in the body, which increases ROS levels, thus raising MDA levels (Nakai & Tsuruta, 2021).

To date, no exact dose has been determined for the minimum and maximum toxicity of PE plastic exposure in either rat or human studies. However, using PE-type equipment exposed to sunlight or heat for 12 days or more can release antimony compounds beyond normal limits, which can be carcinogenic if they enter the body (Tapiory et al., 2019). This is similar to the use of PE plastic containers used to store water or warm or even hot food, which results in the continuous release of antimony compounds (Diningsih & Rangkuti, 2020).

Effect on Histopathological Features of Mammary Glands

Microscopically, the breast consists of ducts, lobules, acinar cells, fat glands, connective tissue, plasma cells, blood vessels, and muscles (Krisdianilo, Sumantri, & Sidabutar 2021). When the body is exposed to micro-nano plastics, it can cause various toxic effects, including on the breast. Exposure to MNPs can lead to excessive free radical exposure, causing oxidative stress. Oxidative stress will initiate cell cycle development, leading to abnormal cell proliferation and resulting in breast cancer (Chen et al., 2022; Park et al., 2023). Histologically, ducts and acini are lined by a single layer of cuboidal epithelial cells. If the cell layer proliferates into two or more layers or if there are changes in the shape of the epithelial cells, this may indicate abnormal cell proliferation activity (Rinjaya & Mardhia, 2022).

In this study, the number of ducts and acini did not differ significantly, indicating no abnormalities in these parts. However, the average number of epithelial cell layers in each group showed significant results. In the control group, both ducts and acini were lined by a single layer of slightly elongated cuboidal epithelial cells, with smooth chromatin and no inflammatory cells. In the PE group, ducts and acini were lined by two layers of rounded cuboidal epithelial cells, which were slightly thickened, had coarse chromatin, and showed no inflammatory cells, with a 4.7-fold increase in duct proliferation and a 3-fold increase in acini compared to the control group.

This study aligns with the findings of Nansi et al., (2015), who administered the chemical benzo(α)pyrene around the cancer area, and with the study by Rinjaya & Mardhia, (2022), who injected 7,12-dimethylbenz (α)anthracene (DMBA) as a cancer-inducing chemical in a breast cancer rat model. These chemicals can be metabolized by the body, causing gene mutations in DNA and having carcinogenic properties that can lead to cancer (Rinjaya & Mardhia, 2022). Both studies support the basic hypothesis in this study that exposure to certain carcinogens can cause significant genetic mutations and have strong carcinogenic properties. These findings provide a strong scientific basis for the mechanism of action of the carcinogens used in this study, although the types of carcinogens used are different.

Another study by (Kim et al., 2021) exposed MNPs of Polypropylene at a dose of 25 mg/kg body weight/day for 4 weeks and showed no significant differences, suggesting that MNP exposure at this dose does not have adverse effects on humans. However, the study by Ijaz et al., (2022), which exposed 50 mg/kg body weight of PE MNPs for 56 days in experimental animals, showed significant results, indicating that high doses and long exposure times can cause damage to various organ systems in the body.

Antimony trioxide contained in PE plays a significant role in the carcinogenesis process in the mammary glands. Continuous exposure to antimony trioxide can cause disturbances in the body's systems, especially the reproductive system in women, as it can interfere with pregnancy and cause abortion (Baharuddin, Asran & Ikhtiar 2023; Tapiory et al., 2019). Additionally, antimony trioxide is potentially a cancer-causing agent in the human body (Dhaka et al., 2022).

4. CONCLUSION

Exposure to micro-nano plastics of PE type at a dose of 15 mg/m³ for 28 days differed significantly in the MDA levels of mammary glands, histopathological picture of mammary glands at cell proliferation events, both in the lactiferous ducts and acini cells. In addition, exposure to PE plastic has the potential to cause health problems in the breast organs if exposed for a long time and in excess doses. For further research, it is better to use inhalation and oral exposure methods with various types of microplastic combinations to better resemble the actual event.

REFERENCES

- Anom, I. D. K., & Lombok, J. Z. (2020). Karakterisasi Asap Cair Hasil Pirolisis Sampah Kantong Plastik sebagai Bahan Bakar Bensin. *Fullerene Journal of Chemistry*, 5(2), 96-101. <https://doi.org/10.37033/fjc.v5i2.206>
- Baharuddin, A., Asran, A., & Ikhtiar, M. (2023). Spasial Analisis Mikroplastik dengan Metode FT-IR (Fourier Transform Infrared) Pada Feses Petani Kerang Hijau. *Window of Health: Jurnal Kesehatan*, 331-343.
- Campanale, C., Massarelli, C., Savino, I., Locaputo, V., & Uricchio, V. F. (2020). A detailed review study on potential effects of microplastics and additives of concern on human health. *International journal of environmental research and public health*, 17(4), 1212. <https://doi.org/10.3390/ijerph17041212>
- Cary, C. M., Seymore, T. N., Singh, D., Vayas, K. N., Goedken, M. J., Adams, S., ... & Stapleton, P. A. (2023). Single inhalation exposure to polyamide micro and nanoplastic particles impairs vascular dilation without generating pulmonary inflammation in virgin female Sprague Dawley rats. *Particle and Fibre Toxicology*, 20(1), 16. <https://doi.org/10.1186/s12989-023-00525-x>
- Chang, X., Xue, Y., Li, J., Zou, L., & Tang, M. (2020). Potential health impact of environmental micro-and nanoplastics pollution. *Journal of Applied Toxicology*, 40(1), 4-15.

- <https://doi.org/10.1002/jat.3915>
- Chen, K., Lu, P., Beeraka, N. M., Sukocheva, O. A., Madhunapantula, S. V., Liu, J., ... & Aliev, G. (2022, August). Mitochondrial mutations and mitoepigenetics: Focus on regulation of oxidative stress-induced responses in breast cancers. In *Seminars in cancer biology*, 83, 556–569. <https://doi.org/10.1016/j.semcancer.2020.09.012>
- Dhaka, V., Singh, S., Anil, A. G., Sunil Kumar Naik, T. S., Garg, S., Samuel, J., ... & Singh, J. (2022). Occurrence, toxicity and remediation of polyethylene terephthalate plastics. A review. *Environmental Chemistry Letters*, 20, 1777–1800. <https://doi.org/10.1007/s10311-021-01384-8>
- Diningsih, A., & Rangkuti, N. A. (2020). Penyuluhan Pemakaian Plastik Sebagai Kemasan Makanan dan Minuman yang Aman Digunakan untuk Kesehatan di Desa Labuhan Rasoki. *Jurnal Education and Development*, 8(1), 17-20. <https://doi.org/10.37081/ed.v8i1.1489>
- Enyoh, C. E., Verla, A. W., Verla, E. N., Ibe, F. C., & Amaobi, C. E. (2019). Airborne microplastics: a review study on method for analysis, occurrence, movement and risks. *Environmental monitoring and assessment*, 191, 668. <https://doi.org/10.1007/s10661-019-7842-0>
- Frommlet, F., & Heinze, G. (2021). Experimental replications in animal trials. *Laboratory animals*, 55(1), 65-75. <https://doi.org/10.1177/0023677220907617>
- Ghatge, S., Yang, Y., Ahn, J. H., & Hur, H. G. (2020). Biodegradation of polyethylene: a brief review. *Applied Biological Chemistry*, 63, 27. <https://doi.org/10.1186/s13765-020-00511-3>
- Helm, J. S., & Rudel, R. A. (2020). Adverse outcome pathways for ionizing radiation and breast cancer involve direct and indirect DNA damage, oxidative stress, inflammation, genomic instability, and interaction with hormonal regulation of the breast. *Archives of toxicology*, 94(5), 1511-1549. <https://doi.org/10.1007/s00204-020-02752-z>
- Ijaz, M. U., Ayaz, F., Mustafa, S., Ashraf, A., Albeshr, M. F., Riaz, M. N., & Mahboob, S. (2022). Toxic effect of polyethylene microplastic on testicles and ameliorative effect of luteolin in adult rats: Environmental challenge. *Journal of King Saud University-Science*, 34(4), 102064. <https://doi.org/10.1016/j.jksus.2022.102064>
- Ilmiawati, I., Mahata, L. E., Aliska, G., Rustam, E., Katar, Y., Rahmatini, R., ... & Usman, E. (2022). Peningkatan Pengetahuan Masyarakat tentang Bahaya Paparan Mikroplastik dan Dampaknya bagi Kesehatan. *Warta Pengabdian Andalas*, 29(3), 305-311. <https://doi.org/10.25077/jwa.29.3.305-311.2022>
- Jelic, M. D., Mandic, A. D., Maricic, S. M., & Srdjenovic, B. U. (2021). Oxidative stress and its role in cancer. *Journal of cancer research and therapeutics*, 17(1), 22-28. https://doi.org/10.4103/jcrt.JCRT_862_16
- Karlsson, T. M., Vethaak, A. D., Almroth, B. C., Ariese, F., van Velzen, M., Hassellöv, M., & Leslie, H. A. (2017). Screening for microplastics in sediment, water, marine invertebrates and fish: Method development and microplastic accumulation. *Marine pollution bulletin*, 122(1-2), 403-408. <https://doi.org/10.1016/j.marpolbul.2017.06.081>
- Kementerian Lingkungan Hidup dan Kehutanan Republik Indonesia. (2022). *Capaian Kinerja Pengelolaan Sampah*. Jakarta: Kementerian Lingkungan Hidup dan Kehutanan Republik Indonesia
- Kementerian Kesehatan Republik Indonesia. (2022). *Kanker Payudara Paling Banyak di Indonesia, Kemenkes Targetkan Pemerataan Layanan Kesehatan*. Retrieved from <https://sehatnegeriku.kemkes.go.id/baca/umum/20220202/1639254/kanker-payudara-paling-banyak-di-indonesia-kemenkes-targetkan-pemerataan-layanan-kesehatan/>

- Kim, J., Maruthupandy, M., An, K. S., Lee, K. H., Jeon, S., Kim, J. S., & Cho, W. S. (2021). Acute and subacute repeated oral toxicity study of fragmented microplastics in Sprague-Dawley rats. *Ecotoxicology and Environmental Safety*, 228, 112964. <https://doi.org/10.1016/j.ecoenv.2021.112964>
- Krisdianilo, V., Sumantri, B., & Sidabutar, R. (2021). Gambaran Sel Epitel Pada Lesi Payudara Dilaboratorium Patologi Anatomi Upt Rsud Deli Serdang Lubuk Pakam. *Jurnal Farmasimed (JFM)*, 3(2), 100-106. <https://doi.org/10.35451/jfm.v3i2.624>
- Maurya, R. P., Prajapat, M. K., Singh, V. P., Roy, M., Todi, R., Bosak, S., ... & Morekar, S. R. (2021). Serum malondialdehyde as a biomarker of oxidative stress in patients with primary ocular carcinoma: impact on response to chemotherapy. *Clinical Ophthalmology*, 871-879. <https://doi.org/10.2147/OPTH.S287747>
- Miao, Y., Rong, M., Li, M., He, H., Zhang, L., Zhang, S., ... & Zeng, Q. (2021). Serum concentrations of organochlorine pesticides, biomarkers of oxidative stress, and risk of breast cancer. *Environmental Pollution*, 286, 117386. <https://doi.org/10.1016/j.envpol.2021.117386>
- Nakai, K., & Tsuruta, D. (2021). What are reactive oxygen species, free radicals, and oxidative stress in skin diseases?. *International journal of molecular sciences*, 22(19), 10799. <https://doi.org/10.3390/ijms221910799>
- Nansi, E. M., Durry, M. F., & Kairupan, C. (2015). Gambaran histopatologik payudara mencit (*Mus musculus*) yang diinduksi benzo (α) pyrene dan diberikan ekstrak kunyit (*Curcuma longa* L.). *eBiomedik*, 3(1), 510-515.
- Park, J. H., Hong, S., Kim, O. H., Kim, C. H., Kim, J., Kim, J. W., ... & Lee, H. J. (2023). Polypropylene microplastics promote metastatic features in human breast cancer. *Scientific reports*, 13(1), 6252. <https://doi.org/10.1038/s41598-023-33393-8>
- Rinjaya, T., & Mardhia, M. (2022). Pengaruh Ekstrak Etanol Daun Sirsak terhadap Histologi Tumor Payudara Tikus Putih Betina Sprague Dawley. *Cermin Dunia Kedokteran*, 49(9), 484-488. <https://doi.org/10.55175/cdk.v49i9.292>
- Rudend, A. J., & Hermana, J. (2021). Kajian pembakaran sampah plastik jenis polipropilena (PP) menggunakan insinerator. *Jurnal Teknik ITS*, 9(2), D124-D130. <https://doi.org/10.12962/j23373539.v9i2.55410>
- Sen, S. K., & Raut, S. (2015). Microbial degradation of low density polyethylene (LDPE): A review. *Journal of Environmental Chemical Engineering*, 3(1), 462-473. <https://doi.org/10.1016/j.jece.2015.01.003>
- Shim, W. J., & Thomposon, R. C. (2015). Microplastics in the ocean. *Archives of environmental contamination and toxicology*, 69, 265-268. <https://doi.org/10.1007/s00244-015-0216-x>
- Tapiory, J. G., Darjati, D., Sari, E., Narwati, N., & Ambarwati, A. (2019). Influence of Sunlight on Drinking Water in Packaging in Plastic Type of Polyethylene Terephthalate Related to Antimon Content. *Jurnal Penelitian Kesehatan "SUARA FORIKES"(Journal of Health Research "Forikes Voice")*, 10(1), 34-41.
- Tirani, M. M., & Haghjou, M. M. (2019). Reactive oxygen species (ROS), total antioxidant capacity (AOC) and malondialdehyde (MDA) make a triangle in evaluation of zinc stress extension. *JAPS: Journal of Animal & Plant Sciences*, 29(4), 1100-1111.
- Vianello, A., Jensen, R. L., Liu, L., & Vollertsen, J. (2019). Simulating human exposure to indoor airborne microplastics using a Breathing Thermal Manikin. *Scientific reports*, 9(1), 8670. <https://doi.org/10.1038/s41598-019-45054-w>

Jurnal Info Kesehatan

Vol. 22, No. 2, June 2024, pp. 272-288

P-ISSN 0216-504X, E-ISSN 2620-536X

DOI: [10.31965/infokes.Vol22.Iss2.1535](https://doi.org/10.31965/infokes.Vol22.Iss2.1535)

Journal homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>



RESEARCH

Open Access

Analysis of the Effect of Black Garlic (*Allium sativum*) Extract on Ovarian Follicular Atresia, Endometrial VEGF Expression, and Fallopian Tube Epithelial Cell Count in Rats (*Rattus norvegicus*) Exposed to Cigarette Smoke

Novalia Kridayanti^{1a*}, Noni Fidya Ayu Anandasari^{1b}, Ni Ketut Devy Kaspirayanti^{1c}, Tatit Nurseta^{2d}, Tri Yudani Mardining Raras^{3e}, Husnul Khotimah^{4f}, Subandi Reksohusodo^{1,2g}, Aina Angelica^{5h}, Kenty Wantri Anita⁵ⁱ, Hendy Setyo Yudhanto^{5j}

¹ Master Program of Midwifery, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia

² Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia

³ Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Brawijaya, East Java, Malang, Indonesia

⁴ Faculty of Medical, Universitas Brawijaya, Malang, East Java, Indonesia

⁵ Department of Pathological Anatomy, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia

^a Email address: novalia.kri@gmail.com

^b Email address: nonifidyaayu@gmail.com

^c Email address: devykaspira@student.ub.ac.id

^d Email address: tns_obg_fk@ub.ac.id

^e Email address: daniraras@ub.ac.id

^f Email address: husnul_farmako_fk@ub.ac.id

^g Email address: desobg@gmail.com

^h Email address: aina.angelina@ub.ac.id

ⁱ Email address: kenty_wa@ub.ac.id

^j Email address: hendy.setyo@ub.ac.id

Received: 23 May 2024

Revised: 11 June 2024

Accepted: 19 June 2024

Abstract

Cigarette smoke exposure significantly impairs reproductive function in *Rattus norvegicus*. This study evaluated the protective effects of black garlic extract against such damage. Using a post-test-only control group design, 25 female Wistar rats were divided into five groups and exposed to cigarette smoke with or without varying doses of black garlic extract. After four weeks, results indicated that black garlic extract significantly increased Vascular Endothelial Growth Factor expression, enhanced fallopian tube secretory epithelial cell counts, and reduced ovarian follicular atresia in rats exposed to cigarette smoke. The group receiving 50 mg/kgBW of black garlic extract showed the most significant improvements. Statistical analysis, including One-way ANOVA, revealed significant differences between groups. Normality was assessed using the Shapiro-Wilk test, and homogeneity was confirmed with the Levene test. Significant decreases in ovarian follicular atresia ($p < 0.05$) and increases in Vascular Endothelial Growth Factor expression ($p < 0.05$) and secretory epithelial cell counts ($p < 0.05$) were observed in the 50 mg/kgBW treatment group compared to controls. In conclusion, black garlic extract offers dose-dependent protection against cigarette smoke-induced reproductive damage, with 50 mg/kgBW being the optimal dose. Further research should explore molecular mechanisms, long-term toxicity, and clinical applications in humans.

Keywords: Black Garlic Extract, Cigarette Smoke, Fallopian Tube Epithelial Cell, Follicular Atresia.

***Corresponding Author:**

Novalia Kridayanti

Master Program of Midwifery, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia

Email: novalia.kri@gmail.com



©The Author(s) 2024. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

1. INTRODUCTION

Cigarette smoke is known to have widespread negative impacts on human health, including increased risk of cardiovascular disease, lung cancer, and reproductive disorders. (Rahma et al., 2019; Widyanti et al., 2020), exposure to cigarette smoke raises the risk of coronary heart disease by 2.4 times in individuals over 45 years old and can reduce fertility by 30% in women who are frequently exposed (Ardiana, 2021). (GATS, 2021) shows an increase in the number of smokers in Indonesia, with 34.5% of the population, or around 70.2 million people, being smokers, most of whom are men.

More than 4800 harmful chemicals and free radicals can be found in cigarette smoke, including nicotine, carbon monoxide, and tar. Research shows that nicotine affects the expression of estrogen and progesterone receptors and vascular endothelial growth factor (VEGF), which play a role in cell survival" (Ardiana, 2021; Totonchi et al., 2016). Cigarette smoke exposure also induces abnormal proliferation of the inner lining of rat uterus and affects endometrial angiogenesis in human and rat endometrial cells (Budani et al., 2021; Lee et al., 2017). Cigarette smoke exposure impairs female fertility through specific mechanisms, including decreased VEGF expression and increased oxidative stress. VEGF is essential for maintaining endometrial thickness and embryo implantation and development (Hanum & Saleha, 2023; Kida et al., 2021).

Cigarette smoke contains various free radicals, such as nicotine and tar, that can cause oxidative stress by disrupting the balance between the body's antioxidant defense mechanisms and the production of reactive oxygen species (ROS) (Awaga et al., 2019). This disrupts the normal function of the hypothalamus-pituitary, ovaries, and endometrium, potentially disrupting the ovulation and implantation process (Susanti et al., 2020).

Oxidative stress, a condition where there is an imbalance between the production of free radicals and the body's ability to neutralize them, can damage DNA and cell membranes, and disrupt the normal function of reproductive cells. This imbalance leads to cell damage and impaired reproductive function, further highlighting the detrimental effects of cigarette smoke on female fertility.

VEGF is essential in the angiogenesis process required for healthy endometrial tissue's growth and development. Decreased VEGF expression due to cigarette smoke exposure can reduce endometrial thickness, which is essential for embryo implantation and normal embryo development. A thin and poorly developed endometrium can reduce the ability of the embryo to attach and survive, thus decreasing the chance of pregnancy.

In addition, oxidative stress caused by free radicals in cigarette smoke can cause damage to ovarian follicles and inhibit the production of reproductive hormones such as estrogen and progesterone. This damage can lead to follicular atresia, a condition where the ovarian follicles that should develop into mature eggs degenerate and die instead. This can significantly reduce the number of eggs available for fertilization, thereby reducing reproductive function and fertility in women.

The increase in the number of smokers globally has led to severe public health problems, including disorders of the female reproductive system. Cigarette smoke contains various harmful chemicals that can damage various organs, including the ovaries, endometrium, and fallopian tubes in women. Recent studies have shown that VEGF expression in the endometrium and the number of endometrial arterioles may decrease due to exposure to cigarette smoke. Chemicals in cigarettes, such as nicotine and free radicals, can induce oxidative stress, leading to cell damage and ovarian follicular atresia, disrupting normal ovarian function and reducing fertility.

Black garlic has been widely studied for its ability to improve health parameters, including overcoming free radical damage. Previous studies have shown various health benefits

of black garlic, such as anti-tumor, antioxidant, anti-allergic, hypocholesterolemic, and hepatoprotective activities (Zhang et al., 2015). The antioxidant properties of black garlic reportedly protect against free radical damage in the body (Chung, 2006). Aged garlic extract (AGE) produced from fresh garlic has also been shown to prevent oxidative damage by counteracting free radicals (Capasso, 2013). Research has also shown that black garlic produced at high temperatures and humidity has strong antioxidant properties (Lu et al., 2017). Black garlic is known to have more potent antioxidant activity than fresh garlic, with additional benefits such as anticancer, anti-allergic, hypocholesterolemic, and hepatoprotective activities (Yuan et al., 2016).

However, previous studies have some limitations. Firstly, many studies focus on general antioxidant properties without identifying the specific molecular mechanisms. Secondly, most studies were conducted in animal or cell models, thus requiring further confirmation through human clinical trials. In addition, variations in black garlic processing techniques, such as fermentation duration and temperature, affect its antioxidant activity (Herlina et al., 2019). However, there is yet to be a widely accepted standard for this process. Another study showed that fermentation with specific bacterial strains can increase antioxidant activity (Setiyoningrum et al., 2018), but this research has yet to be widely applied on a larger scale.

Although previous studies have highlighted the potential health benefits of black garlic, there is still a need for further research that explores specific mechanisms, confirms results through clinical trials, and establishes consistent processing standards to optimize its health benefits. It's important to note that while black garlic is generally considered safe for consumption, it may cause certain side effects such as bad breath, body odor, and heartburn in some individuals. The novelty of this study is that it focuses on evaluating the effects of black garlic on reproductive function affected by cigarette smoke exposure, taking into account the appropriate dosage for optimal protective effects. This makes natural treatment alternatives such as black garlic extract more attractive as they generally have fewer side effects, are safer, and are more affordable. Therefore, there is a growing need to look for safer and more affordable therapeutic alternatives, such as natural ingredients with antioxidant potential. One interesting natural ingredient is black garlic (*Allium sativum*), which is processed from garlic through fermentation, increasing its antioxidant content.

This research presents a novel approach to the negative impact of cigarette smoke exposure on the female reproductive system at the cellular and molecular levels, particularly oxidative stress damage. It addresses issues such as decreased expression of VEGF, vital for angiogenesis in the endometrium, increased follicular atresia indicating damage to the ovaries, and damage to fallopian tube epithelial cells, essential for ovum transportation. The study explores the potential of black garlic extract, a unique and unexplored therapeutic agent, to mitigate these adverse effects. It does so by harnessing the antioxidant properties of black garlic extract to reduce oxidative damage and improve reproductive function.

The study aims to comprehensively examine the therapeutic effects of black garlic extract on damage caused by cigarette smoke to the reproductive system of female rats. Specific objectives include evaluating the ability of black garlic extract to reduce ovarian follicular atresia, increase VEGF expression in the endometrium, and improve the number of secretory epithelial cells in fallopian tubes exposed to cigarette smoke. Through these objectives, the study seeks to provide insight into the potential of black garlic extract as an effective and safe therapeutic alternative to protect female reproductive function from the adverse effects of cigarette smoke.

This study highlights the increasing prevalence of smokers worldwide, particularly in Indonesia, where the number of female smokers and exposure to secondhand smoke has become a severe public health concern. The increased exposure in workplaces, homes, and restaurants necessitates addressing the gap in treatment and prevention of reproductive system damage due to cigarette smoke. By exploring the use of black garlic (*Allium sativum*) extract, which is easily accessible, potentially effective, and affordable, this study aims to offer a viable treatment alternative. Therefore, this research analyzed the effect of black garlic extract on ovarian follicular atresia, endometrial VEGF expression, and fallopian tube epithelial cell count in rats (*Rattus norvegicus*) exposed to cigarette smoke.

2. RESEARCH METHOD

This study used a true experimental method with a post-test-only control group design approach. This study was conducted at the Pharmacology Laboratory, Faculty of Medicine, Universitas Brawijaya, Indonesia, for 28 days. The subjects of this study were female Wistar rats (*Rattus norvegicus*), with an initial population of 25 animals divided into five experimental groups. The sampling technique, which was randomized to determine the experimental and control groups, was chosen for its ability to reduce bias and ensure a representative sample. Each group was exposed to cigarette smoke for five minutes per session, two sessions per day for 28 days. The following is the division of the groups and the duration of exposure:

- 1) CN (Negative Control): This group was not exposed to cigarette smoke and was not given black garlic extract.
- 2) CP (Positive Control): Exposed to cigarette smoke twice a day for five minutes per session for 28 days without black garlic extract.
- 3) P1 (Treatment 1): Exposed to cigarette smoke twice a day for five minutes per session and given a dose of 50 mg/kgBW black garlic extract for 28 days.
- 4) P2 (Treatment 2): Exposed to cigarette smoke twice a day for five minutes per session and given a dose of 100 mg/kgBW black garlic extract for 28 days.
- 5) P3 (Treatment 3): Exposed to cigarette smoke twice a day for five minutes per session and given a 200 mg/kgBW dose of black garlic extract for 28 days.

This duration of exposure was designed to mimic conditions of continuous exposure to cigarette smoke and to test the effectiveness of black garlic extract in reducing the adverse effects of such exposure. Data were collected through direct observation of biological changes in the rats after exposure to cigarette smoke and/or administration of black garlic extract. Each group was exposed to cigarette smoke for five minutes per session, two sessions per day for 28 days.. Specifically, this study measured the expression of VEGF in the endometrium, the number of arterioles in the endometrium, and the number of ovarian follicular atresia. Data were analyzed using parametric statistics and One-way ANOVA to determine significant differences between groups.

Data were presented in tables and graphs to facilitate interpretation of the results. Specifications of the tools used included an Olympus microscope for histological examination, a spectrophotometer for analysis of an image analyzer to calculate VEGF expression and number of arterioles. The materials used included black garlic extracts of specific concentrations prepared at the university's pharmacy laboratory, distilled water for control, and cigarette smoke generated from commercial cigarettes. The experimental conditions, including duration of exposure and dosage, were designed to minimize outside variables and improve the reliability of the results. The entire experimental procedure was approved by the Ethics Committee of the Faculty of Medicine, Brawijaya University, with ethical number 367/EC/KEPK-S2/11/2023.

3. RESULTS AND DISCUSSION

This study examined the effect of black garlic extract (*Allium sativum*) on ovarian follicular atresia, endometrial VEGF expression, and fallopian tube epithelial cell count in

Wistar female rats exposed to cigarette smoke. A total of 25 rats were divided into five groups, each of which was given different treatments: control with distilled water, exposure to cigarette smoke, and three groups that received black garlic extract at doses of 50, 100, and 200 mg/kgBW, and exposure to cigarette smoke.

Table 1. Group Distribution

Group	Cigarette Smoke (2 cigarettes /day for 28 days)	Black garlic Extract
CN	0	0
CP	√	0
P1	√	Dose of 50 mg/kgBW
P2	√	Dose of 100 mg/kgBW
P3	√	Dose of 200 mg/kgBW

Description: CN: Negative Control, CP: Positive Control, P1: Treatment 1, P2: Treatment 2, P3: Treatment 3.

Characteristics of the study subjects showed no significant difference in baseline weight between groups. Univariate analysis showed an overall decrease in body weight in all groups exposed to cigarette smoke, indicating a systemic effect of cigarette exposure.

After completion of the treatment period for four weeks (28 days), rats in the proestrus phase were vaginal swabbed and dissected. Bivariate analysis using the ANOVA test revealed significant differences in endometrial VEGF expression between the control group and the group receiving black garlic extract. In the group given a 50 mg/kg dose of black garlic extract, there was a significant decrease in an increase in VEGF expression compared to the group that was only exposed to cigarette smoke.

The decision criteria, namely $p\text{-value} > 0.05$, then the data is usually distributed, and vice versa; if the $p\text{-value} < 0.05$, then the data is not normally distributed. The Shapiro-Wilk test analysis obtained is shown in the table below:

Table 2. Data normality and homogeneity test results

Assumption Testing	Coefficient	p-value	Data distribution
Atresia Folikel			
Normality		0.765	Normal
Homogeneity		0.051	Homogen
VEGF Expression			
Normality	0.962	0.40	Normal
Homogeneity	1.133	0.36	Homogen
Fallopian Tube Secretory Epithelial Cell Count			
Normality	0.932	0.823	Normal
Homogeneity	2.400	0.087	Homogen

Description: If $p\text{-value} < 0.05$ the data is not normally distributed and if $p\text{-value} > 0.05$ the data is normally distributed.

The Effect of Black Garlic (*Allium sativum*) Extract on Ovarian Follicular Atresia in Rats (*Rattus norvegicus*) Exposed to Cigarette Smoke

The number of follicular atresias with the HE staining method was observed with an Olympus CX23 microscope with a magnification of 30x and 400x to reconfirm the follicles that experienced atresia. In the observation of Figure 1, it can be seen that the most follicular atresia occurs in group 3 (P3) exposed to cigarette smoke and BG extract 200 mg/kgBW. The least atresia was found in Group CN (Negative Control), which was not exposed to cigarette

smoke and only distilled water. In this group, the number of follicular atresia was the lowest compared to other groups exposed to cigarette smoke.

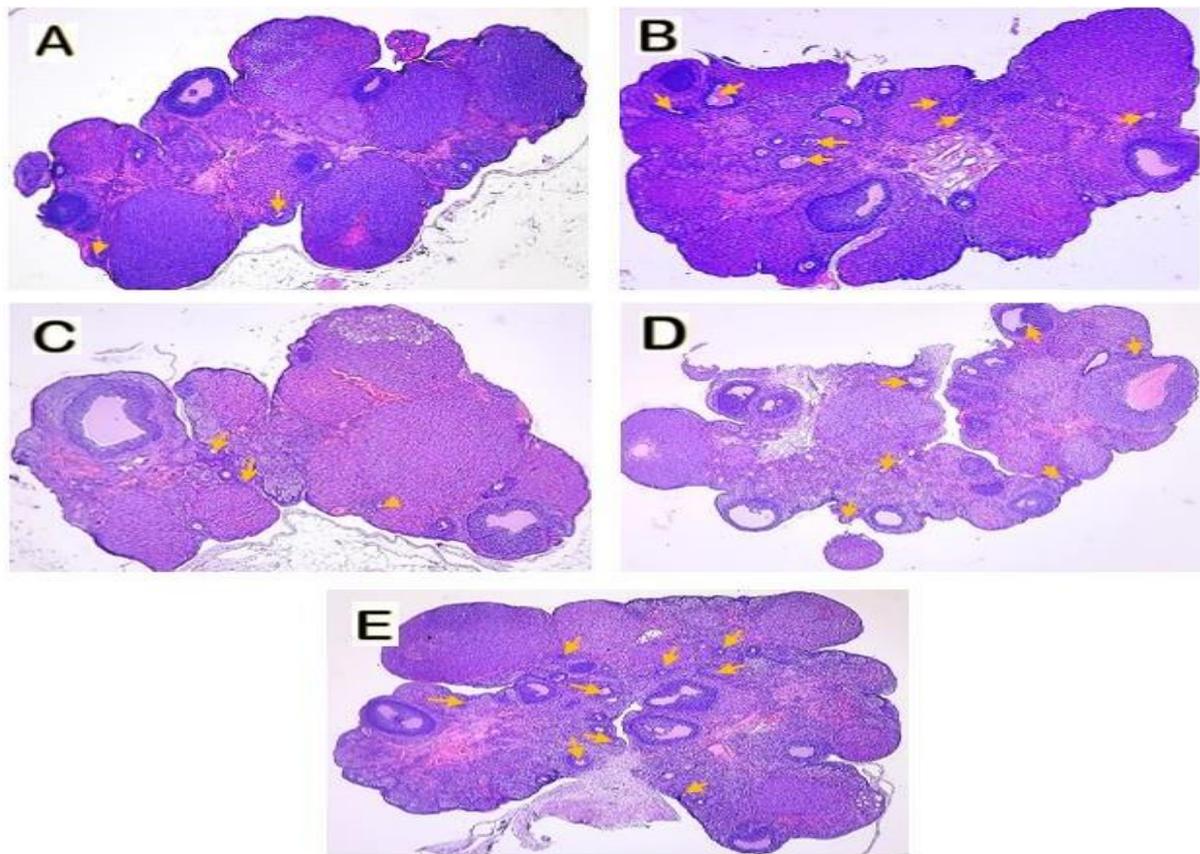


Figure 1. Follicular Histology

Observations in Figure 1 were made with an Olympus SZ51 microscope with 30x magnification. (a) observation on CN (without exposure to cigarette smoke); (b) observation on CP (exposed to cigarette smoke); (c) observation on P1 (cigarette smoke and BG 50 mg/kgBW); (d) observation on P2 (cigarette smoke and BG 100mg/kgBW); (e) P3 (cigarette smoke and 200 mg/kgBW BG extract). Follicular atresia appears more in P3.

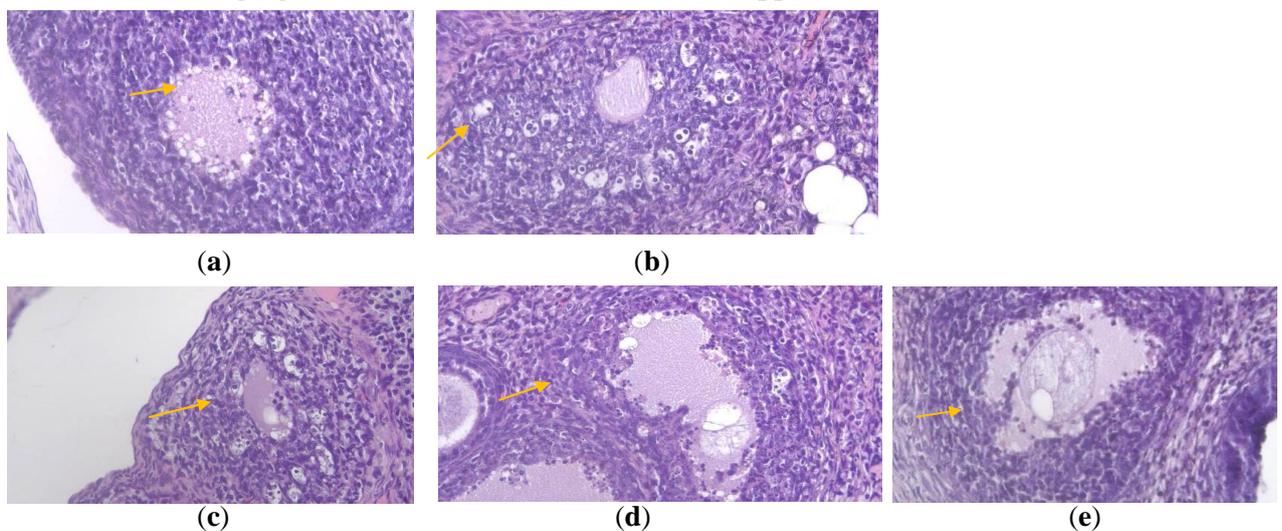


Figure 2. Follicular atresia

Observation of follicular atresia in Figure 2 with Olympus CX23 microscope with 400x magnification. (a) CN group, which was only given a distilled water sonde; (b) only exposed to cigarette smoke (CP); (c) P1 exposed to cigarette smoke and BG extract 50 mg/kgBW; (d) exposed to cigarette smoke and BG 100 mg/kgBW (P2); (e) exposed to cigarette smoke and BG 200 mg/kgBW (P3). Follicular atresia is characterized by apoptotic bodies and necrosis, as the yellow arrows indicate.

Table 3. T-test results of follicular atresia

Group	n	Mean±SD	p-value
CN	5	1.4 ± 1.67 ^a	0.000
CP	5	7.2 ± 3.77 ^b	
P1	5	2.6 ± 1.34 ^a	
P2	5	6.8 ± 0.84 ^b	
P3	5	8.2 ± 1.79 ^b	

Mean ± Standard Deviation indicates descriptive test results. p-value <0.05 indicates a significant difference.

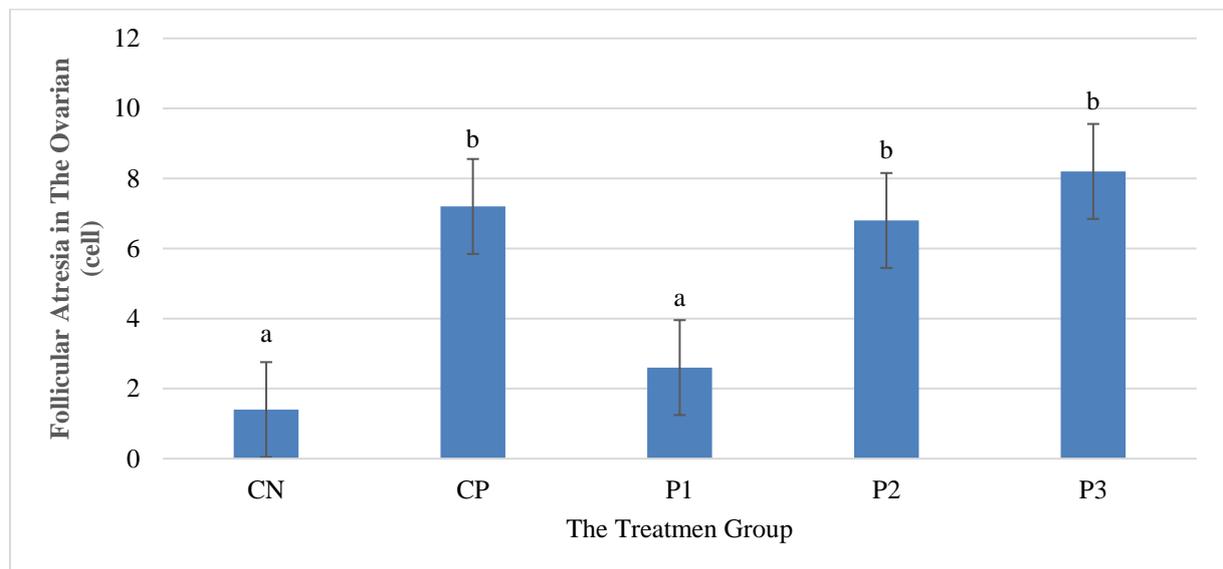


Figure 3. Follicular atresia in the ovarium on rat

Figure 3 shows a histogram of mean follicular atresia in female *Rattus norvegicus* that were not treated (CN) (mean 1.4 ± 1.67 standard deviation), exposed to cigarette smoke (CP) (mean 7.2 ± 3.77 standard deviation), and exposed to cigarette smoke and given BG at doses of 50 mg/kgBW (mean 2.6 ± 1.34 standard deviation), 100 mg/kgBW (mean 6.8 ± 0.84 standard deviation), and 200 mg/kgBW (mean 8.2 ± 1.79 standard deviation) (P1, P2, and P3). The mean follicular atresia appeared lower in group P1; administering BG at a dose of 50 mg/kgBW can reduce the mean follicular atresia in the ovaries. However, in groups P2 and P3, there was an increase in the mean follicular atresia, which was higher than in P1. BG administration with increasing doses can increase the average follicular atresia.

Table 4 shows that there is a significant relationship between BG dose and follicular atresia (p-value = 0.001 < α) in the cigarette smoke exposure and BG administration groups (50 mg/kgBW, 100 mg/kgBW, and 200 mg/kgBW). The test results of the correlation coefficient of dose to follicular atresia resulted in r = 0.791, which indicates a strong relationship or correlation. The correlation is positive, meaning that when the dose of BG is increased, there

will be an increase in follicular atresia in the group exposed to cigarette smoke. Vice versa, when the dose of BG is lowered, there will be a decrease in follicular atresia. Therefore, it can be concluded that there is a relationship between the dose of BG and follicular atresia in *Rattus norvegicus* rats exposed to subacute cigarette smoke, which refers to a level of smoke exposure that is less severe than chronic exposure but more severe than acute exposure.

Table 4. Correlation Test Results with Pearson Correlation

Correlated variables	Correlation coefficient (r)	Meaning	p-value	Description
Dose with follicular atresia	0.791	Strong correlation	0.000	Significant

Description: The correlation coefficient column indicates a strong relationship, and the p-value column indicates a significant relationship between the two variables.

The Effect of Black Garlic (*Allium sativum*) Extract on Endometrial VEGF Expression in Rats (*Rattus norvegicus*) Exposed to Cigarette Smoke

Immunohistochemical images of VEGF expression in the uterus of rats (*Rattus Norwegicus*) in the control group (negative and positive) and the treatment group of black garlic extract at a dose of 50, 100, 200 mg / kgBW in longitudinal sections and observed with an Olympus BX53 microscope with 400x magnification.

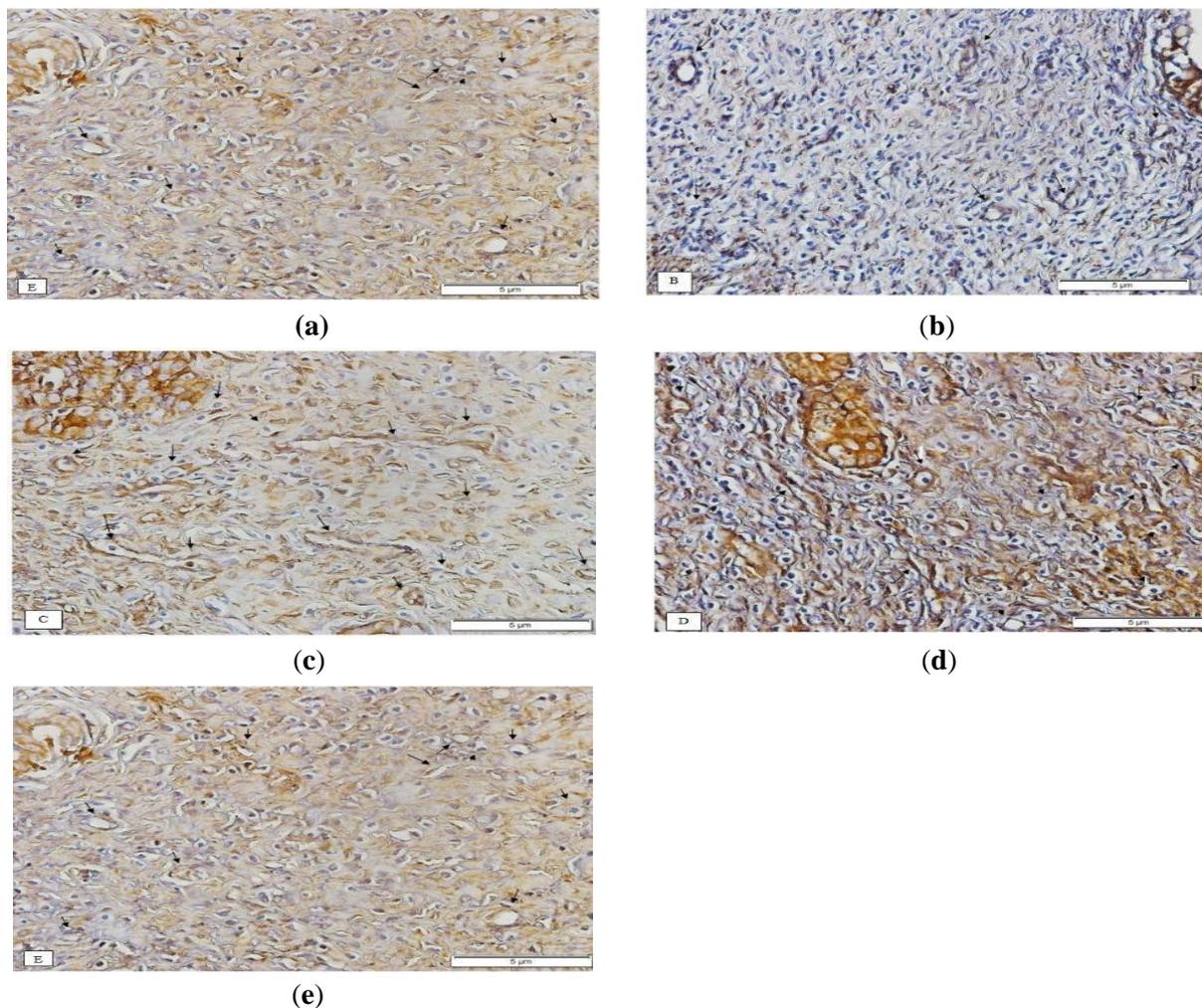


Figure 4. VEGF Expression

In Figure 4, the arrow shows positive VEGF expression, seen in the cytoplasm of endometrial blood vessels that are colored brown. (a) negative control group/no treatment; (b) positive control group/exposed to cigarette smoke; (c) treatment group 1 with cigarette smoke and black garlic 50 mg/kgBW; (d) treatment group with cigarette smoke and black garlic 100 mg/kgBW; (e) treatment group 3 with cigarette smoke and black garlic 200 mg/kgBW.

Table 5. Differential test results of VEGF expression

Group	n	Mean±SD	p-value
CN	5	32.95 ± 7.45 ^a	0.002
CP	5	5.87±1.08 ^a	
P1	5	29.69 ± 15.39 ^b	
P2	5	25.18 ± 8.28 ^b	
P3	5	24.04 ± 9.22 ^b	

Different notations (a,b) give significant meaning to the mean ± sd obtained ($p\text{-value} < \alpha = 0.05$), which means that there is a significant difference, and if the notation makes an unequal letter obtained ($p\text{-value} > \alpha = 0.05$) then there is no significant difference in the HSD post hoc test.

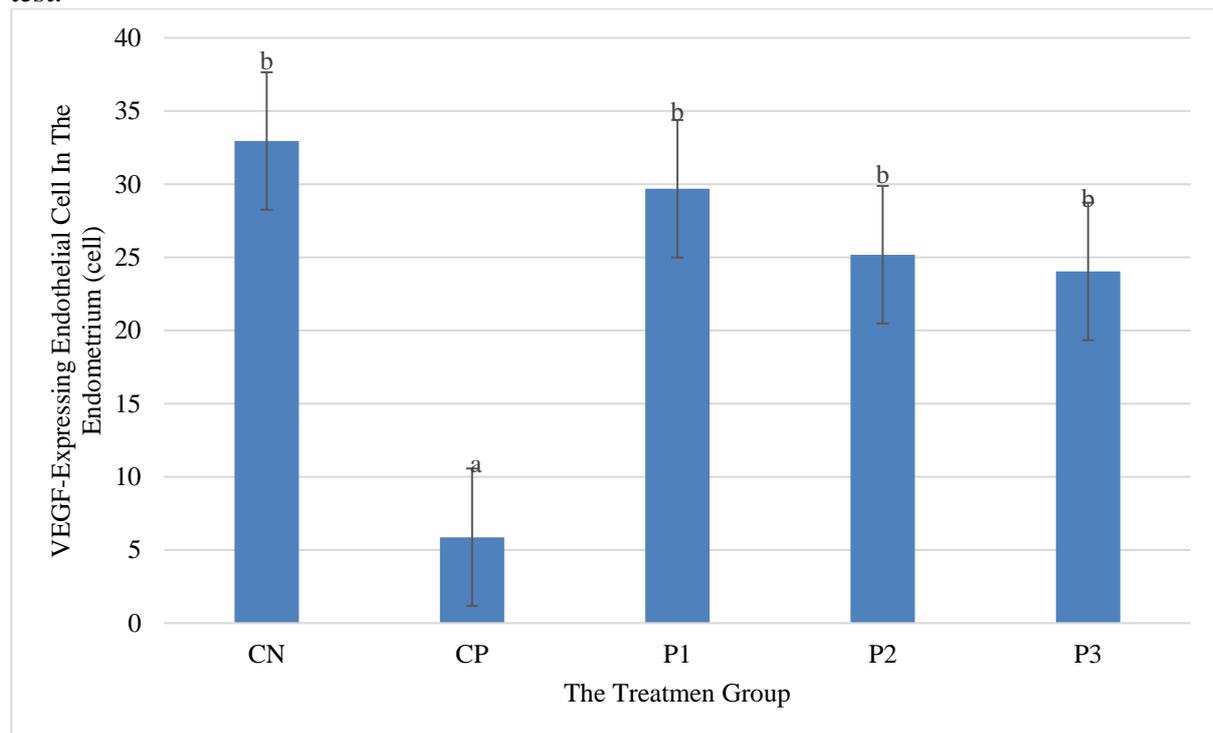


Figure 5. VEGF expressing endothelial cell in the endometrium in mice exposed to cigarette smoke

Figure 5 shows the mean expression of VEGF in the negative control group, positive control group (exposed to cigarette smoke two cigarettes/day), P1 (exposed to cigarette smoke + BG 50 mg/kg/BB), P2 (exposed to cigarette smoke + BG 100 mg/kg/BB), P3 (exposed to cigarette smoke + BG 200 mg/kg/BB). The mean ± sd obtained ($p\text{-value} < \alpha = 0.05$) can mean that there is a significant difference, and if ($p\text{-value} > \alpha = 0.05$), then there is no significant difference.

The Effect of Black Garlic (*Allium sativum*) Extract on Fallopian Tube Secretory Epithelial Cell Count in Rats (*Rattus norvegicus*) Exposed to Cigarette Smoke

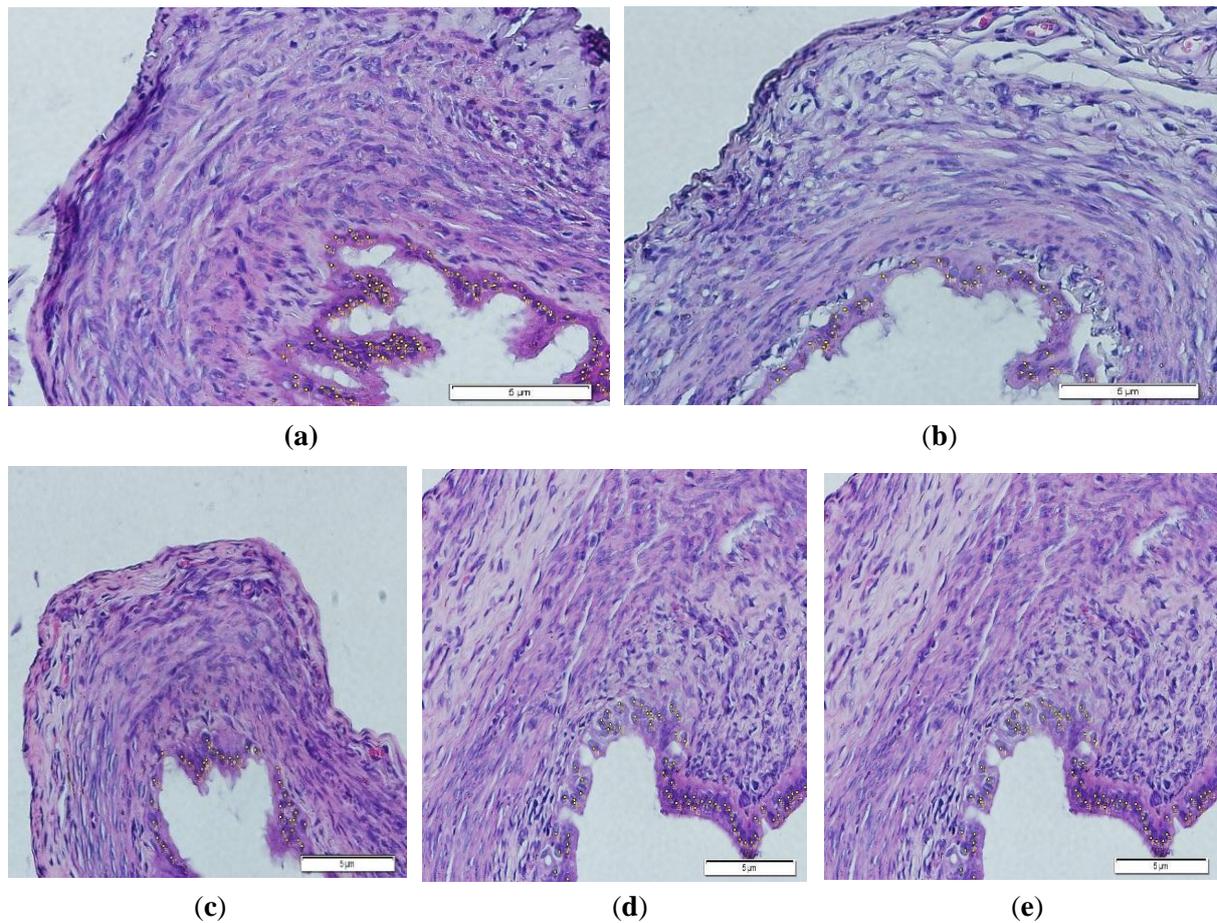


Figure 6. Histopathology of Fallopian tube secretory epithelial cell counts

In Figure 6, (a) negative control group CN; (b) positive control group CP cigarette smoke exposure; (c) treatment group 1 (cigarette smoke exposure and black garlic dose of 50mg/kg/BB); (d) treatment group 2 (cigarette smoke exposure and black garlic dose of 100mg/kg/BB); (e) treatment group 3 (cigarette smoke exposure and dose of 200 mg/kg/BB).

Table 6. Differential test results of VEGF expression

Group	n	Mean±SD	p-value
CN	5	51.240 ± 9.0856 ^b	0,001
CP	5	28.400 ± 3.3166 ^a	
P1	5	43.920 ± 8.1677 ^b	
P2	5	45.960 ± 4.0679 ^b	
P3	5	48.960 ± 10.8898 ^b	

In the mean ± SD if it contains different letters, there is a significant difference ($p < 0.05$) and if it contains the same letter, there is no significant difference ($p > 0.05$).

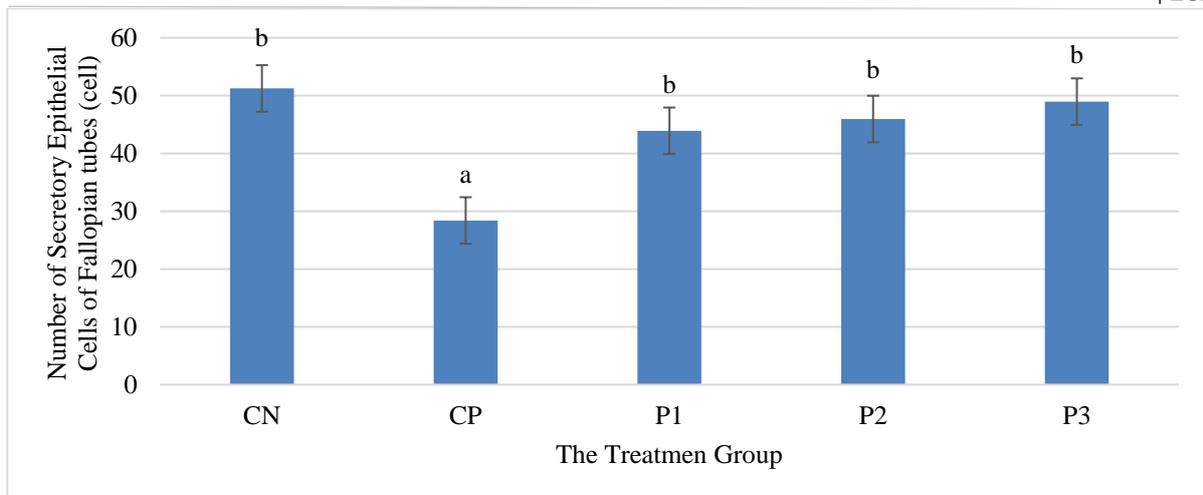


Figure 7. Effect of Black Garlic Extract on Increasing the Number of Secretory Epithelial Cells of Fallopian tubes of White Rats Exposed to Cigarette Smoke

Based on Figure 7, it is known that CN: without exposure to cigarette smoke and administration of black garlic extract; CP: exposed to cigarette smoke and without administration of black garlic extract; P1: exposed to cigarette smoke and given black garlic extract 50 mg/kgBW; P2: exposed to cigarette smoke and given black garlic extract 100 mg/kgBW; P3: exposed to cigarette smoke given black garlic extract 200 mg/kgBW.

Figure 7 above shows that the average number of secretory epithelial cells in the fallopian tubes of the positive control group has an average value of the number of secretory epithelial cells with a smaller value than the negative control group. This indicates that cigarette smoke exposure has an impact on reducing the number of fallopian tube secretory epithelial cells. The treatment group (P1, P2, and P3) had a mean value of the number of secretory epithelial cells with a value greater than the positive control group. This indicates that the administration of black garlic extract can increase the number of fallopian tube secretory epithelial cells.

Here are the calculations for the percentage change in follicular atresia for the doses of 100 mg/kgBW and 200 mg/kgBW compared to the group exposed to cigarette smoke only (CP):

- 1) Dose 50 mg/kgBW (P1):
 - a) Mean follicular atresia in CP: 7.2
 - b) Mean follicular atresia in P1: 2.6
 - c) Percentage change: $\left(\frac{7.2-2.6}{7.2}\right) \times 100 \approx 63.89\%$
- 2) Dose 100 mg/kgBW (P2):
 - a) Mean follicular atresia in CP: 7.2
 - b) Mean follicular atresia in P2: 6.8
 - c) Percentage change: $\left(\frac{7.2-6.8}{7.2}\right) \times 100 \approx 5.56\%$
- 3) Dose 200 mg/kgBW (P3):
 - a) Mean follicular atresia in CP: 7.2
 - b) Mean follicular atresia in P3: 8.2
 - c) Percentage change: $\left(\frac{7.2-8.2}{7.2}\right) \times 100 \approx 13.89\%$

The results indicate that:

- 1) Administration of 50 mg/kgBW reduced follicular atresia by 63.89%.

- 2) Administration of 100 mg/kgBW reduced follicular atresia by 5.56%.
- 3) Administration of 200 mg/kgBW actually increased follicular atresia by 13.89%.

This shows that higher doses of black garlic extract do not provide better effects and may even increase damage to ovarian follicles.

DISCUSSION

The Effect of Black Garlic (*Allium sativum*) Extract on Ovarian Follicular Atresia in Rats (*Rattus norvegicus*) Exposed to Cigarette Smoke

The results indicate that cigarette smoke, as a source of oxidative stress, increases the number of follicles undergoing atresia, triggers granulosa cell apoptosis, and causes oxidative damage. Comparison of the mean follicular atresia between cigarette smoke-exposed and non-exposed groups showed a significant increase in exposed mice, which is in agreement with those who documented that nicotine exposure affects ovarian function by inducing follicular atresia through oxidative stress mechanisms (Li *et al.*, 2020).

Black garlic extract, known for its antioxidant properties, showed protective effects against this oxidative damage, especially at low doses (50 mg/kgBW). The group receiving a low extract dose (50 mg/kgBW) showed the best results compared to the higher doses (100 mg/kgBW and 200 mg/kgBW). The low dose (50 mg/kgBW) gave the best results compared to the higher dose. Administration of this extract decreased the number of follicular atresia, indicating that the black garlic extract ameliorated the free radical damage produced by cigarette smoke. These results support the findings (Chen *et al.*, 2021), which showed that antioxidants in black garlic can mitigate the toxic effects of free radicals on the reproductive system.

However, the study results also showed that increasing the dose of black garlic extract above 50 mg/kgBW did not continue the protective trend and instead may have caused prooxidative effects, as described who stated that high doses of certain compounds in black garlic can have toxic effects (Dutta *et al.*, 2021). Therefore, these findings demonstrate the importance of dosage in the therapeutic application of black garlic extracts, where an optimum point produces protective effects without causing additional damage.

This discussion contributes to a better understanding of the potential of interventions using natural materials in dealing with the negative impact of environmental exposures such as cigarette smoke on the reproductive system. More broadly, this study adds evidence to the corpus of literature regarding the use of antioxidants in preventing oxidative damage to ovarian function and supports the development of safer and more effective therapeutic strategies to maintain reproductive health in the presence of environmental risk factors.

The Effect of Black Garlic (*Allium sativum*) Extract on Endometrial VEGF Expression in Rats (*Rattus norvegicus*) Exposed to Cigarette Smoke

In this study, three doses of black garlic (*Allium sativum*) extract were given to female Wistar rats exposed to cigarette smoke to test its effect on Vascular Endothelial Growth Factor (VEGF) expression in the endometrium. The following is a description of each dose used:

- 1) Dose 50 mg/kgBW (Group P1): This group received 50 mg of black garlic extract per kilogram of rat body weight daily. Based on research, this dose showed the best results in increasing VEGF expression and the number of fallopian tube secretory epithelial cells, as well as reducing ovarian follicular atresia in rats exposed to cigarette smoke.
- 2) Dose 100 mg/kgBW (Group P2): This group received 100 mg of black garlic extract per kilogram of rat body weight daily. This dose was also influential in increasing VEGF expression and the number of fallopian tube secretory epithelial cells, but not as good as the 50 mg/kgBW dose in several parameters measured.

- 3) Dose 200 mg/kgBW (Group P3): This group received 200 mg of black garlic extract per kilogram of rat body weight daily. Although this dose increased VEGF expression and the number of fallopian tube secretory epithelial cells, the results were not as good as the lower dose. They may even lead to increased follicular atresia in some rats.

Exposure of female rats to cigarette smoke of two cigarettes per day was done to trigger a significant decrease in VEGF expression, which was further observed to be affected by the administration of black garlic extract at various doses.

Results showed that administration of black garlic extract significantly increased VEGF expression in mice exposed to cigarette smoke, with the highest increase occurring at the lowest dose (50 mg/kg/BB). This phenomenon can be interpreted through the antioxidant and anti-inflammatory activities of black garlic, which reduce oxidative stress and inflammation induced by cigarette smoke. This reduction in oxidative stress allows for more effective restoration of VEGF expression, which is essential for the maintenance and repair of endometrial tissue through angiogenesis.

Research (Jaleel et al., 2021) also showed a decrease in VEGF expression due to cigarette smoke exposure, strengthening the evidence that cigarette smoke hurts angiogenic factors. However, this study is unique in showing that black garlic extract can reverse this effect at specific doses. Research (You et al., 2019) supports these findings by showing that black garlic reduces molecular activities associated with inflammation and oxidative stress, which may explain its effect on VEGF.

The importance of this study lies in demonstrating the potential of black garlic extract in nutraceutical therapy to protect reproductive tissues from environmental stress, such as cigarette smoke (Wang et al., 2015). In science and technology, these results enrich the literature regarding using natural materials to develop safer and more effective treatment strategies against disorders induced by environmental factors.

Furthermore, this study emphasizes the importance of dosage in phytopharmaceutical use, as higher doses of black garlic extract did not increase VEGF as effectively as lower doses. This suggests the need for further research to explore the specific mechanisms involving black garlic and its interaction with cellular signaling pathways in the context of its effect on VEGF expression (Ahmed & Wang, 2021).

In the context of community development and public health, these findings provide insights for the development of therapies that can reduce the negative impact of cigarette smoke pollution, particularly for populations at high risk of impaired endometrial function. It also offers a natural alternative that may be more acceptable to individuals who avoid using synthetic drugs for health reasons or personal preference.

The Effect of Black Garlic (*Allium sativum*) Extract on Fallopian Tube Secretory Epithelial Cell Count in Rats (*Rattus norvegicus*) Exposed to Cigarette Smoke

Results showed that although there was no significant difference between groups exposed to cigarette smoke and those administered black garlic extract at different doses, there was an increase in the number of secretory epithelial cells. These findings provide insight into the protective potential of black garlic against cigarette smoke-induced damage in the fallopian tubes, which are crucial components in the reproductive system.

In the context of the effects of cigarette smoke, previous studies by (Bala et al., 2021) have shown that cigarette smoke causes impairment of reproductive organ function, including damage to ciliary and secretory epithelial cells (Wang et al., 2015). Cigarette smoke reduces the ability of the fallopian tubes to carry out ovum and sperm transportation functions

effectively, affects hormonal balance, and reduces the quality of the fallopian tube epithelium. These effects are due to oxidative stress induced by nicotine and other harmful components in cigarettes.

Black garlic extract, rich in antioxidants such as S-allyl cysteine (SAC), can potentially reduce these negative impacts. “SAC is known for its powerful antioxidant properties, which help neutralize free radicals and reduce oxidative stress, as described” (Jeong et al., 2016). In the context of this study, the antioxidant activity of SAC likely contributes to the improved condition of the fallopian tube secretory epithelium by reducing the damage caused by cigarette smoke. Antioxidant activity can reduce oxidative stress and improve follicular health, VEGF expression, and epithelial cell function through several essential mechanisms. First, antioxidants such as SAC (S-Allylcysteine) in black garlic extract can capture and neutralize free radicals generated by cigarette smoke. *Free radicals* are highly reactive molecules that can damage DNA, proteins, and lipids in cells, disrupting normal cell function and causing tissue damage. By neutralizing free radicals, antioxidants help protect cells from oxidative damage.

In addition, antioxidants reduce oxidative stress, which occurs when the production of free radicals exceeds the capacity of the body's antioxidant system to neutralize them. The antioxidants in black garlic extract help restore this balance by providing compounds that can interact with free radicals, reducing the amount of free radicals available to cause damage. By reducing oxidative stress, antioxidants also help increase the expression of VEGF (Vascular et al. Factor), a protein essential for angiogenesis, which is the formation of new blood vessels. Oxidative stress can reduce the expression of VEGF, which can disrupt the blood supply to endometrial tissue and affect tissue regeneration and function. Thus, antioxidants help maintain or increase VEGF expression, essential for endometrial tissue health and function.

Furthermore, antioxidants protect epithelial cells in the fallopian tubes, essential for ovum transport and the secretion of factors necessary for fertilization. Oxidative stress can damage these cells, impairing fallopian tube function. The antioxidants in black garlic extract help protect epithelial cells from oxidative damage, ensuring optimal function of the fallopian tubes. In addition, antioxidants reduce follicular atresia, a process in which ovarian follicles degenerate and die before they can mature into ova. Oxidative stress can accelerate this process, reducing the number of follicles available for ovulation. By reducing oxidative stress, antioxidants help protect follicles from damage, increasing the chances of follicles developing into mature ova. Overall, the antioxidant activity of black garlic extract contributes to protecting and repairing cells and tissues affected by cigarette smoke, thus improving overall reproductive health.

Research (Ahmed & Wang, 2021; Diputra et al., 2018) also supports these findings, suggesting that antioxidants in black garlic can effectively decrease the expression of enzymes that produce reactive oxygen species (ROS), repair tissue damage, and support normal cellular function. This is reinforced (Chen et al., 2021; Diputra et al., 2018; Tran et al., 2020), which found that black garlic can inhibit inflammation and histological changes in the context of other diseases.

This study adds evidence to the existing literature that black garlic extract could be a potential nutraceutical intervention to protect reproductive function from the adverse effects of cigarette smoke. This has significant implications for developing reproductive health protection strategies, especially in adverse environmental exposures such as cigarette smoke (Lu et al., 2017; Qiu et al., 2020). Furthermore, this study suggests that adequate doses of black garlic need to be further studied to optimize its benefits without crossing any possible toxicity limits (X. Zhang et al., 2016).

Integrating these findings into science and technology development could give consideration to using black garlic as part of supportive therapy for individuals exposed to

cigarette smoke (Tran et al., 2020), encouraging better health practices and preventive approaches in reproductive health management. Nevertheless, it's crucial to acknowledge the limitations of this study, which include the use of animal models (rats), a relatively small sample size, and a short exposure duration. These factors might restrict the applicability of the results to humans. Therefore, it's imperative to conduct further research in human studies, with larger sample sizes and longer exposure durations, to validate the potential therapeutic effects of black garlic on reproductive health.

4. CONCLUSION

This study demonstrates that black garlic extract (*Allium sativum*) has protective potential against cigarette smoke-induced damage in *Rattus norvegicus*. The extract significantly reduced ovarian follicular atresia increased VEGF expression in the endometrium, and improved the number of secretory epithelial cells in the fallopian tubes. These effects were most pronounced at a low dose (50 mg/kgBW), highlighting the importance of proper dosage. Higher doses (100 mg/kgBW and 200 mg/kgBW) were less effective and may have adverse effects.

Black garlic extract offers a promising therapeutic approach to mitigate the negative impact of cigarette smoke on reproductive health. Further research is needed to explore the mechanisms involved, optimize dosing strategies, and confirm the safety and efficacy of black garlic extract in clinical applications. Integrating black garlic extract into nutraceuticals could provide a preventive and complementary strategy for individuals at risk of exposure to cigarette smoke.

ACKNOWLEDGMENTS

Thanks to the Master of Midwifery at the Faculty of Medicine Brawijaya University for the facilities provided, especially access to literature, research sites, and materials used for experiments.

REFERENCES

- Ahmed, T., & Wang, C. K. (2021). Black garlic and its bioactive compounds on human health diseases: A review. *Molecules*, 26(16). <https://doi.org/10.3390/molecules26165028>
- Ardiana, M. (2021). *Telaah Ilmiah Dan Patologi Paparan Asap Rokok Terhadap Penyakit Jantung*. Airlangga University Press.
- Awaga, H. A., Lympieri, S., Bosdou, J. K., Makedos, A., Mitsoli, A., Bazioti, M. G., Savvaidou, D., Goulis, D. G., Chatzimeletiou, K., Salem, M. N., Ahmed, S. R., Grimbizis, G., Tarlatzis, B. C., & Kolibianakis, E. M. (2019). Addition of procyanidine to semen preserves progressive sperm motility up to three hours of incubation. *Reproductive Biology*, 19(3), 255–260. <https://doi.org/10.1016/j.repbio.2019.07.001>
- Bala, M. M., Peričić, T. P., Zajac, J., Rohwer, A., Klugarova, J., Välimäki, M., Lantta, T., Pingani, L., Klugar, M., Clarke, M., & Young, T. (2021). What are the effects of teaching Evidence-Based Health Care (EBHC) at different levels of health professions education? An updated overview of systematic reviews. *PLoS ONE*, 16(7 July), 1–28. <https://doi.org/10.1371/journal.pone.0254191>
- Budani, M. C., Carletti, E., & Tiboni, G. M. (2021). In Vivo Cigarette Smoke Exposure to Examine the Expression of Genes Involved in the Inflammatory Response in the Mouse Uterus. *Current Protocols*, 1(6), 1–9. <https://doi.org/10.1002/cpz1.172>
- Capasso, A. (2013). Antioxidant action and therapeutic efficacy of *Allium sativum* L.

- Molecules*, 18(1), 690–700. <https://doi.org/10.3390/molecules18010690>
- Chen, C. Y., Tsai, T. Y., & Chen, B. H. (2021). Effects of black garlic extract and nanoemulsion on the deoxy corticosterone acetate-salt induced hypertension and its associated mild cognitive impairment in rats. *Antioxidants*, 10(10). <https://doi.org/10.3390/antiox10101611>
- Chung, L. Y. (2006). The antioxidant properties of garlic compounds: Ayl cysteine, alliin, allicin, and allyl disulfide. *Journal of Medicinal Food*, 9(2), 205–213. <https://doi.org/10.1089/jmf.2006.9.205>
- Diputra, I. M. M., Rai, I. N., & Dharma, I. P. (2018). Isolasi dan Identifikasi Endomikoriza Indigenus pada Perakaran Salak di Kabupaten Karangasem dan Perbanyakannya. *Agrotrop*, 8(1), 56–64.
- Dutta, A., Dahiya, A., Prakash, A., & Agrawala, P. K. (2021). Acute toxicity of diallyl sulfide derived from *Allium sativum* (garlic) in mice and its possible mechanisms. *Phytomedicine Plus*, 1(3), 100084. <https://doi.org/10.1016/j.phyplu.2021.100084>
- GATS. (2021). Gats|Global Adult Tobacco Survey Fact Sheet Indonesia 2021 Gats Objectives. *Fact Sheet Indonesia*, 1–2.
- Hanum, Z., & Saleha, S. (2023). Pengaruh Ekstrak Etanol Bit Merah (*Beta vulgaris* L.) terhadap Jumlah Arteriole Endometrium pada Tikus (*Rattus norvegicus*) yang dipapar Asap Rokok. *Journal of Healthcare Technology and Medicine*, 9(1), 302. <https://doi.org/10.33143/jhtm.v9i1.2810>
- Herlina, H., Lindriati, T., Sulistyani, S., Yunus, M., & Soekarno, S. (2019). Effect of Duration and Temperature of Fermentation on Black Garlic Properties. *Advance Journal of Food Science and Technology*, 17(5), 86–93. <https://doi.org/10.19026/ajfst.17.6033>
- Jaleel, Z., Blasberg, E., Troiano, C., Montanaro, P., Mazzilli, S., Gertje, H. P., Crossland, N. A., Platt, M., & Spiegel, J. (2021). Association of vaping with decreased vascular endothelial growth factor expression and decreased microvessel density in cutaneous wound healing tissue in rats. *Wound Repair and Regeneration*, 29(6), 1024–1034. <https://doi.org/10.1111/wrr.12945>
- Jeong, Y. Y., Ryu, J. H., Shin, J. H., Kang, M. J., Kang, J. R., Han, J., & Kang, D. (2016). Comparison of anti-oxidant and anti-inflammatory effects between fresh and aged black garlic extracts. *Molecules*, 21(4). <https://doi.org/10.3390/molecules21040430>
- Kida, N., Nishigaki, A., Kakita-Kobayashi, M., Tsubokura, H., Hashimoto, Y., Yoshida, A., Hisamatsu, Y., Tsuzuki-Nakao, T., Murata, H., & Okada, H. (2021). Exposure to cigarette smoke affects endometrial maturation including angiogenesis and decidualization. *Reproductive Medicine and Biology*, 20(1), 108–118. <https://doi.org/10.1002/rmb2.12360>
- Lee, H. M., Kim, C. W., Hwang, K. A., Sung, J. H., Lee, J. K., & Choi, K. C. (2017). Cigarette smoke impaired maturation of ovarian follicles and normal growth of uterus inner wall of female wild-type and hypertensive rats. *Reproductive Toxicology*, 73, 232–240. <https://doi.org/10.1016/j.reprotox.2017.06.187>
- Li, F., Ding, J., Cong, Y., Liu, B., Miao, J., Wu, D., & Wang, L. (2020). Trichostatin A alleviated ovarian tissue damage caused by cigarette smoke exposure. *Reproductive Toxicology*, 93(7), 89–98. <https://doi.org/10.1016/j.reprotox.2020.01.006>
- Lu, X., Li, N., Qiao, X., Qiu, Z., & Liu, P. (2017). Composition analysis and antioxidant properties of black garlic extract. *Journal of Food and Drug Analysis*, 25(2), 340–349. <https://doi.org/10.1016/j.jfda.2016.05.011>
- Qiu, Z., Zheng, Z., Zhang, B., Sun-Waterhouse, D., & Qiao, X. (2020). Formation, nutritional value, and enhancement of characteristic components in black garlic: A review for maximizing the goodness to humans. *Comprehensive Reviews in Food Science and Food Safety*, 19(2), 801–834. <https://doi.org/10.1111/1541-4337.12529>

- Rahma, F., Ardiaria, M., & Panunggal, B. (2019). Pengaruh Pemberian Ubi Jalar Ungu (*Ipomoea batatas* L. Poir) Terhadap Kadar Leukosit Total Tikus Wistar Jantan (*Rattus norvegicus*) Yang Dipapar Asap Rokok. *Journal of Nutrition College*, 8(2), 65. <https://doi.org/10.14710/jnc.v8i2.23815>
- Setiyoningrum, F., Priadi, G., Herlina, N., Solikhin, A., & Lisani, N. (2018). Functional properties of *Saccharomyces kluyveri* Y97-fermented solo black garlic. *Asian Journal of Agriculture*, 2(02), 48–51. <https://doi.org/10.13057/asianjagric/g020203>
- Susanti, E., Sudiana, I. K., & Hendarto, H. (2020). Smoke Effects of Disturbances Folliculogenesis (Mda, GnRH, Hsp70, Apoptosis, and Follicles) in Ovarian on Mice Balb/C. *Journal of International Dental and Medical Research*, 13(2), 774–777. <https://www.proquest.com/docview/2428569703?fromopenview=true&pq-origsite=gscholar&sourcetype=Scholarly Journals>
- Totonchi, H., Miladpour, B., Mostafavi-Pour, Z., Khademi, F., Kasraeian, M., & Zal, F. (2016). Quantitative analysis of expression level of estrogen and progesterone receptors and VEGF genes in human endometrial stromal cells after treatment with nicotine. *Toxicology Mechanisms and Methods*, 26(8), 595–600. <https://doi.org/10.1080/15376516.2016.1218578>
- Tran, G.-B., Pham, T.-V., & Trinh, N.-N. (2020). Black Garlic and Its Therapeutic Benefits. *Medicinal Plants - Use in Prevention and Treatment of Diseases*, 1–13. <https://doi.org/10.5772/intechopen.85042>
- Wang, Y., Zhu, Y., Xing, S., Ma, P., & Lin, D. (2015). SIRT5 prevents cigarette smoke extract-induced apoptosis in lung epithelial cells via deacetylation of FOXO3. *Cell Stress and Chaperones*, 20(5), 805–810. <https://doi.org/10.1007/s12192-015-0599-7>
- Widyanti, A. S., Ardiaria, M., & Widyastuti, N. (2020). Pengaruh pemberian ubi jalar ungu (*Ipomoea batatas* L.Poir) terhadap kadar superoksida dismutase (SOD) tikus wistar jantan (*Rattus Norvegicus*) yang dipapar asap rokok. *Jurnal Gizi Indonesia*, 8(1), 45. <https://doi.org/10.14710/jgi.8.1.45-50>
- You, B. R., Yoo, J. M., Baek, S. Y., & Kim, M. R. (2019). Anti-inflammatory effect of aged black garlic on 12-O-tetradecanoylphorbol-13-acetate-induced dermatitis in mice. *Nutrition Research and Practice*, 13(3), 189–195. <https://doi.org/10.4162/nrp.2019.13.3.189>
- Yuan, H., Sun, L., Chen, M., & Wang, J. (2016). The Comparison of the Contents of Sugar, Amadori, and Heyns Compounds in Fresh and Black Garlic. *Journal of Food Science*, 81(7), C1662–C1668. <https://doi.org/10.1111/1750-3841.13365>
- Zhang, X., Li, N., Lu, X., Liu, P., & Qiao, X. (2016). Effects of temperature on the quality of black garlic. *Journal of the Science of Food and Agriculture*, 96(7), 2366–2372. <https://doi.org/10.1002/jsfa.7351>
- Zhang, Z., Lei, M., Liu, R., Gao, Y., Xu, M., & Zhang, M. (2015). Evaluation of Alliin, Saccharide Contents and Antioxidant Activities of Black Garlic during Thermal Processing. *Journal of Food Biochemistry*, 39(1), 39–47. <https://doi.org/10.1111/jfbc.12102>

Jurnal Info Kesehatan

Vol. 22, No. 2, June 2024, pp. 289-299

P-ISSN 0216-504X, E-ISSN 2620-536X

DOI: [10.31965/infokes.Vol22.Iss2.1542](https://doi.org/10.31965/infokes.Vol22.Iss2.1542)Journal homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>**RESEARCH****Open Access****Nurses Experience of Prone Position in The Absence of Positioning Tool, and Suggestion of Prone Position Tool: A Phenomenology Study****Sriyono^{1*}, Hakim Zulkarnain¹, Erna Dwi Wahyuni¹, Wikan Purwihantoro Sudarmaji², Jujuk Proboningsih³, María Pilar Mosteiro-Díaz⁴**¹ Faculty of Nursing, Universitas Airlangga, Surabaya, East Java, Indonesia² Universitas Airlangga Hospital, Surabaya, East Java, Indonesia³ Department of Nursing, Health Polytechnic of Ministry of Health Surabaya, Surabaya, East Java, Indonesia⁴ Faculty of Medicine and Health Sciences, University of Oviedo, Oviedo, Spain^a Email address: sriyono@fkp.unair.ac.id^b Email address: hakim.zulkarnain@fkp.unair.ac.id^c Email address: erna-d-w@fkp.unair.ac.id^d Email address: wikan-p-s@staf.unair.ac.id^e Email address: jujukproboningsih111970@gmail.com^f Email address: pilarmosteiro@gmail.com

Received: 29 May 2024

Revised: 20 June 2024

Accepted: 29 June 2024

Abstract

During the COVID-19 pandemic which is a non-natural disaster, the ARDS cases (Acute Respiratory Disease Syndrome) rocketed. Despite the advantages of prone positioning for ARDS care, ICU staff seldom use it due to the unavailability of positioning tools, making it a challenging task for nurses. This study aims to investigate nurses' experiences with manual prone positioning and propose ICU-friendly prone position tools. This research method used a qualitative phenomenology theory study to 15 ICU nurses who were experienced in prone positioning at least 10 times to intubated patients and had complaints about the intervention. The experience was gathered using structured questions and recorded. The data was analyzed following Colaizzi's thematic method, and all the emerged themes were collected and reported. The research results show that fifteen interviewed nurses noted that prone positioning is physically demanding and requires a coordinated team with a minimum of three staff, 2 handle patient positioning, and 1 leader securing the intubation tube. Before starting, the team assesses the difficulty level. Recommended tools include a slide sheet with handles, a modified Vollman prone positioner, and a crane-like prone harness. The conclusion is nurses find tools highly beneficial for prone positioning but will improvise with available resources like underpads as a ring-shaped pillow if tools are unavailable.

Keywords: Acute Respiratory Distress Syndrome, Critical Care, Prone Position, ICU, Intensive Care Unit, Nursing, Non-Natural Disaster.***Corresponding Author:**

Sriyono

Faculty of Nursing, Universitas Airlangga, Surabaya, East Java, Indonesia

Email: sriyono@fkp.unair.ac.id

©The Author(s) 2024. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

1. INTRODUCTION

Nurses in the ICU can perform prone position on ARDS (Acute Respiratory Disease Syndrome) patients to increase SpO₂ from 85% to 95%, reduce dyspnea, decrease mean hospitalization up to 4.8 days, and significantly reduce mortality (Chui & Craen, 2016; Moghadam et al., 2020). Nurses along with the team can perform prone position on every patient who needs it and does not have any contraindications, regardless of whether the patient is attached to mechanical ventilation or not (Ovayolu et al., 2014), however mechanically ventilated patients are more susceptible to complications during position change (González-Seguel et al., 2021; Wotiye et al., 2022). Scott, et al (2022) said that in addition to the many benefits of prone positioning, complications due to this action can be well mitigated (Scott et al., 2022). However, complications for nurses or staff handling prone positions are sinking from attention due to research too focused on the point of view of patient benefits. Unfortunately, there are barriers for nurses to perform this action related to the nature of the intervention that involves heavy maneuver, loads of manpower, and not all ICU has the specialized tools to perform prone position (Chui & Craen, 2016; González-Seguel et al., 2021; Ovayolu et al., 2014).

Many ICU rooms lack tools for prone positioning, making it a physically demanding task for nurses who must manually position patients, often risking injury (Callihan & Kaylor, 2021; González-Seguel et al., 2021; Wiggermann et al., 2020). Nurses perform the prone position by placing a sheet under the lower part of the patient's body and pulling it to turn the patient from a supine to a prone position (Wiggermann et al., 2020). Nurses need to reposition patients every 2 hours to prevent pressure ulcers and address discomfort, and must quickly return patients to a supine position in case of complications such as bleeding, tubing detachment, or emergencies like prolonged desaturation and hemodynamic instability (Binda et al., 2021; Callihan & Kaylor, 2021). Prone positioning requires a team of 5-8 trained staff, including anesthesiologists, pulmonologists, or respiratory therapists, highlighting the importance of proper training (Bamford et al., 2020; Scott et al., 2022). Despite training, staff shortages and high ICU nurse workloads exacerbate the challenges, especially when untrained or unrelated health workers are involved (Dewi et al., 2019; Scott et al., 2022; Wiggermann et al., 2020).

ICUs often lack specialized prone positioning tools due to cost and limited accessibility. Hospitals face challenges in investing in tools like RotoProne, with a return on investment taking years. Global disparities in access to these resources further compound the issue (Scott et al., 2022; Wiggermann et al., 2020). While solutions exist, such as manual maneuvers or specific machines, they're not universally applicable due to manpower constraints, complications, and financial limitations (Astua et al., 2021). This problem, highlighted over two decades, is underscored by the COVID-19 pandemic (Astua et al., 2021; Berhan, 2020; Chatte et al., 1997; Chui & Craen, 2016; Moghadam et al., 2020; Teklu et al., 2020; Vollman, 1997). According to all data problem, the aim of this study is to explore the nurse experience of manual prone position and suggestion of prone position tool design in ICU.

2. RESEARCH METHOD

This phenomenological study explores nurses' experiences, challenges, and tool recommendations for prone positioning (Chung et al., 2021; Jeffery et al., 2017; Tong et al., 2007; Wirihana et al., 2018). The interviewer consisted of three people, two of them was masters and the other one is PhD. The interviewer and the participant do not have any close relation. The study happened in November – December 2022.

The research was conducted to an ICU nurse of a secondary hospital in Surabaya city Indonesia. This hospital was chosen because during the COVID-19 pandemic, the ICU was full

of patients and the prone positioning was a routine activity. All the participants fulfil the requirements of at least had the experience of performing 10 prone positions to non-intubated and intubated patient.

The participants recruited by contacting the ICU nurse unit manager and requested the population size which was 50 nurses. The target participants were 15 with the consideration of data saturation achieved (Tong et al., 2007). The simple random sampling conducted to the eligible participants and recruited privately to avoid bias related to work hierarchy. All the participant explained the research and filled the informed consent. The interview conducted using virtual meeting software, recorded, and notes were taken. The interview designed for 30 minutes to 1 hour. The transcript was agreed by the participant.

Instrument and data collection. The experience was gathered using structured questions and recorded using a laptop. Each participant asked these questions:

1. What is your experience performing prone positioning?
2. How you do it?
3. Do you have health complaints during the prone positioning?
4. What is your suggestion about a modification tool for the prone positioning?

Additionally, the participants were asked follow-up questions if the answer was new saturation information. The instrument was pilot-tested to nurses of different hospitals and not included in the study.

The data was analyzed using thematic analysis by Colaizzi, all the emerged themes collected from the data and reported (Wirihana et al., 2018). The experience of performing prone position, how the nurse did it, and the health complaints was reported narratively. The data is coded by one coder. The data was triangulated by confirmed the interview finding with available prone position SOP, and expert comment on the finding.

The ethical clearance granted by the Universitas Airlangga Hospital, Indonesia by the record of UA-02-22135

3. RESULTS AND DISCUSSION

The study successfully recruited 15 eligible ICU nurses and consented to join the study, with no participant dropout. The data saturation was achieved on the 14th participants.

The participant's characteristics

Table 1 shows that had a 100% response rate from 15 ICU nurses, with an equal distribution of males and females. The mean age was 29 years, with most holding diplomas or being registered nurses, and one having a master's in nursing. The average ICU experience was 4.3 years, with most having less than 5 years of experience; only one had over 10 years. Participants conducted an average of 82 prone positioning cycles, with half having more than 50 cycles of experience. All had experience with intubated patients and HFNC, 67% with spontaneous breathing, and 20% with NIV. The most common prone cycle duration was 12 hours (47%), and none lasted ≥ 16 hours. Common complaints were waist pain (60%), back pain (47%), shoulder pain (33%), and hand pain (27%), but no knee pain. Most did not report complications (57%), but some reported pressure ulcers (27%), facial edema (27%), accidental extubation (20%), cardiac arrest (7%), and desaturation (7%). Nearly all nurses believed prone positioning is beneficial (93%), though 60% were unsure about its use for non-COVID-19 ARDS patients.

Table 1. The participants characteristics

Demographic Criteria (n = 15)		Frequency	Mean	Percentage (%)
Gender	Male	8		53%
	Female	7		47%
Age	21 - 30	10	29 years	67%
	31 - 40	5		33%
Education	Diploma	7		47%
	Registered Nurse	7		47%
	Master	1		7%
Work in ICU period (years)	< 5 years	9	4,3 years	60%
	5 – 10 years	5		33%
	> 10 years	1		7%
Prone positioning experience (in cycle*)	< 50 cycle	8	82 cycles	53%
	50 – 100 cycle	3		20%
	> 100 cycle	4		27%
Experience to patient breathing with	Spontaneous	10		67%
	NIV (Non Invasive Ventilation)	3		20%
	HFNC (High Flow Nasal Canula)	15		100%
	ETT (Endo Tracheal Tube)	15		100%
Duration of one cycle	1 hours	2		13%
	2 hours	2		13%
	6 hours (Schifino, 2020)	4		27%
	12 hours (Munshi, 2017)	7		47%
	16 hours (Bamford et al, 2022)	0		0%
	> 16 hours (González-Seguel, 2021)	0		0%
Health complaints	Back pain	7		47%
	Knee pain	0		0%
	Shoulder pain	5		33%
	Waist pain	9		60%
	Hand pain	4		27%
Complications	Extubated	3		20%
	Pressure ulcer	4		27%
	Facial Oedema	4		27%
	Arrest	1		7%
	Desaturation	1		7%
	No complications	7		47%
Belief to the benefit of prone position	No	0		0%
	Maybe	1		7%
	Yes	14		93%
Perform to non-COVID ARDS patient	No	1		7%
	Maybe	9		60%
	Yes	4		27%

*Cycle defined as the period of positioned the patient into prone until repositioned into supine (Binda et al., 2021)

Table 2. Emerged themes

Theme	Participants
Nurse experience	
Heavy Maneuver	P1, P5, P6, P7, P9, and P12
Well-coordinated team	P1, P4, P8, P11, P12, P13
Standard procedure of prone position	
Technique for manual prone	P1 – P15
Recommendation Tools	
Special design for prone positioning	P1, P2, P3, P5, P6, P8, P9, P10, P12, and P13

Table 2 shows that at least 3 staff required to handle and flip the patient and an additional 1 staff needed to hold the endotracheal tube. All the nurses suffer complications such as muscle stress after performing prone positions without proper preparation. The patient was susceptible to complication and good preparation is necessary in which participants express the need of briefing prior to prone positioning.

Nurse experience

1) Heavy maneuver

The experience of ICU nurses during manual prone positions requires a lot of energy, especially in patients with more weight. So, it is necessary to pay attention to the ergonomics of the body during the process of prone.

P6: ... We will shift the load, e.g., the patient's load of >90kg and the massive strength used during the actions...

P7: ... especially overweight patients, because it is difficult, usually pain in the back ...

P5: ... we squat causing pain, the bed should level at the waist so that the hands more easily reach the patient's body...

Nurses feel vulnerable to injury during the process of shifting and lifting, so coordination between teams is needed. Because injuries often occur when the process is not coordinated properly.

P9: ... When lifting or moving because it is not right or not ready is usually cause problem in my back, if I flip patient, it's still okay, if I lift the patient, it is a bit difficult.

2) Well-coordinated team

Patient with ETT risk of extubated and desaturated, a team with high precaution is needed. The challenge is to prepare a team with loads of manpower. Multidiscipline team consisted of nurse, doctor, and specialist resident resulted in a safer prone position. The nurses also expressed the needs of advice from anesthesia consultant doctor for special case patients.

P11: The challenge is that nurses can't do it alone, we definitely need a lot of nurses

P8: ETT cannot be alone must be with the team, it is there from the consultant team or specialist residence, anesthesiologist, and the team from the nurse...

P12: if ETT was assisted by PPDS anesthesiologist or consultant anesthesiologist

The nurses categorize the patient based on the clinical status, comorbidities, and overall medical history. Additionally, the team also categorize the needs of manpower according to the patient condition and availability staff.

P4: employed at least 3 people (1 anesthesiologist in the airways, 1 head, 1 side of the patient's body, 1 in the area next to the legs)

P13: ETT 3-4 people, 5 people safer, 1 person to focus area above head, 2 people right and left (shoulders and legs).

P1: 6 and 1 anesthesiologist (who will control airway breathing), nurse 7 on the left and right.

Standard procedure of prone position

1) Technique for manual prone

There is an unwritten standard operational procedure between the nurses to measure the difficulty level of the patient for prone positioning, if it is easy 3 people is needed, normal 3 – 4, and difficulty hard needs 5 – 7 people. The difficulty was measured according to the staff and patient factors.

Staff factors were

1. Experience of the procedure/ work experience
2. Body size, bigger and taller is more prominent
3. Anesthesiologist present

Patient factors were related to the patient's clinical status, comorbidities, and overall medical history, i.e.:

1. Body size
2. Level consciousness
3. Age
4. Head characteristics related to the risk of tube kinked
5. Risk of displaced or accidental extubated
6. Risk of apnea/desaturation
7. Risk of cardiac arrest

Nurses, in coordination with anesthesiologists, assess the difficulty of prone positioning in ICUs. The case manager organizes the team, briefs them on the difficulty and required maneuvers, and assigns staff positions. If a consultant is present, they handle airway tube fixation. For higher-difficulty cases, anesthesiologist consultants are consulted to determine the necessity of prone positioning. The recommended team size is 3-5 personnel for patients of average body weight; however, for overweight patients, 6 or more personnel are recommended. The essential team typically includes 1 intensivist or anesthesiologist and 2 nurses, with additional personnel as needed. This process was highlighted by participants P1, P2, P4, P6, and P8. In detail the step is listed below:

1. Minimum 3 – 5 staffs involved, 1 intensivist or anesthesiologist, and 2 – 4 nurse staff / doctors / health care assistants
2. Staff position:
 - 1) 1 staff is above the head, namely a specialist intensivist or anesthesiologist;
His duties as a leader
And to maintain airway patentability and be ready for reintubation
 - 2) 1 – 2 staff are on the right side of the patient
His job is to pull the patient to slide to the right
Monitor observations and notify the team when vital sign changes are found
 - 3) 1 – 2 is on the right side of the patient
His job is to logroll the patient (positioning the patient supine - lateral - prone)
3. Ensure that all clear, both infusions, other hoses, and ETT are ensured not to shift

- Prone to the left of the patient
4. The nurse on the right slides the patient to the right (right side of the patient) by holding the patient's linen / bed sheet
 5. Once the patient is on the far-right side of the bed, the patient is tilted 90o from the supine towards the left lateral
Position the patient's left leg and hand straight while the patient's right leg flexes
 6. After the position of the left lateral patient, place a pillow in the chest area, under the abdomen (around the perineum)
This opportunity can be used to simultaneously replace the patient's linen and under pad
 7. Position the pillows appropriately and well organized
Can use a donut pillow for the patient's head / use a round shaped under pad
 8. Then turn the patient 90o from the left lateral to prone
Beware that the patient's position is too on the left side of the bed, then the nurse on the right side of the patient must slightly pull the patient to the right so that the patient's position is right in the middle of the bed
 9. Continue by making sure all hoses are in position and functioning properly (CVC, IV Line, Infuse set, Catheter tube, Nasogastric Tube, ETT, etc.)
 10. Turn the patient in a prone position and face tilted to the left / turned to the right depending on the position of ETT fixation. When using a donut pillow, the patient's head is facing down

Recommendation Tools

- 1) Special design for prone positioning
The nurses suggest three tools design for prone position.
 1. A slide sheet that has holder to help hold the patient during the flip.
P2: like the slide pad is more elastic so that it is easier to pull from the left side is not too heavy, like a long linen is used to make the prone easier ...
 2. A modification of Vollman prone positioner with a round base
P12: like block 2 shape letter W, later the tool sleeps, the thigh is lifted, the tool is inserted then the patient is tilted it is easier than we have to position..... lifted 1 side
P3: The first one is needed like a neck pad in a car, to position it on the face when prone so that the tool is not bent or squeezed by the patient, more precisely we put it on the face of the letter c, the connection can be fitted on the ETT, so it will not bend the ETT
P5: a board that can be fixed and can turn the patient over is placed underneath.
P6: idler to support the body, to tilt given like a bolster that can be deflated.
P9: the base is rigid like a slide pad, ... guarantees fixation of the head like a collar brace but the mouth has a hole for ETT
P10: Tools that can fix the neck and head
 3. A harness to hold the patient to the air and flip over
P1: 4 pieces of pipe, stand the end of the bed, above the pipe there is a box pipe then above the box pipe there is a hook that can hook the bed sheet that can slide to the right left, top down, then there is a strong type of bed sheet, the edge has a hole to hook the bed sheet to the pipe
P8: Then also pulleys for up and down,
P13: I once imagined a crane, if for example on a container for big ship,

DISCUSSION

Qualitative findings highlight ICU nurses' experiences with manual prone positioning, emphasizing physical demands and the need for coordinated teamwork. This labor-intensive process, particularly challenging with overweight patients, necessitates a complete team including one anesthesiologist and two nurses to ensure safety. Manual positioning methods align with existing literature, incorporating additional safety measures such as positioning the patient's head in one direction or using a donut-shaped underpad. Recommended tools like the Vollman modification and overhead harness resonate with previous studies, though nurses favor the cost-effective slide pad for its accessibility in hospitals (Vollman, 1997; Wiggermann et al., 2020). This study illuminates crucial aspects of prone positioning in ICUs, impacting both practice and tool selection.

Nurses underscore the importance of physical fitness, especially when handling overweight patients, echoing prior studies on the physical demands of prone positioning (Callihan & Kaylor, 2021; Chui & Craen, 2016). Maintaining ergonomic positions during manual positioning is crucial to prevent discomfort and injury. Effective team coordination, driven by ICU leadership and culture, is essential to mitigate difficulties (Klaiman et al., 2021). Nurses appreciate leaders who communicate the benefits of prone positioning and allocate tasks effectively, stressing the need for collaboration and communication (Elmer et al., 2023). A multidisciplinary team with sufficient manpower is vital, with anesthesiologists or intensivists playing a crucial role in airway management, ultimately leading to better patient outcomes (Cassano et al., 2022; Klaiman et al., 2021; Rezoagli et al., 2021). The study highlights the complex dynamics that influence successful prone positioning in the ICU.

Manual methods for prone positioning are consistent, differing only in patient flipping. The intensive care society recommends strategic pillow placement to prevent displacement and as injury prevention (Bamford et al., 2020). SOPs, crucial for error prevention, detail pillow placement variations in Indonesian settings: head to shoulder, head-only, under diaphragm to iliac crest, and thin side pillows for the abdomen (RS Tasikmalaya, 2011; RSI Sultan Agung, 2020; RSUP Sanglah, 2021). Lower body support involves knee to heel pillows and firm foot support, avoiding upper arm pillow placement to prevent shoulder flexion (RSI Sultan Agung, 2020). Integrating such details into tool design can enhance prone positioning efficacy while minimizing risks.

Nurses in the study use donut-shaped pillows for patient face support, but recent updates advise against it due to increased pressure injury cases (Morata et al., 2023). Alternatives include cushions, inflatable devices, fluidized positioners, and gel pads to distribute body load (Fourie & Beckman, 2020). Guidelines avoid donut shapes, favoring sideways patient face positioning for better airway monitoring, despite the pressure injury risk. Indonesian SOPs vary on face positioning: to the most comfortable side, left side, or no specific regulation (RS Tasikmalaya, 2011; RSI Sultan Agung, 2020; RSUP Sanglah, 2021). With no consensus, nurses adapt available resources, prioritizing patient safety during manual prone positioning.

The strength of this research is the development of recommendation tool to improve the nurse care in the ICU setting. This study is known to be the first in the context of suggestions of nurse of hospital in developing countries. The limitation is present in the single centered study setting. The nurse experience does not represent other hospital. This might affect the difference in the detail of SOP used.

In the absence of prone positioning tools, ICU nurses modify available resources, requiring at least three staff members, ideally five, depending on patient difficulty. Nurses recommend specific tools like a slide sheet with handles, a modified Vollman prone positioner,

and a crane-like harness to ease the process and reduce complications for intubated patients. Research to develop a tool that according on the prone positioning steps expressed by nurses.

4. CONCLUSION

To be concluded, the nurse experience manual prone require lots of energy and needs highly coordinated team. Before the positioning started the nurse assess the patient difficulty level according to staff factor and patient factors, including patient risk of complication. Each prone positioning led by a consultant, anesthesiologist, residence, doctor, or nurse case manager and accompanied by minimum 2 other staffs. The recommended tool design is a sheet to flip the patient modified with a holder, a modification of Vollman prone positioner, and a prone harness. The nurse expressed the present of tool is greatly help the prone position, but if there is no tool at hand then the nurse will modify the available resources such as under pad as a ring-shaped pillow.

ACKNOWLEDGEMENT

The research team thank you to the Universitas Airlangga Hospital management to give the permission for this research and thank you to the ICU nurses to join the study.

REFERENCES

- Astua, A. J., Michaels, E. K., & Michaels, A. J. (2021). Prone during pandemic: development and implementation of a quality-based protocol for proning severe COVID-19 hypoxic lung failure patients in situationally or historically low resource hospitals. *BMC Pulmonary Medicine*, 21(1), 25. <https://doi.org/10.1186/s12890-021-01401-0>
- Bamford, P., Bentley, A., Dean, J., Wilson-Baig, N., Dean, J., & Whitmore, D. (2020). *ICS Guidance for Prone Positioning of the Conscious COVID Patient 2020*. Intensive Care Society.
- Berhan, Y. (2020). Will Africa be Devastated by Covid-19 as Many Predicted? Perspective and Prospective. *Ethiopian Journal of Health Sciences*, 30(3), 459–467. <https://doi.org/10.4314/ejhs.v30i3.17>
- Binda, F., Galazzi, A., Marelli, F., Gambazza, S., Villa, L., Vinci, E., Adamini, I., & Laquintana, D. (2021). Complications of prone positioning in patients with COVID-19: A cross-sectional study. *Intensive and Critical Care Nursing*, 67, 103088. <https://doi.org/10.1016/J.ICCN.2021.103088>
- Callihan, M. L., & Kaylor, S. (2021). Proning Pains: Recognizing the Red Flags of Body Mechanics for Health Care Workers Involved in Prone Positioning Techniques. *Journal of Emergency Nursing*, 47(2), 211–213. <https://doi.org/10.1016/j.jen.2021.01.001>
- Cassano, G., Ingrassia, S., Belotti, M., Marchetti, A., Merletti, L., Iaci, G., Villa, P., & Brambilla, A. M. (2022). Awake self-prone positioning. *Italian Journal of Emergency Medicine*, 10(3). <https://doi.org/10.23736/S2532-1285.21.00117-8>
- Chatte, G., Sab, J. M., Dubois, J. M., Sirodot, M., Gaussorgues, P., & Robert, D. (1997). Prone position in mechanically ventilated patients with severe acute respiratory failure. *American Journal of Respiratory and Critical Care Medicine*, 155(2), 473–478. <https://doi.org/10.1164/AJRCCM.155.2.9032181>
- Chui, J., & Craen, R. A. (2016). An update on the prone position: Continuing Professional Development. *Canadian Journal of Anesthesia*, 63(6), 737–767. <https://doi.org/10.1007/s12630-016-0634-x>
- Chung, L. Y. F., Han, L., Du, Y., & Liu, L. (2021). Reflections on volunteer nurses' work and caring experiences during COVID-19: a phenomenological study. *Journal of Research in Nursing*, 26(5), 457–468. <https://doi.org/10.1177/17449871211007529>

- Dewi, Y. S., Hargono, R., & Rusdi, A. (2019). Factors Correlated to Job Stress among ICU Nurses in Surabaya Indonesia. *Jurnal Ners*, 14(1), 23–27. <https://doi.org/10.20473/JN.V14I1.12125>
- Elmer, N., REIBHAUER, A., Brehm, K., Vockeroth, C., & Liebl, M. E. (2023). Long-term complications of prone position ventilation with relevance for acute and postacute rehabilitation: a systematic review of the literature. *European Journal of Physical and Rehabilitation Medicine*, 59(1), 111–121. <https://doi.org/10.23736/S1973-9087.22.07529-3>
- Fourie, A., & Beckman, D. (2020). PRONect Practical guidance document: Skin Care Considerations for the Patient in Prone Position. *Skin Integrity Research Group*.
- González-Seguel, F., Pinto-Concha, J. J., Aranís, N., & Leppe, J. (2021). Adverse Events of Prone Positioning in Mechanically Ventilated Adults With ARDS. *Respiratory Care*, 66(12), 1898–1911. <https://doi.org/10.4187/RESPCARE.09194>
- Jeffery, A. D., Kennedy, B., Dietrich, M. S., Mion, L. C., & Novak, L. L. (2017). A Qualitative Exploration of Nurses' Information-Gathering Behaviors Prior to Decision Support Tool Design. *Applied Clinical Informatics*, 8(3), 763. <https://doi.org/10.4338/ACI-2017-02-RA-0033>
- Klaiman, T., Silvestri, J. A., Srinivasan, T., Szymanski, S., Tran, T., Oredoko, F., Sjoding, M. W., Fuchs, B. D., Maillie, S., Jablonski, J., Lane-Fall, M. B., & Kerlin, M. P. (2021). Improving prone positioning for severe acute respiratory distress syndrome during the covid-19 pandemic an implementation-mapping approach. *Annals of the American Thoracic Society*, 18(2), 300–307. https://doi.org/10.1513/ANNALSATS.202005-571OC/SUPPL_FILE/DISCLOSURES.PDF
- Moghadam, V. D., Shafiee, H., Ghorbani, M., & Heidarifar, R. (2020). Prone positioning in management of COVID-19 hospitalized patients. *Brazilian Journal of Anesthesiology (English Edition)*, 70(2), 188–190. <https://doi.org/10.1016/j.bjane.2020.05.001>
- Morata, L., Vollman, K., Rechter, J., & Cox, J. (2023). AACN Practice Alert Manual Prone Positioning in Adults: Reducing the Risk of Harm Through Evidence-Based Practices. *Critical Care Nurse*, 43(1), 59–66. <https://doi.org/10.4037/ccn2023174>
- Ovayolu, O., Ovayolu, N., Genc, M., & Col-Araz, N. (2014). Frequency and severity of low back pain in nurses working in intensive care units and influential factors. *Pakistan Journal of Medical Sciences*, 30(1), 70–76. <https://doi.org/10.12669/pjms.301.3455>
- Rezoagli, E., Mariani, I., Rona, R., Foti, G., & Bellani, G. (2021). Difference between prolonged versus standard duration of prone position in COVID-19 patients: a retrospective study. *Minerva Anestesiologica*, 87(12), 1383–1385. <https://doi.org/10.23736/S0375-9393.21.15864-X>
- RS Tasikmalaya. (2011). *SOP Gives Prone Position* (Issue 103/KEP/2011). RS Tasikmalaya.
- RSI Sultan Agung. (2020). *SOP Adjusting the prone position*. 791.1/SPO/RSI-SA/II/2020.
- RSUP Sanglah. (2021). *SOP Prone Position*.
- Scott, J. B., Weiss, T. T., & Li, J. (2022). COVID-19 Lessons Learned: Prone Positioning With and Without Invasive Ventilation. *Respiratory Care*, 67(8), 1011–1021. <https://doi.org/10.4187/RESPCARE.10141>
- Teklu, S., Sultan, M., Azazh, A., Worku, A., Redae, B., Walelegn, M., Tefera, M., Argaw, R., Waganew, W., Yifru, S., Amogne, W., Tssema, N., Bekele, A., Gebregziabher, Y., Araya, H., Birhanu, A., Demoz, G., Tadesse, B., Seman, Y., & Abayneh, A. (2020). Clinical and Socio-demographic Profile of the First 33 COVID-19 Cases Treated at Dedicated Treatment Center in Ethiopia. *Ethiopian Journal of Health Sciences*, 30(5), 645 – 652. <https://doi.org/10.4314/ejhs.v30i5.2>

- Tong, A., Sainsbury, P., & Craig, J. (2007). Consolidated criteria for reporting qualitative research (COREQ): A 32-item checklist for interviews and focus groups. *International Journal for Quality in Health Care*, 19(6), 349–357. <https://doi.org/10.1093/intqhc/mzm042>
- Vollman, K. M. (1997). Prone positioning for the ards patient. *Dimensions of Critical Care Nursing*, 16(4), 184–193. <https://doi.org/10.1097/00003465-199707000-00002>
- Wiggermann, N., Zhou, J., & Kumpar, D. (2020). Proning Patients With COVID-19: A Review of Equipment and Methods. *Human Factors*, 62(7), 1069–1076. <https://doi.org/10.1177/0018720820950532>
- Wirihana, L., Welch, A., Williamson, M., Christensen, M., Bakon, S., & Craft, J. (2018). Using Colaizzi's method of data analysis to explore the experiences of nurse academics teaching on satellite campuses. *Nurse Researcher*, 25(4), 30–34. <https://doi.org/10.7748/NR.2018.E1516>
- Wotiye, A. B., Shimber, E. T., & Ayele, B. A. (2022). Factors Associated with ICU Mortality at Hawassa University Comprehensive Specialized Hospital (HUCSH). *Ethiopian Journal of Health Sciences*, 32(3), 505–512. <https://doi.org/10.4314/ejhs.v32i3.5>

Jurnal Info Kesehatan

Vol. 22, No. 2, June 2024, pp. 300-306

P-ISSN 0216-504X, E-ISSN 2620-536X

DOI: [10.31965/infokes.Vol22.Iss2.1546](https://doi.org/10.31965/infokes.Vol22.Iss2.1546)

Journal homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>



RESEARCH

Open Access

Exploring Key Determinants of Trail Run Athlete's Preparedness to Perform Pre-Hospital First Aid for Ankle Sprain

Hakim Zulkarnain^{1a*}, Galih Indhiantoro^{1b}, Yulis Setiya Dewi^{1c}, Wahyu Sri Astutik^{2d}, Filomena Adelaide de Matos^{3e}

¹ Faculty of Nursing, Universitas Airlangga, Surabaya, East Java, Indonesia

² Department of Nursing, Institute of Health Sciences of Bhakti Wiyata, Kediri, East Java, Indonesia

³ Department of Emergency and Critical Care Nursing, University of Algarve, Faro, Portugal

^a Email address: hakim.zulkarnain@fkp.unair.ac.id

^b Email address: galih.indhiantoro-2019@fkp.unair.ac.id

^c Email address: yulis.sd@fkp.unair.ac.id

^d Email address: wahyu.sri@iik.ac.id

^e Email address: f.matos@ualg.ac.pt

Received: 31 May 2024

Revised: 6 June 2024

Accepted: 29 June 2024

Abstract

Trail runs have a high risk of injury like ankle sprains. Proper ankle sprain first aid requires preparedness to prevent more serious danger and ensure its success in the pre-hospital setting. This study aimed to explore key determinants of trail run athletes' preparedness to perform pre-hospital first aid for ankle sprain using the PRECEDE-PROCEED Model theory. The research method used a descriptive correlational which approached cross-sectionally. The research recruited 120 trail run athletes of two professional clubs who were selected by simple random sampling. Data was collected using questionnaires. The variables measured were knowledge, belief, value, attitude, and confidence. Bivariate analysis used chi-square and spearman-rho tests. Multivariate analysis used a logistic regression test. All of the statistical analyses used $\alpha = 0,05$. The research results show that half of the respondents was 18 – 35 years old. The factors correlated to the trail run preparedness to perform pre-hospital first aid for ankle sprain was as follows. The majority had a medium level of knowledge (54,2%) and a significance level of $p = 0.000$. Similar trends were found between belief, value, and confidence in which there were no low levels of those variables with the same significance value of $p = 0.000$. Lastly, respondent's percentages of attitudes were almost equally distributed between negative (45%) and positive (66%) with a significance level of $p = 0,000$. In multivariate analysis, all the variables had p -values $< 0,05$. The OR was as follows, knowledge 14,713; belief 77,919; value 27,554; attitude 7,213; and confidence 12,408. The conclusion is found that knowledge, belief, value, attitude, and confidence were significantly correlated with the athlete's preparedness. Together all variables were correlated significantly to the athlete's preparedness to perform pre-hospital first aid for ankle sprain. In conclusion, to improve athlete safety during trail runs is by improving the preparedness for any adverse event including trail runs. All of the factors could increase preparedness, but some factors resulted in higher preparedness. The factors that have more effect on preparedness than the others are belief and value.

Keywords: Ankle Sprain, First Aid, Health Promotion, Preparedness, Pre-hospital, Trail Run.

***Corresponding Author:**

Hakim Zulkarnain

Faculty of Nursing, Universitas Airlangga, Surabaya, East Java, Indonesia

Email: hakim.zulkarnain@fkp.unair.ac.id



©The Author(s) 2024. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

1. INTRODUCTION

Trail runners face potential injuries from uneven terrain, climate hazards, and anatomical issues, requiring self or fellow runner first aid. Running experience, race finishing time, and asphalt training contribute to acute running-related injuries (RRIs) (C. Viljoen, Janse van Rensburg, van Mechelen, Verhagen, Silva, et al., 2022; C. T. Viljoen, Janse van Rensburg, et al., 2021). Gradual-onset RRIs (GORRIs), linked to repetitive stress, threaten trail runners, with factors like longer race distance and chronic diseases heightening risks (C. T. Viljoen, Sewry, et al., 2021). Non-professional runners, lacking proper technique knowledge, also face susceptibility (Rachmawaty et al., 2023; C. T. Viljoen, Sewry, et al., 2021). Since ankle sprain is one of the most common sport injuries, especially that involve the excessive use of foot, added by the nature of the trail run terrain, it is concluded that ankle sprain in trail run is common (Sawyer & Sawyer, 2023; C. Viljoen, Janse van Rensburg, van Mechelen, Verhagen, Silva, et al., 2022; C. T. Viljoen, Janse van Rensburg, et al., 2021). Enhancing trail runners' safety involves prioritizing first aid preparedness, addressing training factors, and promoting proper technique. Despite the significance of pre hospital first aid, no research identifies factors improving athletes' preparedness. This study explores behavioral factors influencing trail runner preparedness to perform pre hospital first aid for ankle sprain, using the PRECEDE-PROCEED Model theory.

Statistics of injuries experienced by running athletes amounted to 54.3% with percentage of detail: ankles (49.5%), knees (17.9%), and lower legs (9.5%) (Sanchez-Garcia et al., 2022). The injury survey in Indonesia shows that 6.4% of injuries occur in the wild, while the types of injuries that often occur are abrasions/bruises by 56.1%, lacerations/cuts by 19.7%, sprains by 36.1%, lower limbs by 64.5% and upper limbs by 33.69% (Hardyanto & Nirmalasari, 2020).

Trail run athletes are at high risk of ankle sprain injuries and if the incident happens in the pre hospital setting, he can deliver first aid immediately. The ability of an athlete to perform first aid independently is determined by his preparedness. A systematic review found that a more knowledgeable person will engage in health-related behavior (Kim et al., 2022). Previous research found that knowledge, attitude, and self-efficacy plays as predisposing factor for women performing physical activity (Emdadi et al., 2015). Additionally, a person with diabetes will follow a routine health activity if the person has good knowledge, attitude, and self-efficacy. Unfortunately, there has been no research on the factors that influence the preparedness of trail run athletes to do first aid on ankle sprain. Therefore, there needs to be research on the factors that affect athletes first aid preparedness on ankle sprain. The PRECEDE-PROCEED Model theory by Lawrence Green will be used to explore this phenomenon because knowledge, attitudes, beliefs, values and self-confidence are predisposing factors to behavior change (Kim et al., 2022; Terry, 2021). This study aimed to explore key determinants of trail run athlete's preparedness to perform pre hospital first aid for ankle sprain using the PRECEDE-PROCEED Model theory.

2. RESEARCH METHOD

This quantitative, descriptive-correlational research using a cross-sectional method was carried out on 120 respondents, namely trail run athletes, obtained from a simple random sampling of 166 athletes who were members of the MANTRA club and the BDG Explorer club. Data collection was carried out in April 2023 – May 2023. This study aimed to explore key determinants of trail run athletes' preparedness to perform pre-hospital first aid for ankle sprain using the PRECEDE-PROCEED Model theory.

The sample was calculated using the Slovin formula and adding 10% of the sample size for contingency for respondents who dropped out. Recruited respondents must have the inclusion criteria as active club members for > 1 year, and have done a trail run in the last 3

months. Meanwhile, respondents who were not involved were those who were injured and could no longer do trail runs.

This research investigates 6 variables, namely 5 independent variables and 1 dependent variable. The independent variable consisted of trail runners' knowledge, attitudes, beliefs, values and self-confidence. Furthermore, the dependent variable is the trail runner's preparedness to perform pre-hospital first aid for ankle sprain.

The instrument for this research is a questionnaire. The questionnaire was designed by researchers based on the PRECEDE-PROCEED model and has passed variability and reliability tests. Validity and reliability tests were carried out on 20 trail run athletes who were not members of MANTRA and BDG Explorer. Validity uses Pearson Product Moment (r) with $\alpha = 0.05$ and r table 0.444. The reliability test is based on Crobach's alpha with $\alpha > 0.6$.

The data was collected directly to the respondents using google form. During the survey the researcher accompanied the respondents and took around 5 minutes to complete the form. The data was analyzed through bivariate and multivariate processes. Bivariate analysis used Chi-Square and Spearman Rho tests. Meanwhile, for multivariate analysis using the logistic regression test. All of the statistical analysis using $\alpha = 0,05$. The analysis results were then processed using SPSS (Statistical Package for Social Science) version 26 software.

The study complied with the ethical norm and was granted ethical clearance by the number 2842-KEPK by the Ethical Commission of the Faculty of Nursing Universitas Airlangga on 12 April 2023.

3. RESULTS AND DISCUSSION

Table 2. Demographic Characteristics of Respondents (n=120)

Demographic characteristics	Category	Frequency (f)	Percentage (%)
Trail run clubs	MANTRA	20	16,7
	BDG Explorer	100	83,3
Age	18-25 Years	50	41,7
	26-35 Years	29	24,2
	36-45 Years	24	20
	46-55 Years	15	12,5
	≥ 56 Years	2	1,7
Total		120	100%

Table 2 shows the largest number of respondents came from the BDG Explorer club, 83.3% (100 respondents), while 16.7% (20 respondents) came from the MANTRA club. The majority of respondents, 41.7% (50 athletes) were aged 18 – 25 years. Only 1.7% (2 athletes) were ≥ 56 years old.

Table 3. The significant factor influencing athlete's preparedness to perform pre-hospital first aid

Variable	Category	Preparedness Level % (n)			Total	p-value	r value
		Low	Medium	High			
Knowledge	Low	0% (0)	17,5% (21)	1,7% (2)	19,2% (23)	0,000	
	Medium	0% (0)	24,2% (29)	30% (36)	54,2% (65)		
	High	0% (0)	0,8% (1)	25,8% (31)	26,7% (32)		
Belief	Low	0% (0)	0% (0)	0% (0)	0% (0)	0,000	0,744
	Medium	0% (0)	41,7% (50)	13,3% (16)	55% (66)		
	High	0% (0)	0,8% (1)	44,2% (53)	45% (54)		

		0% (0)	0% (0)	0% (0)	0% (0)		
Value	Medium	0% (0)	41,7% (50)	11,7% (14)	53,3% (64)	0,000	0,770
	High	0% (0)	0,8% (1)	45,8% (55)	46,7% (56)		
Attitude	Negative	0% (0)	38,3% (46)	6,7% (8)	45% (54)	0,000	0,781
	Positive	0% (0)	4,2% (5)	50,8% (61)	55% (66)		
Confidence	Low	0% (0)	0% (0)	0% (0)	0% (0)	0,000	0,723
	Medium	0% (0)	40,8% (49)	13,3% (16)	54,2% (65)		
	High	0% (0)	1,7% (2)	44,2% (53)	45,8% (55)		

Table 3 shows the bivariate analysis employing the Chi-square test yielded a significant value of $p=0.000$ ($p<0.05$), indicating a positive association between knowledge and preparedness. Thus, the knowledge variable qualifies for multivariate analysis using logistic regression. Similarly, Spearman's rho test revealed significant values ($p=0.000$, $p<0.05$) for belief and preparedness, with a strong coefficient of 0.744, meeting the criteria for multivariate analysis. Furthermore, the relationship between scores and preparedness, as indicated by Spearman's rho test ($p=0.000$, $p<0.05$) and a coefficient of 0.770, also qualifies for multivariate analysis. The correlated factors to the trail run preparedness to perform pre hospital first aid for ankle sprain was as follows. Majority had medium level of knowledge (54,2%) and significance level of $p = 0.000$. Similar trends found between belief, value, and confidence in which there were no low levels of those variables with same significance value of $p = 0.000$. Lastly, respondent's percentages of attitude were almost equally distributed between negative (45%) and positive (66%) with a significance level of $p = 0,000$. These findings underscore the robust positive associations between the variables and preparedness for logistic regression analysis.

Table 4. Multivariate analysis (logistic regression)

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1	Knowledge (X1)	2,689	1,175	5,239	1	0,022	14,713
	Belief (X2)	4,356	1,760	6,122	1	0,013	77,919
	Value (X3)	2,865	1,304	4,826	1	0,028	27,554
	Attitude (X4)	1,976	0,917	4,642	1	0,031	7,213
	Confidence (X5)	2,518	1,141	4,871	1	0,027	12,408
	Constant	-27,566	7,170	14,783	1	0,000	0,000

Regression formula $I = -27.566 + 2.689(X1) + 4.356(X2) + 2.865(X3) + 1.976(X4) + 2.518(X5)$

The negative constant value of -27.566 suggests that when all independent variables - Knowledge (X1), Belief (X2), Score (X3), Attitude (X4), and Self-confidence (X5) - are held constant or at zero, the inclination towards first aid preparedness decreases by 27.566 units (table 4). The knowledge variable regarding preparedness to perform pre hospital first aid for ankle sprain shows significance at $p=0.022<0.05$. It denotes that knowledge exerts a significant partial influence on trail runners' preparedness to perform pre hospital first aid for ankle sprain, with a regression coefficient of 2.689. The positive coefficient implies a direct relationship; higher knowledge correlates with increased preparedness. Furthermore, the odds ratio (OR) for knowledge is 14.713, indicating that trail runners with substantial knowledge are 14.713 times more likely to exhibit high preparedness compared to those with lesser knowledge.

Similarly, belief among trail runners regarding preparedness to perform pre hospital first aid for ankle sprain yields significance at $p=0.013<0.05$. Belief significantly influences trail runners' preparedness, with a regression coefficient of 4.356. The positive coefficient suggests a direct relationship; higher belief corresponds to heightened preparedness. The odds ratio (OR) for belief is 77.919, indicating that trail runners with high belief levels are 77.919 times more likely to demonstrate high preparedness compared to those with lower belief levels.

Furthermore, the value related to preparedness for perform pre hospital first aid for ankle sprain among trail runners shows significance at $p=0.028<0.05$. The score variable significantly influences trail runners' preparedness, with a regression coefficient of 2.865. A positive coefficient signifies a direct relationship, indicating that higher scores correlate with increased preparedness. The odds ratio (OR) for the score is 27.554, implying that trail runners with higher scores are 27.554 times more likely to exhibit high preparedness compared to those with lower scores.

Additionally, attitude among trail runners regarding preparedness to perform pre hospital first aid for ankle sprain is significant at $p=0.031<0.05$. Attitude significantly influences trail runners' preparedness, with a regression coefficient of 1.976. The positive coefficient indicates a direct relationship; a more positive attitude corresponds to higher preparedness. The odds ratio (OR) for attitude is 7.213, suggesting that trail runners with a positive attitude are 7.213 times more likely to demonstrate high preparedness compared to those with a negative attitude.

Lastly, self-confidence among trail runners regarding preparedness to perform pre hospital first aid for ankle sprain shows significance at $p=0.027<0.05$. Self-confidence significantly influences trail runners' preparedness, with a regression coefficient of 2.518. The positive coefficient implies a direct relationship; higher self-confidence corresponds to increased preparedness. The odds ratio (OR) for self-confidence is 12.408, indicating that trail runners with high self-confidence are 12.408 times more likely to exhibit high preparedness compared to those with low self-confidence.

2. DISCUSSION

The PRECEDE-PROCEED model is widely used to understand health behavior so a correct health education is possible (Azar et al., 2018; Green & Kreuter, 2005; Monteiro et al., 2014). The study aimed to explore the correlation of knowledge, belief, value, attitude, and self-confidence—among trail runners towards preparedness in perform pre hospital first aid for ankle sprains. The analysis shown that athlete's knowledge, particularly their grasp of complications associated with ankle sprains, as a determinant of preparedness. Those with high preparedness predominantly possess sufficient knowledge (Rostami-Moez et al., 2017). Effective prevention and rehabilitation programs, with the latter noting the high recurrence rate and associated disability. Ankle sprain evaluation, rehabilitation, and prevention could be enhanced by considering the role of athlete knowledge in preparedness (Chen et al., 2019; Pell & Beach, 2022; Tomás & Visco, 2022).

Additionally, the study emphasizes the significant influence of athlete's belief on their preparedness, with high belief levels correlating with increased preparedness. There were no instances of low preparedness coupled with low belief, highlighting a positive correlation between belief and preparedness. Research has shown a positive correlation between an athlete's belief and their preparedness (Emdadi et al., 2015). Despite this, the perceived quality of athlete leaders is positively related to team outcome confidence (Fransen et al., 2014; C. Viljoen et al., 2024; C. Viljoen, Janse van Rensburg, van Mechelen, Verhagen, Korkie, et al., 2022). Additionally, actively do trail running activities at least around 10.5 km / week makes healthier and more prosperous feelings (self-rated wellness and health) (Smiley et al., 2020).

The research found that knowledge, belief, value, attitude, and confidence were significantly correlated with the athlete's preparedness Together all variables were correlated significantly to the athlete's preparedness to perform ankle sprain first aid. To improve athlete's safety during trail run is by improve the preparedness of any adverse event including trail run. All of the factors could increase the preparedness, but some factor resulted higher preparedness. The factors that have more effect to preparedness than the others are belief and value (Fransen et al., 2014; Sawyer & Sawyer, 2023; C. Viljoen et al., 2024; C. Viljoen, Janse van Rensburg,

van Mechelen, Verhagen, Korkie, et al., 2022; C. Viljoen, Janse van Rensburg, van Mechelen, Verhagen, Silva, et al., 2022; C. T. Viljoen, Sewry, et al., 2021).

The limitation of this study only recruited athletes from two trail run clubs, which might cause bias since there are clubs who is not as well prepared as the two recruited clubs.

4. CONCLUSION

In conclusion, the research shown the intricate interplay of knowledge, belief, value, attitude, and self-confidence among trail run athletes, revealing their significant impact on preparedness for ankle sprains first aid. All of the factors could increase the preparedness, but some factor resulted higher preparedness. The factors that have more effect to preparedness than the others are belief and value. Furthermore, high levels of knowledge, belief, perceived value, positive attitude, and self-confidence correlate with heightened preparedness. Athlete should enhance the knowledge, trigger the belief, improve the value, change the attitude, and teach to be confident.

ACKNOWLEDGEMENTS

The research team acknowledge the athletes club, who is willingly join the research and contribute significantly to the findings.

REFERENCES

- Azar, F. E., Solhi, M., Darabi, F., Rohban, A., Abolfathi, M., & Nejhadadgar, N. (2018). Effect of educational intervention based on PRECEDE-PROCEED model combined with self-management theory on self-care behaviors in type 2 diabetic patients. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, 12(6), 1075–1078. <https://doi.org/10.1016/j.dsx.2018.06.028>
- Chen, E. T., McInnis, K. C., Borg-Stein, J., & Finnoff, J. T. (2019). Ankle Sprains: Evaluation, Rehabilitation, and Prevention. *Current Sports Medicine Reports*, 18, 217–223. <https://api.semanticscholar.org/CorpusID:179178041>
- Emdadi, S., Hazavehie, S. M. M., Soltanian, A., Bashirian, S., & Moghadam, R. H. (2015). Predictive factors of regular physical activity among middle-aged women in West of Iran, Hamadan: Application of PRECEDE model. *Journal of Research in Health Sciences*, 15(4), 244–249. <https://www.scopus.com/inward/record.uri?eid=2-s2.0-84951785369&partnerID=40&md5=7e9d08ac2f1d32858fccccd6314eb2cdf>
- Fransen, K., Coffee, P., Vanbeselaere, N., Slater, M., Cuyper, B. De, & Boen, F. (2014). The Impact of Athlete Leaders on Team Members' Team Outcome Confidence: A Test of Mediation by Team Identification and Collective Efficacy. *IEEE Transactions on Signal Processing*, 28, 347–360. <https://api.semanticscholar.org/CorpusID:145065251>
- Green, L., & Kreuter, M. (2005). *Green LW, Kreuter MW. Health Program Planning: An Educational and Ecological Approach. 4th Edition. New York: McGraw-Hill, 2005.*
- Hardyanto, J., & Nirmalasari, N. (2020). Gambaran Tingkat Pengetahuan Tentang Penanganan Pertama Cedera Olahraga Pada Unit Kegiatan Mahasiswa (Ukm) Olahraga Di Universitas Jenderal Achmad Yani Yogyakarta. *Jurnal Kesehatan Mesencephalon*, 6(1). <https://doi.org/10.36053/mesencephalon.v6i1.195>
- Kim, J., Jang, J., Kim, B., & Lee, K. H. (2022). Effect of the PRECEDE-PROCEED model on health programs: a systematic review and meta-analysis. *Systematic Reviews*, 11(1), 1–12. <https://doi.org/10.1186/s13643-022-02092-2>
- Monteiro, S. M. D. R., Jancey, J., & Howat, P. (2014). Physical activity and nutrition intervention for mothers of young children: Process evaluation. *Health*, 06(03), 223–230. <https://doi.org/10.4236/health.2014.63033>

- Pell, E., & Beach, P. (2022). Introducing Trail Running to Young Athletes. *Journal of Physical Education, Recreation and Dance*, 93(9), 57–59. <https://doi.org/10.1080/07303084.2022.2119795>
- Rachmawaty, R., Nurhasana, R., Dirgantari, W., Oktapriana, R., & Nofrida. (2023). Peran Founder Dalam Pengembangan Komunitas Lari. *Media Bina Ilmiah*, 17(17), 2127–2138.
- Rostami-Moez, M., Rezapur-Shahkolai, F., Hazavehei, S. M. M., Karami, M., Karimi-Shahanjarini, A., & Nazem, F. (2017). Effect of educational program, based on PRECEDE and trans-theoretical models, on preventing decline in regular physical activity and improving it among students. *Journal of Research in Health Sciences*, 17(2). <https://www.scopus.com/inward/record.uri?eid=2-s2.0-85018718607&partnerID=40&md5=ae2d914297d6bb631633751f305820f6>
- Sanchez-Garcia, L. F., Penichet-Tomas, A., Pueo, B., & Jimenez-Olmedo, J. M. (2022). Injury Incidence and Pattern in Elite Young Male and Female Trail Runners. *Applied Sciences (Switzerland)*, 12(3). <https://doi.org/10.3390/app12031155>
- Sawyer, T. H., & Sawyer, T. L. (2023). Negligent and Reckless or Part of the Sport: *Megenny v. Dunn* 55 N.E. 3d 367 (Ind. App., 2016). *Journal of Physical Education, Recreation and Dance*, 94(5), 62–63. <https://doi.org/10.1080/07303084.2023.2185001>
- Smiley, A., Ramos, W. D., Elliott, L. M., & Wolter, S. A. (2020). Association between trail use and self-rated wellness and health. *BMC Public Health*, 20(1), 1–10. <https://doi.org/10.1186/s12889-020-8273-0>
- Terry, P. E. (2021). Health Promotion Planning and an Interview With Dr. Lawrence Green. *American Journal of Health Promotion*, 35(6), 760–765. <https://doi.org/10.1177/08901171211022560>
- Tomás, R., & Visco, C. J. (2022). Management of Acute Ankle Sprains in the Athlete. *Current Physical Medicine and Rehabilitation Reports*, 10(1), 27–37. <https://doi.org/10.1007/s40141-021-00336-1>
- Viljoen, C., du Toit, E., van Niekerk, T., Mashaba, S., Ndaba, Z., Verster, M., Bellingan, A., Ramagole, D., Jansen van Rensburg, A., Botha, T., & Janse van Rensburg, D. C. (2024). Training for shorter ultra-trail races results in a higher injury rate, a more diverse injury profile, and more severe injuries: 2022 Mac ultra races. *Physical Therapy in Sport*, 65(July 2023), 7–13. <https://doi.org/10.1016/j.ptsp.2023.10.004>
- Viljoen, C., Janse van Rensburg, D. C., van Mechelen, W., Verhagen, E., Korkie, E., & Botha, T. (2022). Development of a trail running injury screening instrument: A multiple methods approach. *Physical Therapy in Sport*, 56, 60–75. <https://doi.org/10.1016/j.ptsp.2022.06.003>
- Viljoen, C., Janse van Rensburg, D. C., van Mechelen, W., Verhagen, E., Silva, B., Scheer, V., Besomi, M., Gajardo-Burgos, R., Matos, S., Schoeman, M., van Rensburg, A. J., van Dyk, N., Scheepers, S., & Botha, T. (2022). Trail running injury risk factors: a living systematic review. *British Journal of Sports Medicine*, 56(10), 577–587. <https://doi.org/10.1136/bjsports-2021-104858>
- Viljoen, C. T., Janse van Rensburg, D. C., Verhagen, E., van Mechelen, W., Tomás, R., Schoeman, M., Scheepers, S., & Korkie, E. (2021). Epidemiology of Injury and Illness Among Trail Runners: A Systematic Review. *Sports Medicine*, 51(5), 917–943. <https://doi.org/10.1007/s40279-020-01418-1>
- Viljoen, C. T., Sewry, N., Schweltnus, M. P., Janse van Rensburg, D. C., Swanevelder, S., & Jordaan, E. (2021). Independent Risk Factors Predicting Gradual Onset Injury in 2824 Trail Running Race Entrants: SAFER XVIII Study. *Wilderness and Environmental Medicine*, 32(3), 293–301. <https://doi.org/10.1016/j.wem.2021.04.002>

Jurnal Info Kesehatan

Vol. 22, No. 2, June 2024, pp. 307-316

P-ISSN 0216-504X, E-ISSN 2620-536X

DOI: [10.31965/infokes.Vol22.Iss2.1553](https://doi.org/10.31965/infokes.Vol22.Iss2.1553)Journal homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>**RESEARCH****Open Access****The Effect Flavonoids *Phaleria macrocarpa* Fruit Extract on Thickness of Trabeculae, Cortex Ratio Femoral Bone and Aortic Intima-Media in Mice Menopause Model****R.A. Rahmawati Nurul Fadilah^{1a*}, Ani Khoirinda^{1b}, Sutrisno^{2c}, Yahya Irwanto^{2d}, Kenty Wantri Anita^{3e}, R.A. Rose Khasana Dewi^{3f}**¹ Master Program of Midwifery, Department of Midwifery, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia² Department of Obstetric and Gynecology, Brawijaya University, Malang, East Java, Indonesia³ Department of Anatomical Pathology, Brawijaya University, Malang, East Java, Indonesia^a Email address: rahmawatinurulfadilah@gmail.com^b Email address: anikhoirinda@gmail.com^c Email address: snospog@gmail.com^d Email address: yahyairwanto50@gmail.com^e Email address: kentywa72@gmail.com^f Email address: rosenade.dr@gmail.com

Received: 04 June 2024

Revised: 29 June 2024

Accepted: 30 June 2024

Abstract

A deficiency of the hormone estrogen at menopause can lead to an increased rate of the destruction of the bone tissue that leads to bone loss, which can lead to osteoporosis and impaired fat metabolism, which increases the risk of atherosclerosis. Phytoestrogens from flavonoid extract *P. Macrocarpa*, having effects similar to endogenous estrogens themselves, prevent osteoporosis and atherosclerosis in menopausal women. The purpose of this research is to assess the influence of flavonoids from *P. Macrocarpa* fruit extract on trabeculae cortex thickness, ratio of femoral bone, and aortic IMT (A-IMT) in a menopausal mouse model. The study was conducted in a true experimental-posttest-only control group design. Using 32 mice; namely KN (normal mice with no treatment), KP (OVX with no treatment), P1(OVX and given flavonoid 3.75 mg/mice/day), P2 (OVX and given flavonoid 7.5 mg/mice/day), P3(OVX and given flavonoid 11.25 mg/mice/day), P4 (OVX and given flavonoid 15 mg/mice/day), the treatment given within 14 days. Then the thickness of the trabeculae, cortex, and intima-media aorta with Hematoxylin-Eosin (HE) staining. In the trabeculae, cortex thickness ratio obtained KN results meaningfully dissimilar to the KP group and the P3 and P4 groups were meaningfully dissimilar from the KP. The A-BMI in KP is meaningfully dissimilar to P1, P2, P3 and P4. The conclusion of the study is flavonoid fruit extract *P. Macrocarpa* can increase the thickness ratio of trabeculae, and cortex femoral bone of mice menopausal model in groups P3, P4 and can decrease A-IMT starting in groups P1 to P4.

Keywords: Flavonoid, *Phaleria macrocarpa*, Menopause, Osteoporosis, Atherosclerosis.***Corresponding Author:**

R.A. Rahmawati Nurul Fadilah

Master Program of Midwifery, Department of Midwifery, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia

Email: rahmawatinurulfadilah@gmail.com

©The Author(s) 2024. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

1. INTRODUCTION

Although literally, menopause means the cessation of menstruation, in a broader sense, it means the menstrual cycle terminating permanently as a result of ovarian follicular activity declining and estrogen levels falling (Silva et al., 2021; WHO, 2022). The regularity and length of a woman's menstrual cycle vary widely throughout her reproductive life, although the age of natural menopause commonly occurs in women globally aged 45 to 55 (WHO, 2022). 4.3 million women in Indonesia aged 45 to 55 years. In 2017, Indonesia's population reached 261.89 million, consisting of 130.31 million women aged 45 to 55 years, and it is estimated that there are 15.8 million women who are menopausal in 2020. This indicates that there will be more menopausal women in Indonesia by 2020 (BPS, Bappenas dan UNFPA Indonesia, 2008). Menopause is referred to as failure and the beginning of disease (Macpherson & Quinton, 2022).

Osteoporosis is one of the most frequent disorders among postmenopausal women because a lack of the hormone estrogen after menopause can accelerate bone resorption and disrupt the bone-rebuilding process. 40% of Indonesian women are susceptible to osteoporosis (Juwita & Fatma, 2021). Osteoporosis is a condition characterized by reduced bone density and deterioration of the microscopic structure of bone tissue, resulting in increased vulnerability to fractures and an increased risk of fractures (Liu, 2020; NIH, 2022). Osteoporosis is primarily influenced by aging and the reduction in steroid hormone levels (Geng et al., 2019).

Cardio Vascular disease (CVD) caused by atherosclerosis, also known as Atherosclerotic Cardiovascular Disease (ASCVD), is more commonly found in Canadian women with an average age of 69 years (Hopper et al., 2021; Newson, 2018). Estrogen levels can also be associated with impaired fat metabolism that has a risk of lipid peroxidation, leading to atherosclerosis (Newson, 2018). Hypercholesterolemia can be caused by a decrease in estrogen that occurs in menopausal women. Low Density Lipoprotein (LDL) levels increase in this condition. Monocytes enter the tunica intima and become macrophages due to increased LDL in the subendothelium (Javadifar et al., 2021; Pratiwi & Damayanty, 2020). To produce atheromatose plaques, scavengers on macrophages can identify Ox-LDL and engulf it into foam cells (Khatana et al., 2020; Sargowo, 2015).

Hormone replacement therapy, (HRT), is given to individuals experiencing hormonal disorders, one of which is menopausal women receiving estrogen hormone therapy (Nayak et al., 2022). Several studies have shown that HRT lowers fracture risk and may be used to prevent or treat osteoporosis in postmenopausal women (de Villiers, 2023; Women Health Concern, 2021). In addition, it is known that estrogen hormone therapy can also reduce atherosclerosis and fat accumulation in menopausal women (Khoudary et al., 2020). However, long-term side effects such as breast cancer, uterine cancer, impaired liver function, and vaginal bleeding are still a debate about the use of HRT (Yousefzadeh et al., 2020). In addition, menopausal women are afraid to accept prescriptions and undergo HRT therapy (Macpherson & Quinton, 2022).

Therefore, additional preventive alternatives are needed, especially natural compounds that have minimal side effects, namely phytoestrogens (Bacciottini et al., 2007). Flavonoids of *Phaleria macrocarpa* extract, known as *mahkota dewa*, are widely found in nature, especially in Indonesia, and are one of the phytoestrogens (Ahmad et al., 2023). The bioactive compounds of the *Phaleria macrocarpa* plant include flavonoids (Stephenus et al., 2023). Flavonoids found in *Phaleria macrocarpa* fruit function as antimicrobial, antibacterial, antifungal, antiallergic, antioxidant, and vasodilator (Fitriana et al., 2023). The highest relative levels of flavonoids were found in the 70% ethanol extract of the flesh of the *Phaleria macrocarpa* fruit, which reached 45.734 g/mg (Maharani et al., 2021). Phytoestrogens in plants have the ability to bind to estrogen receptors (ER) to mimic or modulate endogenous estrogen activity. This

endogenous estrogen is 17 β -estradiol, mainly by binding to the ER. Aside from impacting estrogen receptors, phytoestrogens can also function as antioxidants (Forslund & Andersson, 2017; Hasanah et al., 2020; Kuhnle et al., 2009).

The novelty of this research is to see the effect of giving *Phaleria macrocarpa* fruit extract where flavonoids isolated specifically can be one of the active substances for phytoestrogens. Other studies on the effects of flavonoids of *Phaleria macrocarpa* fruit extract have given good results such as research on endometriosis, diabetes, and other diseases, but until now there have been no studies related to menopause. Therefore, this research aims to ascertain how flavonoids from *Phaleria macrocarpa* fruit extract affect the ratio of trabecula thickness and femoral bone cortex and can reduce the thickness of the intima-media aorta of mice in menopausal models. Through this research, it is hoped that flavonoids of *Phaleria macrocarpa* fruit extract can be an alternative preventive innovation in reducing the incidence of osteoporosis and atherosclerosis, especially in women with menopause.

2. RESEARCH METHOD

This study uses a true experiment research design conducted on female mice (*Mus musculus*). Randomized Post-Test Only Control Group Design was used as the research design. The research also used in vivo methods to see how flavonoids of *Phaleria macrocarpa* fruit extract impacted menopausal models. A place for ovariectomy treatment in mice, where the treatment is given and the tissue collection process is carried out at the Embryology Laboratory, Faculty of Veterinary Medicine, Airlangga University, Surabaya. Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Brawijaya Malang as a place for making and observing research preparations. In this study, 32 mice (*Mus musculus*) were used as experimental animals; 2 mice were used as samples to test FSH levels to ensure that the mice were in menopause and the allocation of 30 mice was partitioned into six groups, with each group including five mice.

The negative control group (KN) comprises subjects receiving no treatment, while the positive control group (KP) consists of subjects undergoing ovariectomy treatment, where before the division of treatment groups 1 to 4, 2 samples of mice were taken to ensure that mice were in a state of menopause characterized by increased FSH results on the 28th day after ovariectomy. The experimental groups designated as P1, P2, P3, and P4 were groups that carried out ovariectomy treatment and were given flavonoids *P. macrocarpa* fruit extract at doses of 3.75 mg/mice/day, 7.5 mg/mice/day, 11.25 mg/mice/day, and 15 mg/mice/day for 14 days. The dosage in this study is based on previous research by Maharani et al (2021) which investigated the impact of flavonoids derived from *P. macrocarpa* fruit extract on endometriosis mice. The study stated that flavonoids of god's crown fruit extract were obtained through herbal extraction techniques using 96% ethanol solvent because 96% ethanol is semi-polar resulting in higher levels can be caused by flavonoids containing more is nonpolar so that flavonoid levels are obtained (Maharani & Sutrisno, 2021; Pujiastuti & El'Zeba, 2021).

Making flavonoids *Phaleria macrocarpa* fruit extract is a ripe fruit washed, the seeds are removed, and the fruit is dried not in the sun to be free from water protection. Simplisia powder is made by blending dried *Phaleria macrocarpa*. Simplisia powder is soaked in 96% ethanol for 30 minutes, stirred well, and allowed to stand for 5 days until settled. Using a funnel of bunches, strain the liquid. To obtain flavonoid-rich extracts, ethanol extracts are partitioned/liquid-liquid fractionated using polar and nonpolar solvents, namely n-hexane and n-butanol.

After being treated for 14 days. Then surgery was performed and examination of thickness of trabeculae, cortex ratio femoral bone, and aortic intima-media in the menopausal mice model with Hematoxylin-Eosin (HE) staining. In this study, the ratio of the thickness of trabeculae and cortex, namely the thickness of the trabeculae in the metaphysic area and the thickness of the cortex in the diapise area, was observed by making a thickness of 3-4 μ m, using

1 field of view with 10 measures and calculating the average of each preparation. Measurement of intima-media thickness seen from 4 viewing lights (directions at 3, 6, 9, and 12 o'clock) descending thoracic aorta, organs that have been stained and then scanned using Aperio CS2 Leica and calculations using the ImageScopex64 application.

The thickness of trabeculae, cortex ratio, femoral bone, and aortic intima-media in the menopausal mice model were statistically analyzed using IBM SPSS Statistics 27.0 for Windows. The tests employed in this work encompass data normality assessments utilizing the Shapiro-Wilk test, data homogeneity evaluations employing the Levene Test method, One Way ANOVA Test, and Post Hoc Test.

The procedures employed in this inquiry strictly complied with the applicable norms and regulations and obtained clearance from the Health Research Ethics Committee of the Faculty of Medicine, University of Brawijaya Malang, Indonesia, under ethics code numbers: 108/EC/KEPK-S2/05/2024 and 26/EC/KEPK-S2/01/2024.

3. RESULTS AND DISCUSSION

a. Ratio of Trabeculae and Cortex Thickness in Femoral Bone of Mice Menopause Model

The results of staining the thickness of trabeculae and femoral bone cortex of mice menopausal models carried out Hematoxylin-Eosin (HE) staining and observations were made using Aperio ImageScopex64 software with a magnification of 50-100X with the treatment of giving flavonoids *Phaleria macrocarpa* fruit extract with 4 different doses, the following results were obtained:

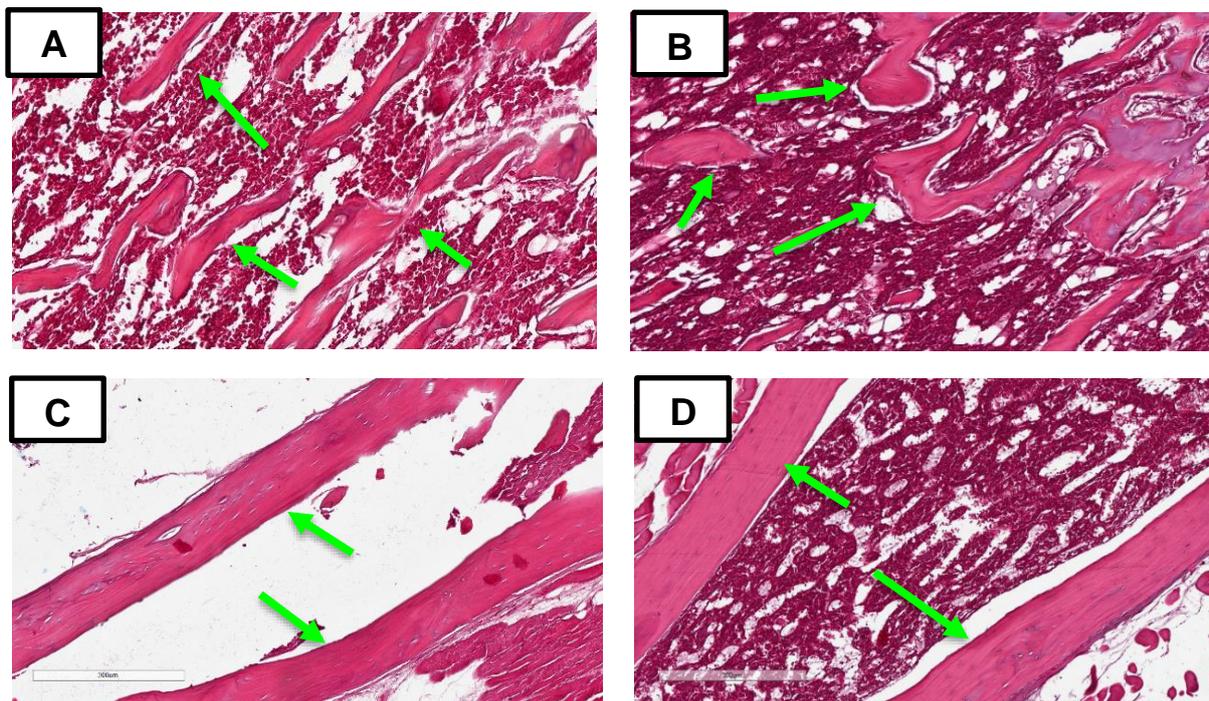


Figure 1. Histopathology of the femoral bone of mice. Histopathological picture of trabeculae bone in the area of the metaphysis (A, B), cortex bone in the area of the diaphysis (C, D). Image with 50-100x magnification with HE coloring

Table 1. One-way ANOVA Test Results of Trabeculae and Cortex Thickness Ratio in Femoral Bone

Treatment Group	Mean \pm SD (Thickness (μm))	p-value
KN	0.426 \pm 0.157	0.020
KP	0.156 \pm 0.010	
P1	0.311 \pm 0.054	
P2	0.327 \pm 0.174	
P3	0.399 \pm 0.087	
P4	0.408 \pm 0.161	

Table 1 illustrates that the positive control group (KP), which is a group that was only given ovariectomy treatment to make mice menopause model, the average ratio of trabeculae and cortex thickness decreased to 0.156 μm compared to the negative control group (KN) or normal mice, which is 0.426 μm , where the significance value $p = 0.022$ ($p < 0.05$), this indicates that there is a significant difference in each treatment group (P1-P4) after administering flavonoids of *Phaleria macrocarpa* fruit extract with different doses for 14 days.

This study has also proven that using 4 doses of flavonoids in *Phaleria macrocarpa* fruit extract can increase the ratio of trabeculae and cortex thickness. There was an increase in the ratio of trabeculae and cortex thickness in the intervention group 1 (P1) treated with flavonoid *Phaleria macrocarpa* fruit extract at a dose of 3.75 mg/mice/day and intervention group 2 (P2) with a dose of 7.5 mg/mice/day with an average result of 0.311 μm and 0.327 μm , the results increased when contrasted with the average of the KP group yield of 0.156 μm . In intervention group 3 (P3) and intervention group (P4), there was a statistically meaningful increase compared to the KP group. The mean values for P3 and P4 were 0.399 μm and 0.408 μm , respectively.

Table 2. Tukey HSD Test Results Against Trabeculae and Cortex Thickness Ratio Data

p-value	KN	KP	P1	P2	P3	P4
KN	-	0.022*	0.680	0.797	0.999	1.000
KP	-	-	0.380	0.278	0.047*	0.037*
P1	-	-	-	1.000	0.863	0.811
P2	-	-	-	-	0.936	0.901
P3	-	-	-	-	-	1.000
P4	-	-	-	-	-	-

*p-value < 0.05 is significant

Table 2, from which one can infer notable distinctions between the KN and KP groups. Consistent with research investigating the impact of administering chitosan from white shrimp shells on the thickness of the trabecular bone of female mice femurs following ovariectomy, the ovariectomized rat group demonstrated an average trabecular thickness value of 59.53 μm while the group of ovariectomy mice had a smaller average trabecular thickness value of 32.44 μm (Rizalah et al., 2016). This indicates that the trabecular thickness of the ovariectomy group will decrease after ovariectomy. These results are echoed by research that states it is projected that women will undergo a decrease of around 50% in trabecular bone and 30% in cortical bone over their lifespan, with around half of this decline happening within the first ten years after menopause (de Villiers, 2023). During menopause, the primary effect of low estrogen levels is heightened cellular activity, leading to both bone resorption and formation. However, there's an imbalance where bone formation doesn't adequately counteract bone loss, resulting in accelerated bone loss and structural alterations such as disarray, thinning, and breakage of bone trabeculae. These changes significantly heighten the risk of fractures, primarily attributed to intense bone remodeling (Gosset et al., 2021).

These results are supported by other studies that state that *Phaleria macrocarpa* fruit meat contains flavonoid compounds, as the highest antioxidant substance, and flavonoids *Phaleria macrocarpa* fruit extract not only has one type of flavonoid but has six kinds of flavonoids, which causes *Phaleria macrocarpa* fruit to have high antioxidant and phytoestrogen potential that is good for the body (Dumanauw et al., 2022; Maharani et al., 2021). Phytoestrogen chemicals exert their effects by attaching to their receptors, which can occur through either ER-dependent or ER-independent routes. However, the ER-dependent route directly triggers its actions by attaching to the Estrogen Receptor α (ER- α) (Mirza et al., 2021). Additionally, a study has found that immunohistochemistry reveals a greater expression of ER β in trabecular bone compared to cortical bone (cortex). ER α was shown to have an inverse pattern, with higher levels observed in cortical bone compared to trabecular bone, as indicated by the same study. Studies have demonstrated a correlation between variations in ER α and ER β genes and bone mass in people (Biaison-Lauber & Lang-Muritano, 2022).

b. Thickness of Intima-Media Aortic in Mice Menopausal Model

The results of the intima-media thickness of the aorta of mice *Mus musculus* are shown in figure 2 using the Hematoxylin-eosin (HE) method.

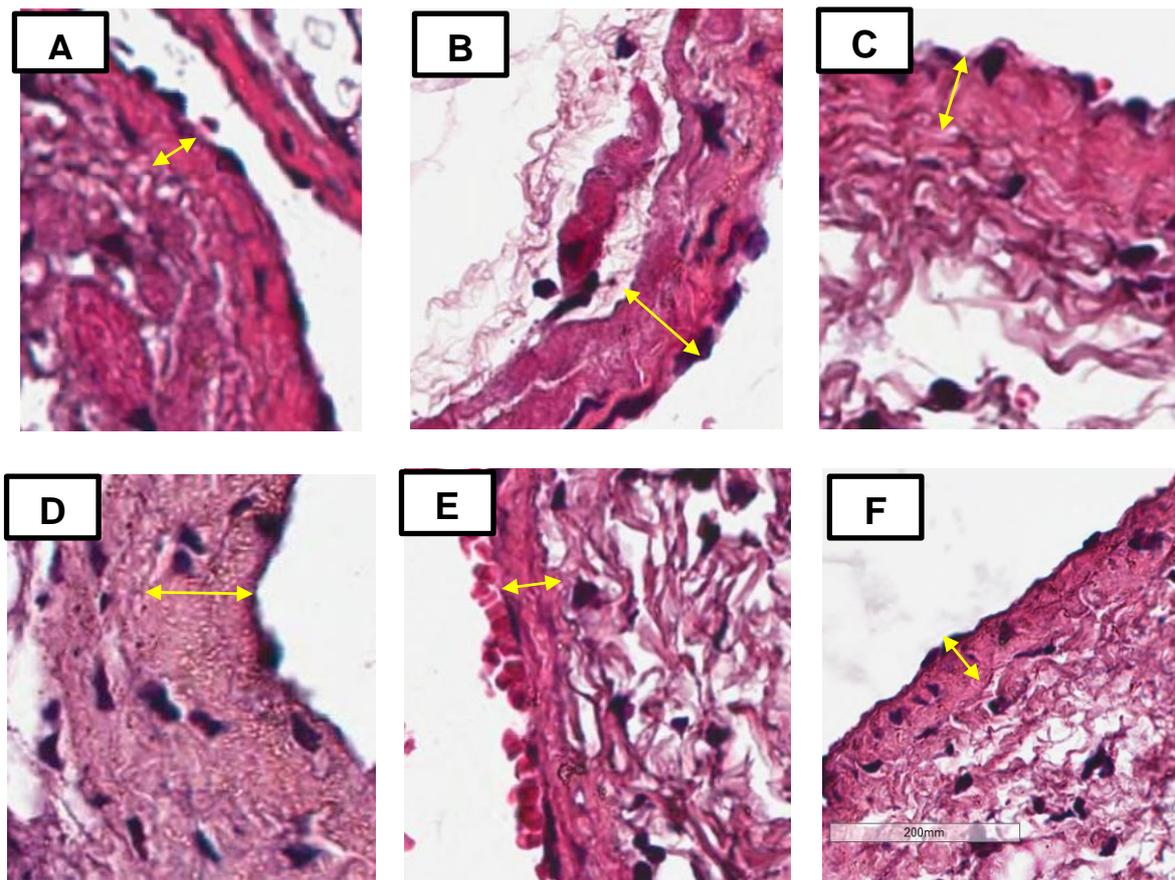


Figure 2. Examination of the thickness of the intima-media aorta of mice with the method of Hematoxylin-eosin

Table 3. One-Way ANOVA Test of Aortic Intima-media Thickness

Treatment Group	Mean (μm)	Standard Deviation (SD)	p-value
KN	12.2500	2.21736	0.000
KP	49.9375	13.42786	
P1	27.4375	8.71391	
P2	24.0625	6.21951	
P3	22.3750	7.25862	
P4	14.1875	1.79554	

Table 3 illustrates that the maximum mean intima-media thickness is in the positive group of 49.9375 ± 13.42786 and the negative group of 12.2500 ± 2.21736 , significant results were obtained between the KP and the KN, namely $\text{sig} = 0.000$ ($\text{sig} < 0.05$). This study can mean that the thickness of the aortic intima-media in the KN and the KP (ovariectomy) a meaningfully dissimilar. The effect of decreasing estrogen can affect the profile of cholesterol in the blood, one of which is the occurrence of memory in LDL (Low-Density Lipoprotein) (Pratiwi & Damayanty, 2020). Increased LDL in the blood can penetrate the endothelium and then accumulate in the nucleus cells (Huff et al., 2021). LDL that enters the nucleus of a cell will bind to free radicals or local reactive oxygen species (ROS), resulting in its transformation into LDL oxidation (Ox-LDL) (Jebari-Benslaiman et al., 2022). Ox-LDL prompts endothelial cells and smooth muscle to produce monocyte chemoattractant protein 1 (MCP-1), leading to heightened recruitment of monocytes to the sub-endothelium. Upon reaching the sub endothelium via MCP-1, circulating monocytes transform into macrophages. These macrophages possess scavenger receptors, which identify and engulf Ox-LDL, transforming into foam cells (Goo, 2019). Foam cells generate Platelet-Derived Growth Factor (PDGF), this process causes the movement of smooth muscle cells from the middle layer of the blood vessel wall to the inner layer, leading to the thickening of the inner layer (Erizon & Karani, 2020).

Table 4. HSD Post Hoc Test Results on Intima-media Thickness Data of Aortic Mice Menopausal Model after One Way ANOVA Test

p-value	KN	KP	P1	P2	P3	P4
KN	-	0.000*	0.105	0.298	0.456	0.999
KP	-	-	0.007*	0.002*	0.001*	0.000*
P1	-	-	-	0.988	0.933	0.197
P2	-	-	-	-	1.000	0.482
P3	-	-	-	-	-	0.666
P4	-	-	-	-	-	-

*p-value < 0.05 is significant

Table 4 shows that the are significant distinctions between the treatment group (KP) and treatment groups (P1-P4). Specifically, there's a noteworthy variance between KP and P1, with a p-value of 0.007, KP and P2 with a p-value of 0.002, KP and P3 with a p-value of 0.001, and KP and P4 with a p-value of 0.000. Hence, it can be concluded that administering doses of flavonoids from *Phaleria macrocarpa* fruit extract ranging from 3.75 mg/mice/day to 15 mg/mice/day has a significant impact.

Phaleria macrocarpa fruit contains flavonoids, where flavonoids have a function as antioxidants. Antioxidants found in *Phaleria macrocarpa* fruit can reduce free radicals and lipid peroxidase so that it can make macrophages carry out their functions as cells that transport fat normally and can keep lipid levels in the blood remain at normal limits (Rochmah, 2008). In addition, flavonoids work as antioxidants by donating or releasing hydrogen ions to free radicals to become more stable. This activity blocks the reaction of OX-LDL (Low-Density Lipoprotein Oxidation), thus way, inhibiting the accumulation of fat on the blood vessel walls. Antioxidants can convert free radicals into low reactivity, so there is no reaction with fat and

there is no accumulation of foam cells (Athiroh & Permatasari, 2012). Flavonoids have antioxidant activity that can increase the synthesis of Nitric oxide (NO) in the endothelium. Synthesized NO will cause vasodilation in vascular smooth muscle and can lower blood pressure. Nitric oxide is known to be the main regulator of smooth muscle. NO is one of the relaxation factors. Decreased bioavailability of NO due to endothelial dysfunction of blood vessels (Sadik, & Saiful Bachri, 2021).

4. CONCLUSION

The flavonoid extract from *Phaleria macrocarpa* fruit shows promise as a beneficial phytoestrogen and a potential alternative preventive measure against osteoporosis and atherosclerosis among menopausal women. Intervention with flavonoid extract from *Phaleria macrocarpa* fruit enhances the ratio of trabecular thickness and femoral bone cortex in a mice menopause model, particularly notable in intervention group 3 (P3) at a dosage of 11.25 mg/mice/day and intervention group 4 (P4) at a dosage of 15 mg/mice/day. Moreover, administering flavonoids from *Phaleria macrocarpa* fruit extract reduces the thickness of the intima-media aorta in the menopausal mice model, starting from the intervention group (P1) to P4, with dosages ranging from 3.75 to 15 mg/mice/day. Future studies could explore additional variables concerning other menopause conditions such as calcium and LDL levels.

REFERENCES

- Ahmad, R., Mazlan, M. K. N., Aziz, A. F. A., Gazzali, A. M., Rawa, M. S. A., & Wahab, H. A. (2023). *Phaleria macrocarpa* (Scheff.) Boerl.: An updated review of pharmacological effects, toxicity studies, and separation techniques. *Saudi Pharmaceutical Journal*, 31(6), 874-888. <https://doi.org/10.1016/j.jsps.2023.04.006>
- Athiroh AS, N., & Permatasari, N. (2012). Mekanisme Kerja Benalu Teh pada Pembuluh Darah: Mechanism of Tea Mistletoe Action on Blood Vessels. *Jurnal Kedokteran Brawijaya*, 27(1), 1–7. <https://doi.org/10.21776/ub.jkb.2012.027.01.1>
- Bacciottini, L., Falchetti, A., Pampaloni, B., Bartolini, E., Carossino, A. M., & Brandi, M. L. (2007). Phytoestrogens: food or drug. *Clin Cases Miner Bone Metab*, 4(2), 123-130.
- Biason-Lauber, A., & Lang-Muritano, M. (2022). Estrogens: Two nuclear receptors, multiple possibilities. *Molecular and Cellular Endocrinology*, 554(November 2020), 111710. <https://doi.org/10.1016/j.mce.2022.111710>
- BPS, Bappenas dan UNFPA Indonesia. (2008). *Proyeksi Penduduk Indonesia 2005-2025*. Jakarta: BPS, Bappenas dan UNFPA Indonesia
- de Villiers, T. J. (2023). Bone health and menopause: Osteoporosis prevention and treatment. *Best Practice and Research: Clinical Endocrinology and Metabolism*, 38(1), 101782. <https://doi.org/10.1016/j.beem.2023.101782>
- Dumanauw, J. M., Minggu, R. E., Rintjap, D. S., Rumagit, B., & Maramis, R. N. (2022). Efek Farmakologi Tanaman Mahkota Dewa (*Phaleria Macrocarpa* (Scheff .) Boerl) (Studi Literatur). *E-Prosding Seminar Nasional Poltekkes Kemenkes Manado*, 1(2), 157–167.
- Erizon, E., & Karani, Y. (2020). HDL dan Aterosklerosis. *Human Care Journal*, 5(4), 11–23. <https://doi.org/10.32883/hcj.v5i4.851>
- Fitriana, S., Andarini, S., Sutrisno, S., Nawangtantrini, G., & Maharani, M. (2023). Hepatotoxicity Oral Administration of Flavonoids Rich Extract from *Phaleria Macrocarpha* in Mice. *Asian Journal of Health Research*, 2(2), 36-41. <https://doi.org/10.55561/ajhr.v2i2.109>
- Forslund, L. C., & Andersson, H. C. (2017). *Phytoestrogens in foods on the Nordic market: A literature review on occurrence and levels*. Nordic Council of Ministers.

- Geng, Q., Gao, H., Yang, R., Guo, K., & Miao, D. (2019). Pyrroloquinoline Quinone Prevents Estrogen Deficiency-Induced Osteoporosis by Inhibiting Oxidative Stress and Osteocyte Senescence. *International journal of biological sciences*, *15*(1), 58–68. <https://doi.org/10.7150/ijbs.25783>
- Goo, Y. H. (2019). Cholesterol metabolism in atherosclerosis development. *The Molecular Nutrition of Fats*, 299–306. <https://doi.org/10.1016/B978-0-12-811297-7.00023-8>
- Gosset, A., Pouillès, J. M., & Trémollières, F. (2021). Menopausal hormone therapy for the management of osteoporosis. *Best Practice & Research Clinical Endocrinology & Metabolism*, *35*(6), 101551. <https://doi.org/10.1016/j.beem.2021.101551>
- Hasanah, M., Bahri, S., & Merta, I. W. (2020). Effect of Red Bean Extract (*Phaseolus vulgaris*, L) on the Development of Female Mice Eggs (*Mus musculus*) Balb/C strains. *Jurnal Penelitian Pendidikan IPA*, *6*(2), 227-231. <https://doi.org/10.29303/jppipa.v6i2.390>
- Hopper, S. E., Cuomo, F., Ferruzzi, J., Burris, N. S., Roccabianca, S., Humphrey, J. D., & Figueroa, C. A. (2021). Comparative Study of Human and Murine Aortic Biomechanics and Hemodynamics in Vascular Aging. *Frontiers in Physiology*, *12*(October), 1–14. <https://doi.org/10.3389/fphys.2021.746796>
- Huff, T., Boyd, B., & Jialal, I. (2021). *Physiology, Cholesterol*. Statpearls Publishing. Retrieved from <https://www.ncbi.nlm.nih.gov/books/NBK470561/>
- Javadifar, A., Rastgoo, S., Banach, M., Jamialahmadi, T., Johnston, T. P., & Sahebkar, A. (2021). Foam cells as therapeutic targets in atherosclerosis with a focus on the regulatory roles of non-coding RNAs. *International Journal of Molecular Sciences*, *22*(5), 1–27. <https://doi.org/10.3390/ijms22052529>
- Jebari-Benslaiman, S., Galicia-García, U., Larrea-Sebal, A., Olaetxea, J. R., Alloza, I., Vandebroek, K., Benito-Vicente, A., & Martín, C. (2022). Pathophysiology of Atherosclerosis. *International Journal of Molecular Sciences*, *23*(6), 1–38. <https://doi.org/10.3390/ijms23063346>
- Juwita, D. A., & Fatma, R. M. (2021). Effect of Propolis on Bone Quality and Cortical Bone Thickness of Ovariectomized Female Wistar White Rats as A Model for Osteoporosis. *Pharmaceutical Sciences and Research*, *8*(3), 121–127. <https://doi.org/10.7454/psr.v8i3.1214>
- Khatana, C., Saini, N. K., Chakrabarti, S., Saini, V., Sharma, A., Saini, R. V., & Saini, A. K. (2020). Mechanistic insights into the oxidized low-density lipoprotein-induced atherosclerosis. *Oxidative medicine and cellular longevity*, *2020*(1), 5245308. <https://doi.org/10.1155/2020/5245308>
- Khoudary, S. R., Venugopal, V., Manson, J. E., Brooks, M., Santoro, N., Black, D. M., Harman, M., Hodis, H. N., Brinton, E. A., Miller, V. M., Taylor, H. S., & Budoff, M. J. (2020). Heart Fat and Carotid Artery Atherosclerosis Progression in Recently Menopausal Women: Impact of Menopausal Hormone Therapy. *The KEEPS Trial*, *27*(3), 255–262. <https://doi.org/10.1097/GME.0000000000001472.Heart>
- Kuhnle, G. G. C., Dell'Aquila, C., Aspinall, S. M., Runswick, S. A., Joosen, A. M. C. P., Mulligan, A. A., & Bingham, S. A. (2009). Phytoestrogen content of fruits and vegetables commonly consumed in the UK based on LC-MS and ¹³C-labelled standards. *Food Chemistry*, *116*(2), 542–554. <https://doi.org/10.1016/j.foodchem.2009.03.002>
- Liu, X. (2020). Crucial molecular mechanisms of phytoestrogen regulation in osteoporosis. *Front Endocrinol (Lausanne)*, *11*(1), 608–634. <https://doi.org/10.3389/fendo.2020.608634>
- Macpherson, B. E., & Quinton, N. D. (2022). Menopause and healthcare professional education: A scoping review. *Maturitas*, *166*(August), 89–95. <https://doi.org/10.1016/j.maturitas.2022.08.009>
- Maharani, M., Lajuna, L., Yuniwati, C., Sabrida, O., & Sutrisno, S. (2021). Phytochemical

- characteristics from *Phaleria macrocarpa* and its inhibitory activity on the peritoneal damage of endometriosis. *Journal of Ayurveda and Integrative Medicine*, 12(2), 229–233. <https://doi.org/10.1016/j.jaim.2020.06.002>
- Maharani, & Sutrisno. (2021). Pengaruh Flavonoid Ekstrak Mahkota Dewa (*Phaleria Macrocarpa*) Terhadap Peningkatan Indeks Apoptosis Pada Peritoneal Mencit Model Endometriosis Abstract Effect of Flavonoid Extract From Mahkota Dewa (*Phaleria Macrocarpa*) on Increasing Cell Apoptotic Index . *Jurnal Kebidanan Malahayati*, 7(4), 652–657. <https://doi.org/10.33024/jkm.v7i4.4606>
- Mirza, D. M., Purbosari, I., Hardjono, S., & Agil, M. (2021). Prediksi Aktivitas Fitoestrogenik Senyawa Golongan Flavonoid terhadap Receptor Estrogen α (ER- α) dengan pendekatan In Silico Prediction. *Jurnal Sains Dan Kesehatan*, 3(4), 512–519.
- Nayak, T., Freaney, P. M., & Maganti, K. (2022). *Atherosclerotic Cardiovascular Disease Risk Assessment and Menopause: Current Evidence*. American College of Cardiology.
- Newson, L. (2018). Menopause and cardiovascular disease. *Post Reproductive Health*, 24(1), 44–49. <https://doi.org/10.1177/2053369117749675>
- NIH. (2022). *Structure of Bone Tissue*. Seer Training Moduls Natioanal Institute of Health. Retrieved from <https://training.seer.cancer.gov/anatomy/skeletal/tissue.html>
- Pratiwi, M., & Damayanty, A. E. (2020). Pengaruh Pemberian Susu Kedelai (Glicine Max L. Merr) terhadap Kadar HDL dan LDL pada Wanita Menopause (Studi pada Ibu-Ibu Pengajian Aisyiyah Ranting Melati Medan). *JURNAL ILMIAH KOHESI*, 4(4), 132-137.
- Pujiastuti, E., & El'Zeba, D. (2021). Perbandingan Kadar Flavonoid Total Ekstrak Etanol 70% dan 96% Kulit Buah Naga Merah *Hylocereus Polyrhizus*) dengan Spektrofotometri. *Cendekia Journal of Pharmacy*, 5(1), 28–43. <https://doi.org/10.31596/cjp.v5i1.131>
- Rizalah, S., Hasan, M., & Wahyudi, S. S. (2016). Pengaruh Pemberian Kitosan Cangkang Udang Putih (*Penaeus merguensis*) terhadap Ketebalan Trabekular Femur Tikus Wistar Betina Pasca Ovariectomi. *E-Jurnal Pustaka Kesehatan*, 4(1), 146–151.
- Rochmah, K. (2008). Potensi Ekstrak Buah Mahkota Dewa (*Phaleria macrocarpa*) Sebagai Antioksidan Dalam Pengaturan Profil Lipid Darah Mencit. *Jurnal Faal Indonesia*, 7(3), 155–242.
- Sadik, F., & Saiful Bachri, M. (2021). Uji Efektivitas Ekstrak Etanol Daun Jarak Pagar (*Jatropha Curcas. L*) Sebagai Antihipertensi Pada Tikus. *Kieraha Medical Journal*, 3(2), 2686–5912. <https://doi.org/10.33387/kmj.v3i2.3949>
- Sargowo, D. (2015). *Disfungsi Endotel*. UB Press.
- Silva, T. R., Oppermann, K., Reis, F. M., & Spritzer, P. M. (2021). Review nutrition in menopausal women: A narrative review. *Nutrients*, 13(7), 1–14. <https://doi.org/10.3390/nu13072149>
- Stephenus, F. N., Benjamin, M. A. Z., Anuar, A., & Awang, M. A. (2023). Effect of Temperatures on Drying Kinetics, Extraction Yield, Phenolics, Flavonoids, and Antioxidant Activity of *Phaleria macrocarpa* (Scheff.) Boerl. (Mahkota Dewa) Fruits. *Foods*, 12(1), 1–19. <https://doi.org/10.3390/foods12152859>
- WHO. (2022). *Menopause*. World Health Organization.
- Women Health Concern. (2021). *Osteoporosis – Bone Health Following the Menopause*. Women Health Concern.
- Yousefzadeh, N., Kashfi, K., Jeddi, S., & Ghasemi, A. (2020). Ovariectomized rat model of osteoporosis: A practical guide. *EXCLI Journal*, 19, 89–107. <https://doi.org/10.17179/excli2019-1990>

Jurnal Info Kesehatan

Vol. 22, No. 2, June 2024, pp. 317-325

P-ISSN 0216-504X, E-ISSN 2620-536X

DOI: [10.31965/infokes.Vol22.Iss2.1554](https://doi.org/10.31965/infokes.Vol22.Iss2.1554)Journal homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>**RESEARCH****Open Access****The Effect of Flavonoids of *Phaleria macrocarpa* Fruit Extract on Aortic Diameter Mice Menopause Model****Ani Khoirinda^{1a*}, R.A. Rahmawati Nurul Fadilah^{1b}, Sutrisno^{2c}, Yahya Irwanto^{2d}, R.A. Rose Khasana Dewi^{3e}**¹ Master Program of Midwifery, Department of Midwifery, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia² Department of Obstetric and Gynecology, Brawijaya University, Malang, East Java, Indonesia³ Department of Anatomical Pathology, Brawijaya University, Malang, East Java, Indonesia^a Email address: anikhoirinda@gmail.com^b Email address: rahmawatinurulfadilah@gmail.com^c Email address: snospog@gmail.com^d Email address: yahyairwanto50@gmail.com^e Email address: rosenade.dr@gmail.com

Received: 4 June 2024

Revised: 29 June 2024

Accepted: 30 June 2024

Abstract

Menopause is a phase experienced by women with an age range of 45-55 years. Menopause is a condition where menstruation stops for a minimum of 12 consecutive months due to the decline in ovarian function, leading to a reduction in estrogen levels. A decrease in estrogen can lead to impaired fat metabolism resulting in atherosclerosis. This study aimed to illustrate the influence of flavonoid extract derived from *Phaleria Macrocarpa* on the enlargement of the aorta's diameter of mice with a menopause condition. The method of this study is a genuine experimental laboratory setting with a research design of a Randomized Post Test Only Control Group setting. Using 32 female mice divided into 6 groups: K- (without ovariectomy and flavonoid extract *Phaleria Macrocarpa*), K+ (ovariectomy without treatment), P1 (ovariectomy + dose 3.75 mg/mice/day), P2 (ovariectomy + dose 7.5 mg/mice/day), P3 (ovariectomy + dose 11.25 mg/mice/day), and P4 (ovariectomy + dose 15 mg/mice/day). Administration of flavonoid extract *Phaleria Macrocarpa* was carried out for 14 days. Data analysis using statistical analysis. The results showed that in a post-hoc test, namely the administration of *Phaleria macrocarpa* flavonoid extract at a dose of 11.25 mg/mice/day and 15 mg/mice/day showed that it could increase the dilation of the aortic diameter of mice model menopause. The study concludes that the flavonoid fruit extract from *Phaleria Macrocarpa* has the ability to increase the width of the aorta in mice with a menopause condition. In future studies, it is recommended to investigate various variables in order to identify the factors that contribute to the narrowing of the aorta. Additionally, it is suggested to perform further research specifically focusing on women going through menopause.

Keywords: Menopause, *Phaleria macrocarpa*, Diameter.***Corresponding Author:**

Ani Khoirinda

Master Program of Midwifery, Department of Midwifery, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia

Email: anikhoirinda@gmail.com

©The Author(s) 2024. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

1. INTRODUCTION

Menopause is a physiological state that occurs in women. Menopause is characterized by the cessation of menstruation for a continuous period of 12 months and is attributed to the irreversible decline in ovarian follicular activity (WHO, 2022; Zhu et al., 2020). According to WHO, the average age at which women experience menopause ranges from 45 to 55 years. While the age in early menopause women is <40 years which can be caused by abnormalities such as chromosomes, or immune or other unknown causes (WHO, 2022). The number of menopause women in Indonesia increased from 15.8 million people in 2017 to 30.3 million people in 2020 (BPS, Bappenas dan UNFPA Indonesia, 2008). This suggests that the number of menopause women in Indonesia is increasing. Menopause is a condition that marks the start of the disease's onset (Macpherson & Quinton, 2022).

Ovarian follicular dysfunction results in a reduction in estrogen or hypoestrogenemia. Hypoestrogens are associated with fat metabolism disorders that have a risk of lipid peroxidation so that it can cause atherosclerosis as a risk of Cardio Vascular disease (CVD) (Newson, 2018). The incidence rate of CVD caused by atherosclerosis or so-called Therosclerotic Cardiovascular Disease (ASCVD) in women with an average age of 69 years in Canada from April 1, 2002, to March 31, 2018, was 432,300 from 1,042,621 (Hopper et al., 2021).

Menopause women may experience hypercholesterolemia due to a reduction in estrogen, resulting in elevated levels of low density lipoprotein (LDL) (Pratiwi & Damayanty, 2020). The progressive elevation of LDL levels and its interaction with reactive oxygen species (ROS) leads to the oxidation of LDL, resulting in the formation of Ox-LDL. Continuous increase in ROS results in oxidation stress or tissue damage such as endothelial dysfunction and decreased nitrite (NO) oxidation (Javadifar et al., 2021; Sargowo, 2015). Oxidized low-density lipoprotein (Ox-LDL) can stimulate the expression of lectin-1 (LOX-1) receptors for oxidized LDL (Li & Mehta, 2009). The binding of oxidized low-density lipoprotein (Ox-LDL) to lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) causes an upregulation in the production of adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), and cytokines, including monocyte-chemoattractant-protein-1 (MCP-1). This, in turn, leads to the attachment of monocytes to endothelial cells (Kattoor et al., 2019). The increase in monocyte recruitment to the tunica intima and differentiation into macrophages is due to an increase in LDL in the subendothelium (Javadifar et al., 2021). Scavengers found in macrophages can recognize Ox-LDL and engulf it into foam cells. So that the pile of foam cells will become atheromatous plaques. An increase in atheromatous plaques causes thickening of the tunica intima media and narrowing of the diameter of the aorta which leads to atherosclerosis (Khatana et al., 2020; Sargowo, 2015).

Hormone Replacement Therapy (HRT) is a treatment given to someone who has hormonal disorders, namely a decrease in estrogen levels, one of which is menopause women (Khoudary et al., 2020). Hormone Replacement Therapy (HRT) has the capacity to decelerate the process of adipose accumulation and atherosclerosis in women experiencing menopause. HRT in postmenopause women remains controversial in the prevention of Therosclerotic Cardiovascular Disease (ASCVD) due to long-term side effects, including increased incidence of stroke, lipid metabolism, pulmonary embolism, breast and uterine cancer, vaginal bleeding, and impaired liver function (Goldštajn et al., 2023; Nayak et al., 2022; Yousefzadeh et al., 2020).

Thus, it is necessary to explore alternate preventive measures, particularly natural substances that have minimal adverse effects and function similarly to estrogen. One such group of molecules is phytoestrogens. Chemically, phytoestrogens are phenolic or polyphenolic phytochemicals. It is the largest and most widely distributed phytochemical category in the

plant world (Bacciottini et al., 2007). One of the phytoestrogens is the flavonoid extract *Phaleria macrocarpa* which is widely found in nature, especially in Indonesia (Ahmad et al., 2023). Flavonoids in *Phaleria macrocarpa* fruit have potential as anti-microbial, anti-bacterial, antifungal, anti-allergic, antioxidant, and vasodilator (Fitriana et al., 2023). In the flesh of *Phaleria macrocarpa*, there are six kinds of flavonoid compounds. 70% ethanol extract of the *Phaleria macrocarpa* fruit has the largest relative flavonoid content of 45.734 µg/mg (Maharani & Sutrisno, 2021). By attaching to oestrogen receptors, phytoestrogens in plants can replicate or alter the effects of endogenous oestrogens. The endogenous estrogen is 17β-estradiol, mainly by binding to the estrogen receptor (ER). Phytoestrogens have an impact on oestrogen receptors, but they can also have antioxidant properties (Forslund & Anderson, 2017; Hasanah et al., 2020; Kuhnle et al., 2009).

Novelty in this study is looking at the effect of giving *Phaleria macrocarpa* fruit extract where flavonoids isolated specifically can be one of the active substances for phytoestrogens. Other studies on the effects of flavonoids of *Phaleria macrocarpa* fruit extract gave good results such as endometriosis and diabetes but until now there have been no studies related to menopause. The objective of this study was to examine the impact of flavonoids derived from the extract of *Phaleria macrocarpa* on the aortic diameter of menopause mice.

2. RESEARCH METHOD

This study employed a real experimental laboratory design with a research design of Randomized Post Test Only Control Group Design. The sample used was mice (*Mus musculus*) with a menopause model. Healthy mice were obtained from the Farma Veterinary Center (Pusvetma) of East Java Province. The number of mice used can be determined by counting the sample size using the formula $(t-1)(n-1) \geq 15$, where t represents the number of groups and n represents the number of mice used. This study utilizes a total of 32 mice, with 30 of them being allocated to 6 different treatment groups. The remaining 2 mice will be used for FSH checking, with one mice assigned to the control group and the other to the treatment group. FSH checking aims to check whether mice are already in menopause or not, if there is an increase in FSH in mice in the treatment group compared to the control group, then mice can be tied already in menopause (Rodríguez-Landa, 2022). The place to conduct research is the Embryology Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga as a place for maintaining mice (*Mus musculus*), making menopause model mice, and giving flavonoid treatment of *P. macrocarpa* extract and the Anatomical Pathology Laboratory of the Faculty of Medicine, Universitas Brawijaya for aortic diameter examination.

Six groups in this study consisted of K- (control group, mice were not ovariectomy and were not given flavonoid extract *P. macrocarpa*), K+ (positive group, mice ovariectomy and not given flavonoid extract *P. macrocarpa*), P1 (ovariectomy and given flavonoid extract *P. macrocarpa* dose 3.75 mg/mice/day), P2 (ovariectomy and given flavonoid extract *P. macrocarpa* dose 7.5 mg/mice/day), P3 (ovariectomy and given flavonoid extract *P. macrocarpa* dose 11.25 mg/mice/day), and P4 (ovariectomy and given flavonoid extract *P. macrocarpa* dose 15 mg/mice/day) (Maharani & Sutrisno, 2021). Mice were ovariectomy in the 8th with a recovery period of 28 days. Ovariectomy is done by removing both ovaries of mice or called bilateral ovariectomy. After the recovery period, 2 mice were surgically removed for FSH examination. The mice are positioned on the underpad and then observed to assess the complete drying of the sutures. Once dry, the underpad is substituted with husks, the mice are treated with antibiotics, and they are provided with daily food and water. After FSH rose, mice were given treatment, namely *P. macrocarpa* flavonoid extract with a dose according to the group for 14 days. After treatment for 14 days, the termination and removal of the aortic and femoral organs was carried out.

P. macrocarpa fruit is obtained from Batu City, East Java and processed in the Batu materia medica laboratory. The part of the fruit used in this study is the peel and the ripe flesh is characterized by a maroon color on the skin of the fruit, from which part of the seed is removed. After the *P. macrocarpa* fruit is mashed and soaked in 96% ethanol for \pm 30 minutes and allowed to stand for 5 days until settled. Next, filter or filter the marinade with a *buncher* funnel to get maserat. Maserat from the *P. macrocarpa* which still contains ethanol solvent, then the solvent is evaporated with an evaporator for 8 hours with a temperature of 60 ° C to obtain a thick extract. It is then fractionated by a liquid-liquid process using n-hexane and n-butanol partitions (Maharani et al., 2021).

After 14 days of administration of *P. macrocarpa* extract, the next stage is termination for organ harvesting and preparation making. Before surgery, mice are injected with ketamine. Then dissected and cut the *descending thoracic aorta*, the organs are placed in different bottles containing 10% formalin for 7-24 hours and make sure all the organs are submerged. After that, the organ is cut in the aorta. Checking to see the diameter of the aorta is done by *Hematoxylin-Eosin* (HE) staining. Each organ will be cut with a thickness of 3-4 μ m. Aortic measurement is done by drawing a line from the intima to the intima by measuring the largest and smallest diameters. The colored organs were scanned using Aperio CS2 Leica and calculated using the ImageScopex64 application.

Analysis statistics the data from the comparison of the treatment group and the control group's aorta diameter in menopause mice model. Normality test using the Shapiro-wilk Test, homogeneity test using *Leven Test* method, One-way ANOVA Test, and *Post hoc* test using tukey HSD method.

Research actions are in accordance with the applicable research code of ethics and have been approved by the Faculty of Medicine, Brawijaya University, Malang, Indonesia, by issuing an ethics number: 26/EC/KEPK-S2/01/2024.

3. RESULTS AND DISCUSSION

a. Diameter of mice in a menopause model

The results of staining the thickness of the diameter of the aorta of mice menopause model with Hematoxylin-Eosin staining and observations were made using the ImageScopex64 application with a magnification of 400X given flavonoid treatment of *P. macrocarpa* fruit extract with 4 different doses, the following results were obtained:

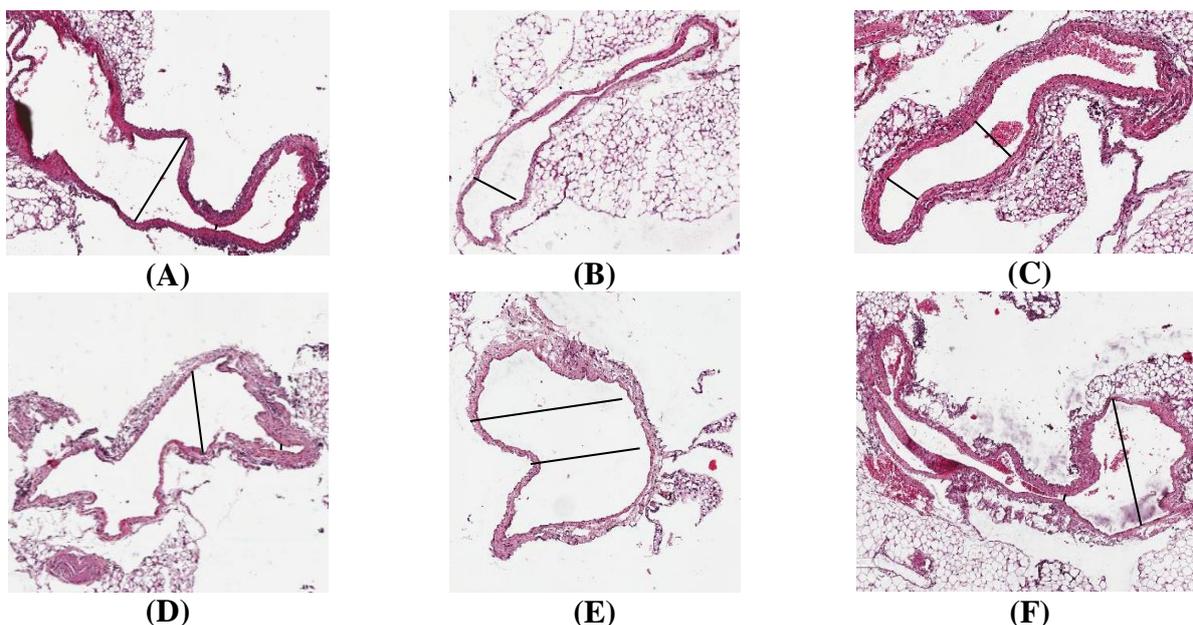


Figure 1. Histopathology of the diameter of the mice aorta. Image with 40x magnification with HE coloring. (A) K- (without ovariectomy and without administration of flavonoid extract *P. macrocarpa*), (B) K+ (ovariectomy and without administration of flavonoid extract *P. macrocarpa*), (C) P1 (ovariectomy and administration of flavonoid extract *P. macrocarpa* dose of 3.75 mg/mice/day), (D) P2 (ovariectomy and administration of flavonoid extract *P. macrocarpa* dose 7.5 mg/mice/day), (E) P3 (ovariectomy and administration of flavonoid extract *P. macrocarpa* dose 11.25 mg/mice/day), (F) P4 (ovariectomy and administration of flavonoid extract *P. macrocarpa* dose 15 mg/mice/day).

Table 1. Normality and Homogeneity Test on Aortic Diameter Mice Menopause Model

Group	N	<i>p-value</i> <i>Shapiro-Wilk test</i>	Data distribution	<i>p-value</i> <i>Levene test</i>	Data Homogeneity
KN	4	0.403	Normal	0.226	Homogen
KP	4	0.422			
P1	4	0.158			
P2	4	0.764			
P3	4	0.198			
P4	4	0.444			
Total	24	0.365			

The Shapiro-Wilk test was conducted to assess the normality of the data from the six groups. The obtained result, with a significance level of 0.365 ($p > 0.05$), indicates that the data can be considered normally distributed. After the data was distributed normally, the homogeneity test continued with the *Levene test* method and obtained the result sig = 0.226 ($p > 0.05$) which showed that the data variant was homogeneous. Both tests are a requirement to perform the *One-way ANOVA test*.

The average results of aortic diameter from untreated (K-) and treated mice samples in the positive control group and treatment group with 4 different doses of *P. macrocarpa* flavonoid extract are shown in the table as follows:

Table 2. One-Way ANOVA Test of Aortic Diameter in Mice Menopause Model

Treatment Group	Average \pm SD (Thickness (μ m))	<i>p-value</i> One-way ANOVA
K-	298.500 \pm 132.8100	0.000
K+	47.625 \pm 13.1617	
P1	119.875 \pm 33.5941	
P2	182.375 \pm 70.1134	
P3	218.000 \pm 33.5286	
P4	282.875 \pm 54.2776	

In table 1 of the results of this study with the Hematoxylin and eosin (HE) test, the average aortic diameter in the negative control group (K-) was 298,500 \pm 132.8100 or in mice without higher treatment when compared to the average aortic diameter in the positive control group (K+) of 47.625 \pm 13.1617 or in mice that were varietomized without being given *P. macrocarpa* extract. This study is in line with other studies that explain that there was a decrease in the positive control group (K+) of 101.93 \pm 12.27 compared to the negative control group (K-) of 152.42 \pm 5.66 (Yuliawati & Astuti, 2021). In addition, another study explained that there was a difference in aortic diameter in the ovariectomy group with the negative control group (K-) with values of 1.31 \pm 0.05 and 1.28 \pm 0.07 respectively (Halim et al., 2021).

This is because mice that are divariectomy experience differences in estrogen and FSH levels in menopause mice, namely estrogen in a decreased state and FSH in an increased state. Both of these things are very influential on vascularity, namely narrowing the diameter of the

aorta. The decrease in estrogen has a significant impact on the lipid profile, namely on the levels of HDL and LDL. When there is a reduction in HDL and an elevation in LDL (Moiety et al., 2015). Increased LDL in the blood can cause a buildup of LDL in the intima cells by penetrating the endothelium. LDL can affect endothelial permeability. LDL contained in the intima and binding to local ROS (reactive oxygen species) makes Ox-LDL. The overproduction of Ox-LDL stimulates the activation of LOX-1 on VSMC (Wu et al., 2017). Ox-LDL interacts with LOX-1, leading to the activation of CAM such as VCAM-1 and MCP-1. VCAM-1 and MCP-1 cause monocytes to be attached to endothelial cells and monocytes differentiate into macrophages (Kattoor et al., 2019).

Macrophages that enter the intima will eat Ox-LDL with the help of surface receptors called scavengers to recognize Ox-LDL. Ox-LDL eaten by macrophages will turn into foam cells. The buildup of foam cells will become atheroma plaques or called fatty streaks. Atheroma plaque is one of the causes of the thickening of the tunica intima, causing the narrowing of the diameter of the aorta (Khatana et al., 2020; Sargowo, 2015).

The test yielded a p-value of 0.000 ($p < 0.05$), showing that there are statistically significant variations in aortic diameter among the six treatment groups. The administration of flavonoid extract *P. macrocarpa* affects 4 treatment groups, namely P1, P2, P3, and P4. However, these results could not determine which group differed significantly among the 6 observation groups. Consequently, the analysis proceeded with the implementation of the Post Hoc Test, specifically utilizing the Tukey HSD test.

Table 3. Post hoc HSD Test of Aorta Diameter in Mice Menopause Model

p-value	K-	K+	P1	P2	P3	P4
KN	-	0.001*	0.017*	0.205	0.567	0.999
K+	-	-	0.670	0.104	0.024*	0.001*
P1	-	-	-	0.784	0.362	0.033*
P2	-	-	-	-	0.974	0.338
P3	-	-	-	-	-	0.757
P4	-	-	-	-	-	-

*p-value < 0.05 is significant

The HSD post hoc test indicated a statistically significant distinction (p -value = 0.024) between the K+ treatment group and the P3 treatment group. When comparing K+ with P4, there is a notable distinction with a p-value of 0.001. It may be inferred that administering quantities of *P. macrocarpa* fruit extract containing 11.25 mg/mice/day in treatment group 3 (P3) and a dose of 15 mg/mice/day in group (P4) of flavonoids has a notable impact. Furthermore, along with prior research, it has been demonstrated that administering *Vigna unguiculata*, a substance rich in antioxidants, to mice who have undergone ovariectomy can substantially augment the size of their aortas (Yulinda et al., 2014). Flavonoids consumed from *P. macrocarpa* fruit can help endogenous antioxidants by preventing cell damage caused by excess free radicals in the aorta. Radical clearance by flavonoids that inhibit LDL oxidation for the formation of atherosclerosis (Simanjuntak, 2012).

4. CONCLUSION

Flavonoids *P. macrocarpa* fruit extract, which includes phytoestrogens, is a botanical remedy that may serve as a substitute for reducing the likelihood of atherosclerosis in women experiencing menopause. The administration of a dose of 11.25 mg/mice/day in the treatment group 3 (P3) and a dose of 15 mg/mice/day in group (P4) can lead to an increase in the diameter of the aorta. Future research should focus on examining the amounts of flavonoid antioxidants in *P. macrocarpa* extract, as these antioxidants play a crucial role in influencing the diameter

of the aorta. Additionally, it is suggested to perform further research specifically focusing on women going through menopause.

REFERENCES

- Ahmad, R., Mazlan, M. K. N., Aziz, A. F. A., Gazzali, A. M., Rawa, M. S. A., & Wahab, H. A. (2023). *Phaleria macrocarpa* (Scheff.) Boerl.: An updated review of pharmacological effects, toxicity studies, and separation techniques. *Saudi Pharmaceutical Journal*, 31(6), 874–888. <https://doi.org/10.1016/j.jsps.2023.04.006>
- Bacciottini, L., Falchetti, A., Pampaloni, B., Bartolini, E., Carossino, A. M., & Brandi, M. L. (2007). Phytoestrogens: Food or drug? *Clinical Cases in Mineral and Bone Metabolism*, 4(2), 123–130. <https://doi.org/10.1201/b14631-25>
- BPS, Bappenas dan UNFPA Indonesia. (2008). *Proyeksi Penduduk Indonesia 2005-2025*. Jakarta: BPS, Bappenas dan UNFPA Indonesia
- Fitriana, S., Andarini, S., Sutrisno, S., Nawangtantrini, G., & Maharani, M. (2023). Hepatotoxicity Oral Administration of Flavonoids Rich Extract from *Phaleria Macrocarpha* in Mice. *Asian Journal of Health Research*, 2(2), 36-41. <https://doi.org/10.55561/ajhr.v2i2.109>
- Forslund, L. C., & Andersson, H. C. (2017). Phytoestrogens in foods on the Nordic market: A literature review on occurrence and levels. Denmark: Nordic Council of Ministers. Retrieved from [https://books.google.co.id/books?hl=en&lr=&id=r8o1DwAAQBAJ&oi=fnd&pg=PA1&dq=Forsslund,+L.+C.,+%26+Anderson,+H.+C.+\(2017\).+Phytoestrogens+in+foods+on+the+Nordic+market.+&ots=xCZLacz6aP&sig=LN-7rUhkV7Dxp7vQPr8aQ6T7O3A&redir_esc=y#v=onepage&q&f=false](https://books.google.co.id/books?hl=en&lr=&id=r8o1DwAAQBAJ&oi=fnd&pg=PA1&dq=Forsslund,+L.+C.,+%26+Anderson,+H.+C.+(2017).+Phytoestrogens+in+foods+on+the+Nordic+market.+&ots=xCZLacz6aP&sig=LN-7rUhkV7Dxp7vQPr8aQ6T7O3A&redir_esc=y#v=onepage&q&f=false)
- Goldštajn, M. Š., Mikuš, M., Alberto, F., Mariachiarra, F., Stefano, B., Noventa, M., Török, P., Terzic, S., Simone, A., & Simone, L. (2023). Effects of transdermal versus oral hormone replacement therapy in postmenopause : a systematic review. *Archives of Gynecology and Obstetrics*, 307(6), 1727–1745. <https://doi.org/10.1007/s00404-022-06647-5>
- Halim, R. M., Kamisah, Y., Aziz, N. F., Sudirman, U. N., Ahmad, N. A. N., Yong, C. K., & Zainalabidin, S. (2021). S-Allylcysteine Supplementation Effects on Vascular and Bone Health in Ovariectomized Wistar Rats. *Pakistan Veterinary Journal*, 41(4), 507–512. <https://doi.org/10.29261/pakvetj/2021.118>
- Hasanah, M., Bahri, S., & Merta, I. W. (2020). Effect of Red Bean Extract (*Phaseolus vulgaris*, L) on the Development of Female Mice Eggs (*Mus musculus*) Balb/C strains. *Jurnal Penelitian Pendidikan IPA*, 6(2), 227-231. <https://doi.org/10.29303/jppipa.v6i2.390>
- Hopper, S. E., Cuomo, F., Ferruzzi, J., Burris, N. S., Roccabianca, S., Humphrey, J. D., & Figueroa, C. A. (2021). Comparative study of human and murine aortic biomechanics and hemodynamics in vascular aging. *Frontiers in physiology*, 12, 746796. <https://doi.org/10.3389/fphys.2021.746796>
- Javadifar, A., Rastgoo, S., Banach, M., Jamialahmadi, T., Johnston, T. P., & Sahebkar, A. (2021). Foam cells as therapeutic targets in atherosclerosis with a focus on the regulatory roles of non-coding RNAs. *International Journal of Molecular Sciences*, 22(5), 1–27. <https://doi.org/10.3390/ijms22052529>
- Kattoor, A. J., Goel, A., & Mehta, J. L. (2019). LOX-1: regulation, signaling and its role in atherosclerosis. *Antioxidants*, 8(7), 218. <https://doi.org/10.3390/antiox8070218>
- Khatana, C., Saini, N. K., Chakrabarti, S., Saini, V., Sharma, A., Saini, R. V., & Saini, A. K. (2020). Mechanistic insights into the oxidized low-density lipoprotein-induced atherosclerosis. *Oxidative medicine and cellular longevity*, 2020(1), 5245308. <https://doi.org/10.1155/2020/5245308>
- Khoudary, S. R. El, Venugopal, V., Manson, J. E., Brooks, M., Santoro, N., Black, D. M.,

- Harman, M., Hodis, H. N., Brinton, E. A., Miller, V. M., Taylor, H. S., & Budoff, M. J. (2020). *Heart Fat and Carotid Artery Atherosclerosis Progression in Recently Menopausal Women: Impact of Menopausal Hormone Therapy. The KEEPS Trial*. 27(3), 255–262. <https://doi.org/10.1097/GME.0000000000001472>.Heart
- Kuhnle, G. G. C., Dell'Aquila, C., Aspinall, S. M., Runswick, S. A., Joosen, A. M. C. P., Mulligan, A. A., & Bingham, S. A. (2009). Phytoestrogen content of fruits and vegetables commonly consumed in the UK based on LC-MS and ¹³C-labelled standards. *Food Chemistry*, 116(2), 542–554. <https://doi.org/10.1016/j.foodchem.2009.03.002>
- Li, D., & Mehta, J. L. (2009). Intracellular signaling of LOX-1 in endothelial cell apoptosis. *Circulation Research*, 104(5), 566-568. <https://doi.org/10.1161/CIRCRESAHA.109.194209>
- Macpherson, B. E., & Quinton, N. D. (2022). Menopause and healthcare professional education: a scoping review. *Maturitas*, 166, 89-95. <https://doi.org/10.1016/j.maturitas.2022.08.009>
- Maharani, M., Lajuna, L., Yuniwati, C., Sabrida, O., & Sutrisno, S. (2021). Phytochemical characteristics from P.macrocarpa and its inhibitory activity on the peritoneal damage of endometriosis. *Journal of Ayurveda and Integrative Medicine*, 12(2), 229–233. <https://doi.org/10.1016/j.jaim.2020.06.002>
- Maharani, M., & Sutrisno, S. (2021). Pengaruh Flavonoid Ekstrak Mahkota Dewa (P. macrocarpa) Terhadap Peningkatan Indeks Apoptosis Pada Peritoneal Mencit Model Endometriosis. *Jurnal Kebidanan Malahayati*, 7(4), 652–657. <https://doi.org/10.33024/jkm.v7i4.4606>
- Moiety, F. M., Salem, H. A., Mehanna, R. A., & Abdel-Ghany, B. S. (2015). Comparative study on induction and effects of surgical menopause in a female rat model: a prospective case control study. *International journal of clinical and experimental medicine*, 8(6), 9403–9411.
- Nayak, T., Freaney, P. M., & Maganti, K. (2022). *Atherosclerotic Cardiovascular Disease Risk Assessment and Menopause: Current Evidence*. American College of Cardiology. Retrieved from <https://www.acc.org/latest-in-cardiology/articles/2022/04/18/12/39/atherosclerotic-cardiovascular-disease-risk-assessment-and-menopause>
- Newson, L. (2018). Menopause and cardiovascular disease. *Post Reproductive Health*, 24(1), 44–49. <https://doi.org/10.1177/2053369117749675>
- Pratiwi, M., & Damayanty, A. E. (2020). Pengaruh Pemberian Susu Kedelai (Glicine max L. merr) Terhadap Kadar HDL Dan LDL Pada Wanita Menopause (Studi Pada Ibu - Ibu Pengajian Aisyiyah Ranting Melati Medan, 4(4), pp. 132–137.
- Rodríguez-Landa, J. F. (2022). Considerations of timing post-ovariectomy in mice and rats in studying anxiety-and depression-like behaviors associated with surgical menopause in women. *Frontiers in Behavioral Neuroscience*, 16, 829274. <https://doi.org/10.3389/fnbeh.2022.829274>
- Sargowo, D. (2015). *Disfungsi Endotel*. Malang: UB Press. Retrieved from https://www.google.co.id/books/edition/Disfungsi_Endotel/t0JRDwAAQBAJ?hl=id&gbpv=1&dq=sindrom+koroner+akut+adalah&printsec=frontcover
- Simanjuntak, K. (2012). Peran antioksidan flavonoid dalam meningkatkan kesehatan. *Bina Widya*, 23(3), 135-140.
- WHO. (2022). *Menopause*. World Health Organization. <https://www.who.int/news-room/factsheets/detail/menopause>
- Wu, M. Y., Li, C. J., Hou, M. F., & Chu, P. Y. (2017). New insights into the role of inflammation in the pathogenesis of atherosclerosis. *International journal of molecular*

- sciences*, 18(10), 2034. <https://doi.org/10.3390/ijms18102034>
- Yuliawati, D., & Astuti, W. W. (2021). Efek Glycine Soja Terhadap Angiogenesis dan Histologi Aorta Tikus Ovariectomi. *Jurnal Kesehatan Andalas*, 9(4), 392. <https://doi.org/10.25077/jka.v9i4.1506>
- Yulinda, D., Yueniwati, Y., & Nurseta, T. (2014). Vigna unguiculata reduces aortic intima-media thickness and increases aortic diameter and angiogenesis in ovariectomized rats. *Journal of Experimental and Integrative Medicine*, 4(2), 85-88.
- Yousefzadeh, N., Kashfi, K., Jeddi, S., & Ghasemi, A. (2020). Ovariectomized rat model of osteoporosis: A practical guide. *EXCLI Journal*, 19, 89–107. <https://doi.org/10.17179/excli2019-1990>
- Zhu, D., Chung, H. F., Dobson, A. J., Pandeya, N., Brunner, E. J., Kuh, D., Greenwood, D. C., Hardy, R., Cade, J. E., Giles, G. G., Bruinsma, F., Demakakos, P., Simonsen, M. K., Sandin, S., Weiderpass, E., & Mishra, G. D. (2020). Type of menopause, age of menopause and variations in the risk of incident cardiovascular disease: Pooled analysis of individual data from 10 international studies. *Human Reproduction*, 35(8), 1933–1943. <https://doi.org/10.1093/humrep/deaa124>

Jurnal Info Kesehatan

Vol. 22, No. 2, June 2024, pp. 326-334

P-ISSN 0216-504X, E-ISSN 2620-536X

DOI: [10.31965/infokes.Vol22.Iss2.1630](https://doi.org/10.31965/infokes.Vol22.Iss2.1630)

Journal homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>



RESEARCH

Open Access

The Influence of Providing Information and Educational Media in Efforts to Prevent Stroke

Cindy Eka Wijaya^{1a*}, Irza Haicha Pratama^{1b}, Ermi Girsang^{1c}

¹ Department of Medicine, Faculty of Medicine, Dentistry and Health Sciences, Universitas Prima Indonesia, Medan, North Sumatra, Indonesia

^a Email address: cindyekawijaya@gmail.com

^b Email address: irzahp12@gmail.com

^c Email address: ermigirsang@unprimdn.ac.id

Received: 26 June 2024

Revised: 29 June 2024

Accepted: 29 June 2024

Abstract

Stroke is the leading cause of physical and mental disability worldwide. In Indonesia, the incidence of stroke is alarmingly high, affecting approximately 10.9% of the population annually, with around 713,783 individuals experiencing stroke each year. Understanding how to prevent stroke is crucial. Clear and regular information, along with effective educational media, are essential for increasing public awareness of stroke. This study aims to determine the impact of social media and knowledge on stroke prevention. This type of study is a non-experimental quantitative study using a cross-sectional. This study is conducted in portions, with patients who are willing and able to talk effectively. Univariate, bivariate, and multivariate analyses were conducted using Pearson correlation tests and linear regression tests. The results show that among the 100 respondents, most were male, aged 41-60 years, and engaged in the best stroke prevention efforts. The Pearson correlation test showed significant correlations between information accuracy, frequency, and educational media for stroke prevention (Pearson $p=0.89$; $p=0.62$; $p=0.75$). The linear regression test revealed that the clarity of information, frequency of information, and educational media together increased stroke prevention efforts ($p=0.037$), with the clarity of information being the most significant factor. The conclusion is the study concludes that educational media and accurate information are crucial in preventing stroke. It is recommended that the Kasih Sayang Clinic conducts comprehensive stroke education and screening every six months to enhance prevention efforts.

Keywords: Stroke, Prevention, Social Media, Educational Media, Public Awareness, Indonesia

*Corresponding Author:

Cindy Eka Wijaya

Department of Medicine, Faculty of Medicine, Dentistry and Health Sciences, Universitas Prima Indonesia, Medan, North Sumatra, Indonesia

Email: cindyekawijaya@gmail.com



©The Author(s) 2024. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

1. INTRODUCTION

Stroke is a medical condition that causes physical and mental problems worldwide. According to a World Health Organization report, stroke is the second leading cause of death after heart disease (Helboe, Eddelien, & Kruuse, 2023). In the United States, stroke significantly impacts society, being the leading cause of long-term disability and the second highest cause of death. It is estimated that about 7 million adults in the United States have had a stroke, representing about 3% of the adult population, with 600,000 new stroke cases occurring each year. The majority of these strokes (87%) are ischemic infarctions, 10% are primary hemorrhagic strokes, and 3% are subarachnoid strokes. Although less common, strokes also occur in children, with about 1 to 2.5 per 100,000 children experiencing a stroke each year, of which 50 to 75 percent are hemorrhagic strokes (Saini, Guada, & Yavagal, 2021).

In Indonesia, the 2018 Basic Health Survey conducted by the Ministry of Health of the Republic of Indonesia showed that approximately 10.9% of the population experiences a stroke each year, with the number of cases reaching 713,783 annually. East Kalimantan has the highest incidence of stroke in Indonesia, with 14.7% of the total population or 9,696 cases. Most stroke cases in Indonesia occur in individuals over the age of 75, the age group with the highest risk factors (Venketasubramanian, Yudiarto, & Tugasworo, 2022). Various conditions and behaviors such as hypertension, diabetes, dyslipidemia, unhealthy diet, smoking, lack of physical activity, alcohol consumption, and other health conditions like primary hypertension and cardiovascular disease are stroke risk factors. Managing these risk factors through a healthy diet, regular exercise, weight control, and a healthy lifestyle is crucial for stroke prevention (Aini, et al., 2022).

Stroke complications that can occur after a brain attack are very serious and can affect other body functions, including severe brain injury, cognitive impairments, speech and walking difficulties, and other health problems such as heart disease and liver failure (Buckley et al., 2022). Public knowledge and understanding of risk factors are one of the effective ways to prevent stroke (Talango & Kusdhiarningsih, 2024). However, more research is needed to assess how effectively information and educational media are used for stroke prevention (Firmawati, Rochmawati, & Setyopranoto, 2023). According to Dewi, et al., (2022), many patients who experience recurrent strokes are unaware of the risk factors that caused the attacks. Therefore, education on stroke prevention needs to be provided to the general healthy population to reduce the risk of recurrent stroke attacks (Mone, et al., 2023).

Research by Ali et al., (2023) shows that education can significantly improve public understanding of stroke risk factors. Before education, only 31% of respondents correctly answered questions about stroke risk factors, but this number increased to 72.4% after the educational program. This highlights the importance of educational programs in raising public awareness. To enhance awareness and understanding of stroke prevention, medical professionals should use clear and easily understandable tools for patients and their families. The study by Bhattad & Pacifico, (2022) indicates that the physical and psychosocial quality of life of patients can be improved with personalized patient education and verbal instructions from healthcare providers, which also increases overall patient satisfaction. This study aims to determine the impact of social media and knowledge on stroke prevention.

2. RESEARCH METHOD

This research method uses a quantitative, non-experimental study employing a cross-sectional design to evaluate the impact of information provision and educational media on stroke prevention. The study was conducted at Kasih Sayang Clinic in Medan, North Sumatra, starting in February 2024. The population included all patients receiving medical care at the clinic, with a total sample size of 100 respondents selected through total sampling.

A representative sample of patients who were willing and able to answer a validated and reliable questionnaire was selected. Primary data were collected through direct surveys, while secondary data were obtained from literature and journals. The study investigated three independent variables: frequency of information delivery, clarity of information delivery, and educational media, which were hypothesized to impact the dependent variable, stroke prevention.

Data analysis included univariate analysis to describe the characteristics of the variables. Bivariate analysis using Pearson or Spearman rho correlation tests to examine relationships between independent variables and stroke prevention. Multivariate analysis employing ordinal regression to evaluate the influence of independent variables on stroke prevention. Variable selection was based on p-values <0.25, with the elimination of variables with p-values >0.05. This comprehensive approach aimed to identify significant factors contributing to effective stroke prevention efforts. This research has also received ethical approval from the Prima Indonesia University health research ethics commission with number: 009/KEPK/UNPRI/V/2024.

3. RESULTS AND DISCUSSION

Table 1. Participant characteristics and demographics

Gender	n	Percentage (%)
Male	56	56
Female	44	44
Age	n	Percentage (%)
20 - 40	22	22
41 - 60	52	52
61 - 80	26	26

Table 1 shows that the characteristics of respondents include age and gender, with the majority being aged 41-60 (52%) and male (56%).

Table 2. Pearson correlation matrix in the study of stroke prevention efforts

Frequency of Information		Clarity of Information		Educational Media	Stroke Prevention
Frequency of Information	Pearson Correlation	1	.721**	.682**	.619**
	Sig. (2-tailed)		.000	.000	.000
	N	100	100	100	100
Clarity of Information	Pearson Correlation	.721**	1	.682**	.883**
	Sig. (2-tailed)	.000		.000	.000
	N	100	100	100	100
Educational Media	Pearson Correlation	.682**	.682**	1	.750**
	Sig. (2-tailed)	.000	.000		.000
	N	100	100	100	100
Stroke prevention	Pearson Correlation	.619**	.883**	.750**	1
	Sig. (2-tailed)	.000	.000	.000	
	N	100	100	100	100

** . Correlation is significant at the 0.01 level (2-tailed).

Table 2 shows that the bivariate analysis using Pearson's test indicated a normal data distribution ($p=0.200$, $p > 0.05$). Positive and significant correlations were found between the frequency ($r=0.619$, $p=0.005$) and clarity of information ($r=0.883$, $p=0.005$) with stroke

prevention efforts. Educational media also showed a significant positive correlation with stroke prevention efforts ($r=0.750$, $p=0.005$), indicating that higher frequency, clarity, and effectiveness of educational media correspond to better stroke prevention efforts.

Information frequency refers to how often information on stroke risks, signs, symptoms, and prevention actions is conveyed. While 92% of respondents felt the information frequency was adequate, the correlation between information frequency and stroke prevention was not statistically significant ($r=0.62$, $p>0.05$). Nonetheless, this study aligns with others indicating that frequent information can enhance stroke prevention knowledge and behaviors (Sahirah, Ikhsan, & Nadira, 2023).

Information clarity pertains to how clear and accurate the provided information on stroke risks and prevention actions is. Ninety-four percent of respondents found the received information clear, with a significant positive correlation ($r=0.883$, $p=0.005$), highlighting the pivotal role of information clarity in stroke prevention (Hariadi, Pamungkas, & Sidharta, 2020).

Educational media encompasses various resources for disseminating information about stroke and its prevention. Ninety-five percent of respondents felt satisfied with educational media, correlating significantly ($r=0.75$, $p=0.005$). Effective educational media can boost awareness and influence stroke prevention behaviors (Gandolfo, et al., 2022).

Table 3. Linear regression matrix in the study of stroke prevention efforts.

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	3.632	1.715		2.118	.037
Frequency of Information	-.224	.086	-.170	-2.609	.011
Clarity of Information	.665	.056	.777	11.940	.000
Educational Media	.564	.103	.336	5.451	.000

Multivariate analysis evaluated the joint impact of information frequency, clarity, and educational media on stroke prevention efforts. Variables with $p < 0.25$ were included. Results of linear regression showed that collectively, these factors significantly influenced stroke prevention efforts ($p=0.037$). Among them, information clarity had the most significant impact (beta=0.665), indicating its dominance in enhancing stroke prevention compared to frequency and educational media.

Overall, this study emphasizes the importance of clear and frequent information dissemination, along with the effective use of educational media, in stroke prevention efforts. Clear information enhances patient understanding of risks and preventive actions, while educational media boosts public awareness and knowledge about stroke prevention. These findings can guide the development of more effective health education programs at Kasih Sayang Clinic and other healthcare facilities, focusing on improving information clarity and utilizing appropriate educational media.

Multivariate analysis indicates that information frequency, clarity, and educational media collectively influence stroke prevention ($p=0.037$). Information clarity has the strongest influence, with a beta value of 0.665. High information frequency and educational media aid understanding and effective prevention actions (Wardhani Firdaus, & Mayasari, 2023).

Information clarity emerges as the most dominant factor affecting stroke prevention. Clear information aids understanding of symptoms and preventive actions. Structured information use assists individuals in managing stroke risks, such as reducing alcohol consumption and stress (Lonini et al., 2022). Clear information dissemination enhances public awareness of stroke risks and their ability to address these risks. General information can elevate awareness and preventive actions (Romadoni, 2023).

The Role of Information Frequency in Stroke Prevention Efforts at the Kasih Sayang Clinic

In efforts to prevent stroke, the frequency of information refers to how often or repetitive the information about risk factors, signs and symptoms, and preventive measures of stroke are conveyed to the public. The term "frequency of information" refers to how often or repeatedly the information is delivered to individuals or communities within a certain period of time (Pomalango, 2022).

This study is in line with a study by Sahirah et al., (2023), The results show that people who receive information about stroke risk regularly have a lower likelihood of stroke than those who do not receive such information. This indicates that frequent dissemination of information can increase people's awareness of stroke risk and influence how they act when facing such risk.

One important factor in stroke prevention is the high frequency of information. Individuals who receive information regularly can understand stroke risk factors such as high cholesterol, hypertension, and diabetes, and can take effective preventive actions to prevent stroke. Therefore, a high frequency of information can increase people's awareness of stroke risk and influence how they behave when facing such risk. This can help prevent recurrent strokes and expedite physical and psychological recovery after an attack (Firmawati, Rochmawati & Setyopranoto, 2023).

In various ways, the use of a high frequency of information can help prevent stroke. For example, information regularly distributed on social media can increase people's awareness of stroke risk and how they treat such risk (Garg, et al., 2023). Health education programs with a high level of information can also help people understand stroke risk factors and take effective preventive actions. A high frequency of information during consultations with doctors can help people better understand stroke diagnoses and treatments, as well as influence how they behave in the face of risk (Rakhma et al., 2023).

The Role of Information Clarity in Stroke Prevention Efforts at the Kasih Sayang Clinic

Stroke causes brain nerve cells to no longer function normally, resulting in cognitive, mental, paralysis, or death impairments (Rahayu, 2023). Therefore, public awareness is crucial in preventing this disease by providing information about stroke. Thus, people can take preventive actions to reduce the risk of stroke before it's too late (Boehme, Esenwa, & Elkind, 2017).

In stroke prevention efforts, information clarity focuses on the importance of providing clear and accurate information to individuals about stroke risks and methods to avoid them (Hariadi, Pamungkas, & Sidharta, 2020). This is based on the idea that clearly received information can help people understand stroke risk factors and take effective preventive actions to prevent stroke.

Providing clear and accurate information about stroke disease, including symptoms, diagnosis, and treatment, is essential. Regularly received information can help people better understand stroke disease and choose appropriate treatment (Pomalango, 2022). Providing clear information can be used in various ways to prevent stroke, such as through health education programs, social media, or consultations with doctors (Romadoni, 2023). Therefore, providing clear information can increase people's awareness of stroke risk and influence how they act when facing such risk.

The Role of Educational Media in Stroke Prevention Efforts at the Kasih Sayang Clinic

In stroke prevention efforts, educational media focuses on the importance of using various resources to provide clear and accurate information about stroke disease and how to

prevent it from occurring. According to [Pomalango et al., \(2022\)](#), this statement is based on the idea that people can learn more about stroke risk factors and take effective preventive actions.

Educational media states that clear and accurate information about stroke disease can be provided through various media, such as social media, health education programs, and consultations with doctors ([Gandolfo et al., 2022](#)).

Educational media can be used to prevent stroke in various ways, such as through health education programs and consultations with doctors ([Romadoni, 2023](#)). Thus, educational media can increase people's awareness of stroke risk and change how they act when facing such risk. Therefore, educational media can help raise individuals' awareness of stroke risk and influence their behavior when facing such risk.

In Indonesia, various parties, including the government, health institutions, and medical institutions, have implemented programs to prevent stroke. Among them is a program by the Ministry of Health of the Republic of Indonesia called GATROK SEDI (Early Stroke Prevention) and CERDIK (Stroke Prevention through Risk Detection and Education). Both programs focus on raising public awareness about the dangers of stroke and how to prevent it ([Laili, Heni, & Tanoto, 2023](#)).

At Pertamina Jaya Hospital, various types of educational media are also used, such as leaflets, videos, and consultations with doctors, with a focus on providing education about stroke and providing comprehensive health services to prevent and manage stroke ([Lonini et al., 2022](#)).

The Influence of Information Frequency, Information Clarity, and Educational Media on Stroke Prevention Efforts

As part of stroke prevention efforts, increasing the amount of information provided, the clarity of information provided, and the use of educational media can help raise public awareness about stroke risk and how to avoid it ([Murthy, Thomas, & Dasgupta, 2019](#)). The clarity of information received regularly can help people understand stroke risk factors and effective preventive actions. A high frequency of information can also help people understand stroke disease and its treatment. To increase public awareness about stroke risk and how to avoid it, educational media can take the form of videos, leaflets, or consultations with doctors ([Wardhani, Firdaus & Mayasari, 2023](#)).

A high frequency of information can help individuals understand stroke risk factors and develop effective preventive actions, while the clarity of information received regularly can help individuals understand stroke disease and how to treat it ([Rupasinghe et al., 2022](#)). The educational media used can help raise public awareness about stroke risk and how to avoid it, as well as influence their behavior when facing such risk ([Azali, et al., 2023](#)).

This study has similar findings to a study found by [Kusumawaty & Nurapandi, \(2023\)](#), which showed that individuals who received education about stroke through educational media had a lower likelihood of stroke compared to those who did not receive such education. The simultaneous use of information frequency, information clarity, and educational media in stroke prevention efforts can help raise public awareness about stroke risk and how to avoid it, as well as influence behavior when facing such risk ([Khan et al., 2021](#)).

Factors Influencing Stroke Prevention Efforts at the Kasih Sayang Clinic

The use of information in an organized structural format can also help individuals understand stroke risk factors and how to manage them. Therefore, information presented in this format can help individuals understand how to reduce stroke risk, such as reducing alcohol consumption, reducing caloric intake, and reducing stress ([Prabhakaran & Chong, 2014](#)). Thus,

information presented in this format can help individuals understand how to reduce stroke risk and how to treat it (Firmawati, Rochmawati & Setyopranoto, 2023).

Based on the results of several studies, the dissemination of clear information can increase public awareness about stroke risk and improve their ability to face such risk. Therefore, clear information can help raise public awareness about stroke risk and improve their actions in facing such risk (Sahirah, Ikhsan & Nadira, 2023).

4. CONCLUSION

This research concludes that the frequency and clarity of information, along with educational media, play crucial roles in stroke prevention efforts at the Kasih Sayang Clinic. Clarity of information emerged as the most influential factor, highlighting the need for easily understandable public health information. The recommendation is to enhance stroke prevention, Kasih Sayang Clinic should increase educational activities and promotive information, such as regular seminars on healthy lifestyles. Participants are encouraged to continue exploring the latest prevention strategies. Future research should include additional variables like family support and individual knowledge to deepen the understanding of factors supporting stroke prevention efforts. These conclusions and recommendations can guide improved stroke prevention and overall community health.

REFERENCES

- Aini, N., Mashfufa, E. W., Setyowati, L., Freeska, O., & Marta, D. (2022). The Effect of Education on Self-Management and Stroke Prevention Behavior on Recurrence. *Jurnal Multidisiplin Madani (MUDIMA)*, 2(3), 1477–1488. Retrieved from <https://journal.formosapublisher.org/index.php/mudima/article/view/251>
- Ali, M., Berbudi Bl, A., Robbani, F. Y., Hanafi, I., Anugrah, M. R., Ansari, N. V., & Wijaya, S. P. (2023). Peningkatan Kesadaran Masyarakat Terhadap Pentingnya Pencegahan Dini Stroke. *Jurnal Pengabdian Masyarakat Fisioterapi dan Kesehatan Indonesia*, 2(01), 65-71. <https://doi.org/10.59946/jpmfki.2023.199>
- Azali, L. M. P., Sulistyawati, R. A., Saelan, S., & Putri, D. S. R. (2023). Edukasi Management Pengelolaan Faktor Penyebab sebagai Upaya Pencegahan Serangan Stroke dan Serangan Berulang. *Jurnal Kreativitas Pengabdian Kepada Masyarakat (PKM)*, 6(11), 5140–5152. <https://doi.org/10.33024/jkpm.v6i11.12081>
- Bhattad, P. B., & Pacifico, L. (2022). Empowering Patients: Promoting Patient Education and Health Literacy. *Cureus*, 14(7), e27336. <https://doi.org/10.7759/cureus.27336>
- Boehme, A. K., Esenwa, C., & Elkind, M. S. (2017). Stroke risk factors, genetics, and prevention. *Circulation research*, 120(3), 472-495. <https://doi.org/10.1161/CIRCRESAHA.116.308398>
- Buckley, B. J. R., Harrison, S. L., Hill, A., Underhill, P., Lane, D. A., & Lip, G. Y. H. (2022). Stroke-Heart Syndrome: Incidence and Clinical Outcomes of Cardiac Complications Following Stroke. *Stroke*, 53(5), 1759–1763. <https://doi.org/10.1161/STROKEAHA.121.037316>
- Dewi, N. L. P. T., Wati, N. M. N., Jayanti, D. M. A. D., Lestari, N. K. Y., & Sudarma, I. N. (2022). Edukasi Metode CERDIK Dan PATUH Modifikasi Gaya Hidup Sehat Dalam Upaya Mencegah Kejadian Stroke Berulang. *Jurnal Empathy Pengabdian Kepada Masyarakat*, 3 (1), 42–52. <http://jurnalempathy.com/index.php/jurnalempathy/article/view/91>

- Firmawati, E., Rochmawati, E., & Setyopranoto, I. (2023). Deteksi Risiko Stroke Dan Edukasi Sebagai Upaya Pencegahan Primer Terjadinya Stroke. *Jurnal SOLMA*, 12(2), 705-712. <https://doi.org/10.22236/solma.v12i2.11834>
- Gandolfo, C., Alberti, F., Del Sette, M., & Reale, N. (2022). Stroke prevention and therapy awareness in a large sample of high school students: results of an educational campaign in the Northern-Western Italy. *Neurological Sciences*, 43(12), 6847–6854. <https://doi.org/10.1007/s10072-022-06372-6>
- Garg, D., Agarwal, A., Srivastava, M. P., & Vishnu, V. Y. (2023). Use of Social Media in Stroke: A Systematic Review. *Annals of Indian Academy of Neurology*, 26(3), 206-212. https://doi.org/10.4103/aian.aian_58_23
- Helboe, K. S., Eddelien, H. S., & Kruuse, C. (2023). Visual symptoms in acute stroke—a systematic review of observational studies. *Clinical Neurology and Neurosurgery*, 229, 107749. <https://doi.org/10.1016/j.clineuro.2023.107749>
- Khan, F., Gaowgzeh, R. A. M., Saif, A. A., Chevidikunnan, M. F., Soman, A., Mazi, A., ... & Anjamparuthikal, H. (2021, November). Effect of community education program on stroke symptoms and treatment on school and college students from south india: a longitudinal observational study. *Healthcare*, 9(12), 1637. <https://doi.org/10.3390/healthcare9121637>
- Hariadini, A.L., Pamungkas, S.S.A., & Sidharta, B. (2020). Pengaruh Pemberian Informasi Obat Antihipertensi terhadap Tingkat Pengetahuan dan Kepatuhan Pasien Peserta PROLANIS di Puskesmas Gedangan Kabupaten Malang. *Pharmaceutical Journal Of Indonesia*, 2020(1), 63–68. <https://doi.org/10.21776/ub.pji.2020.006.01.10>
- Kusumawaty, J., & Nurapandi, A. (2022). Edukasi Dan Mobilisasi (ROM) pada Lansia Penderita Stroke dengan Audio Visual di Panti Jompo Welas Asih Tasikmalaya. *Kolaborasi: Jurnal Pengabdian Masyarakat*, 2(1), 45-51. <https://doi.org/10.56359/kolaborasi.v2i1.42>
- Laili, N., Heni, S., & Tanoto, W. (2023). Optimalisasi Program Edukasi Pencegahan Stroke ‘Cerdik’ pada Penderita Hipertensi. *Jurnal Abdi Kesehatan Dan Kedokteran*, 2(2), 53–65. <https://doi.org/10.55018/jakk.v2i2.40>
- Lonini, L., Moon, Y., Embry, K., Cotton, R. J., McKenzie, K., Jenz, S., & Jayaraman, A. (2022). Video-Based Pose Estimation for Gait Analysis in Stroke Survivors during Clinical Assessments: A Proof-of-Concept Study. *Digital Biomarkers*, 6(1), 9–18. <https://doi.org/10.1159/000520732>
- Mone, B. Y., Uly, A., Balarminus, P., & Santoso, S. D. R. P. (2023). Fulfillment Of Psychosocial Needs (Empowerment) In Stroke Patients In The Internal Room Of The Waikabubak General Hospital, West Sumba District. *Jurnal Keperawatan Sumba*, 2(1), 21–31. <https://doi.org/doi.org/10.31965/jks.v2i1.1294>
- Murthy, MeenaK. S., Thomas, P., & Dasgupta, M. (2019). Potential for a comprehensive stroke education: Assessing awareness about stroke among community health workers - A qualitative study from Urban Bangalore, Karnataka, India. *Journal of Family Medicine and Primary Care*, 8(7), 2424. https://doi.org/10.4103/jfmpe.jfmpe_303_19
- Pomalango, Z. B., & Jusuf, H. (2022). Pengaruh Pendidikan Kesehatan pada Pengetahuan Keluarga tentang Gejala dan Pencegahan Resiko Stroke. *Care Journal*, 1(2), 68-73. <https://doi.org/10.35584/carejournal.v1i2.85>
- Pomalango, Z. B. (2022). Pengaruh Edukasi Deteksi Dini Stroke dengan Metode Fast Terhadap Tingkat Pengetahuan Keluarga dengan Risiko Tinggi Stroke di Wilayah Kerja Puskesmas Suwawa Kabupaten Bone Bolango. *Care Journal*, 1(1), 20-26. <https://doi.org/10.35584/carejournal.v1i1.22>

- Prabhakaran, S., & Chong, J. Y. (2014). Risk factor management for stroke prevention. *CONTINUUM: Lifelong Learning in Neurology*, 20(2), 296-308. <https://doi.org/10.1212/01.CON.0000446102.82420.64>
- Rahayu, T. G. (2023). Analisis Faktor Risiko Terjadinya Stroke Serta Tipe Stroke. *Faletehan Health Journal*, 10(1), 48–95. <https://doi.org/10.33746/fhj.v10i01.410>
- Rakhma, T., Dewi, L. M., Putri, N. M., Ruspita, W. S., Madania, M., Khusna, S. A., ... Feriyanto, D. D. (2023). Penyuluhan Pencegahan Stroke dan Faktor Risikonya pada Lansia. *Jurnal Pengabdian Masyarakat Medika*, 23–28. <https://doi.org/10.23917/jpmmedika.v3i1.1316>
- Romadoni, S. (2023). Pengaruh Edukasi Media Booklet Terhadap Pengetahuan Keluarga Tentang Deteksi Dini Stroke. *Masker Medika*, 11(2), 403–413. <https://doi.org/10.52523/maskermedika.v11i2.580>
- Rupasinghe, C. D., Ammar Bokhari, S., Lutfi, I., Noureen, M., Islam, F., Khan, M., Amin, F., & Muthanna, F. M. S. (2022). Frequency of Stroke and Factors Associated With It Among Old Age Hypertensive Patients in Karachi, Pakistan: A Cross-Sectional Study. *Cureus*, 14(3), e23123. <https://doi.org/10.7759/cureus.23123>
- Sahirah, R., Ikhsan, M., & Nadira, C. S. (2023). Gambaran Tingkat Pengetahuan Paramedis tentang Pencegahan Primer Stroke di Rumah Sakit Umum Cut Meutia Aceh Utara. *GALENICAL: Jurnal Kedokteran dan Kesehatan Mahasiswa Malikussaleh*, 2(6), 102-113. <https://doi.org/10.29103/jkkmm.v2i6.12436>
- Saini, V., Guada, L., & Yavagal, D. R. (2021). Global epidemiology of stroke and access to acute ischemic stroke interventions. *Neurology*, 97(20_Supplement_2), S6-S16. <https://doi.org/10.1007/s13311-011-0053-1>
- Talango, F., & Kusdhiarningsih, B. (2024). The Influence of Counseling Education Based on the Health Belief Model Theory on Knowledge of Preventing Hypertension Complications in Karanganyar. *Jurnal Keperawatan Sumba*, 2(2), 79–90. <https://doi.org/10.31965/jks.v2i2>
- Venkatasubramanian, N., Yudiarto, F. L., & Tugasworo, D. (2022). Stroke Burden and Stroke Services in Indonesia. *Cerebrovascular Diseases Extra*, 12(1), 53–57. <https://doi.org/10.1159/000524161>
- Wardhani, F. A., Firdaus, A.W., & Mayasari, S. (2023). Peningkatan Edukasi Stroke Masyarakat dengan Video Edukasi Stroke – “CHERIE” (Cerebral Hemorrhage and Ischemic Educational Video). *Jurnal Pengabdian Masyarakat (JUDIMAS)*, 1(2), 228–233. <https://doi.org/10.54832/judimas.v1i2.163>

Jurnal Info Kesehatan

Vol. 22, No. 2, June 2024, pp. 335-346

P-ISSN 0216-504X, E-ISSN 2620-536X

DOI: [10.31965/infokes.Vol22.Iss2.1488](https://doi.org/10.31965/infokes.Vol22.Iss2.1488)Journal homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>**RESEARCH****Open Access****Vibration Stimulation to Increase Milk Production in Puerperal Mothers****Rhela Panji Raraswati^{1a*}, Krisdiana Wijayanti^{1b}, Heni Hendriyani^{1c}**¹ Midwifery Study Program, Applied Master Program, Poltekkes Kemenkes Semarang, Semarang, Central Java, Indonesia^a Email address: relapanji@gmail.com^b Email address: wijayanti.k@hotmail.com^c Email address: heni_sahid@yahoo.com

Received: 12 May 2024

Revised: 29 June 2024

Accepted: 30 June 2024

Abstract

Breast milk is the best food for babies, but insufficient production can hinder breastfeeding. Many mothers face challenges with milk production, preventing optimal breastfeeding. This study aims to innovate and develop a vibration-based electric massage device to enhance breast milk production and analyze its effectiveness based on baby weight indicators. The research employs a quantitative method with a Quasi-Experimental design, divided into an intervention group and a control group. A random sampling technique selected 46 respondents, split evenly between the two groups. The intervention group received breast care using the vibration-based device, while the control group received standard breast care for 14 days. Breast care was administered twice daily for 2-3 minutes. Baby weight was measured at 14, 21, and 28 days. Tools to measure energy intake, protein intake, and fluid intake utilized food recall, and breastfeeding frequency was measured by a scale. Psychological status was assessed using the DASS questionnaire. Data analysis included Repeated Measure ANOVA and Independent Simple T-Test. Results indicated a significant difference in breast milk production based on baby weight between the intervention and control groups ($p=0.000$). The mean weight difference between the groups was 200.21 grams, favoring the intervention group. Increased breast milk production was not influenced by confounding variables such as energy intake, protein intake, fluid intake, breastfeeding frequency, and psychological status. In conclusion, the developed vibration-based electric massage device effectively increased breast milk production, evidenced by significant weight gain in babies within the intervention group compared to the control group ($p=0.000$).

Keywords: Massage Equipment, Postpartum, Vibration, Breast Milk Production.

***Corresponding Author:**

Rhela Panji Raraswati

Midwifery Study Program, Applied Master Program, Poltekkes Kemenkes Semarang, Semarang, Central Java, Indonesia

Email: relapanji@gmail.com

©The Author(s) 2024. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

1. INTRODUCTION

Mother's milk (ASI) is the best food option aimed at babies because of the large nutritional content in breast milk needed for babies such as protein, white blood cells, and immune substances that are suitable for optimal growth and development. Exclusive breastfeeding refers to giving breast milk to babies for 6 months unbroken and does not provide other food or drinks, including water, except under certain circumstances such as the use of ORS (Linda, 2019).

Mother's milk (ASI) has nutrients, antioxidants, hormones, and antibodies, and both children such as fat, carbohydrates, protein, minerals, and vitamins are needed. ASI also has protective substances namely IG A, IG E, IG M, Lactobacillus Bifidus, Lactoferrin, and Lisozyme, Cellular Immunity does not cause allergies, so it can neutralize bacteria, fungi, viruses, and parasites. Providing breast milk (ASI) continuously or exclusively for 6 months brings a number of important benefits, including the presence of antibodies and macrophage cells contained in colostrum and ASI, which provides special protection for infants. Children who are given exclusive breast milk tend to be lower in contagious diarrhea, necrotizing enterocolitis, respiratory infections and ears (Handayani & Pujiastuti, 2016).

The World Health Organization (WHO) in the global strategy of feeding for infants and children states that exclusive breastfeeding for six months is effective in preventing child death. The World Health Organization (WHO) promotes actively that ASI is the best nutrition and exclusive ASI in 2025 at least the first 6 months as much as 50% (WHO, 2022). In Indonesia, the percentage of exclusive breastfeeding of 77.41% still does not meet the target set, which is 80% including under the target, while for the Central Java region in 2022 there are 78.71% of children who get exclusive breastfeeding less than 6 months of age (BPS, 2022). In addition, based on the preliminary results in the Magelang District Health Office, exclusive ASI was obtained 82.1% with the highest Pakis Pakis Puskesmas at 95% while the low ASI coverage in the Secang 2 Puskesmas area of 37% that had not yet received exclusive breastfeeding.

Exclusive breastfeeding is the provision of breast milk alone without additional liquids, whether formula milk, water, orange juice or other additional foods to babies aged 0-6 months, which will have extraordinary benefits for the baby's development and growth in addition to increasing the bond of affection. mother and baby (Hipson, Handayani & Erwanda, 2023). Problems that often arise in mothers during breastfeeding can start before delivery (antenatal period), in the early postpartum period, and in the late postpartum period (Rahayuningsih, 2020). Exclusive breastfeeding and the correct breastfeeding process are reliable means of building quality human resources. Apart from that, in the correct breastfeeding process, babies will get good physical, emotional, and spiritual development in their lives (Pratama et al., 2023).

Breast milk needs to be given because it has several uses for babies, namely providing a better life for the development and growth of the baby, and having antibodies so that it protects the baby from several viral, bacterial, parasitic, and fungal infections. Breast milk has a complex composition according to the baby's needs, which increases the baby's intelligence, and avoids the risk of allergies due to formula milk, direct breastfeeding can provide love to the baby and reduce the risk of metabolic diseases such as Type II diabetes mellitus, hypertension, obesity as an adult (Cynthia et al., 2019). One of the best investments to improve health, survival, economic improvement and social development of individuals and nations is breastfeeding. Optimal breastfeeding according to guidelines can overcome more than 20,000 maternal deaths and 823,000 child deaths every year (Hasan, & Saputra, 2023).

One of the problems that can arise from not giving exclusive breast milk to babies is inhibiting growth and development, such as stunting. The impact of stunting itself on children has the potential to reduce their intelligence level, increase children's vulnerability to disease, and potentially result in reduced productivity in the future. According to research conducted by Louis, babies who are not exclusively breastfed are 61 times more likely to suffer from stunting

than babies who are exclusively breastfed at the age of six months. In addition, not getting exclusive breast milk has a high risk of developing upper respiratory tract infections (ARI) (Louis, Mirania, & Yuniarti, 2022).

In reality there are still many mothers experiencing problems in breastfeeding, including the lack of smooth milk production so that breastfeeding cannot be done optimally. Before delivery, a mother must have imagined the first moment carrying her baby in her arms and began to prepare the main basic needs for her baby, namely breastfeeding. However, in reality, the stage of breastfeeding is not always in line with expectations. Most mothers, especially those who are experiencing experience for the first time become mothers, have difficulty when they have to breastfeed their babies for the first time. Some even face situations where their breasts do not produce breast milk, even after two days after giving birth (Chomaria, 2020).

The scope of exclusive breastfeeding that is still low is considered to play an important role in more than one number of children's deaths each year and has a bad impact on baby development, so that it can affect the quality of life of the nation's next generation. In addition, exclusive breastfeeding cannot be separated from the success of breast milk produced by breastfeeding mothers.

There are several factors including those that are closely related to the production of breast milk such as food consumed by the mother, the way the baby suckles seen from the baby's suction, the frequency of breastfeeding, the mother's rest patterns, the mother's nutritional intake, mother's psychological and breast care. Breast care can be started from the third trimester of pregnancy until after giving birth. Breasts according to the period of development both from pregnancy until after labor will experience changes. This change makes breast care considered important to do, because breast care is very influential in milk production (Katuuk & Kundra, 2018).

The results of the research described in the study conducted by Emilda on breast care in controlling the smooth flow of breast milk show that breast care has an influence on the smooth flow of breast milk in mothers after giving birth (Emilda, 2022). This is consistent with the findings found in research conducted by Mukarramah on breast care for breast milk production. This study concluded that there was a significant difference in breast milk production between the group that received breast care and the control group, with higher milk production in mothers who received breast care compared to those who did not. These results confirm that breast care has a positive impact on postpartum maternal breast milk production at the Kassi-Kassi Health Center, Makassar City (Mukarramah, Nurdin & Ahmad, 2021).

Breast care can be interpreted as a breast care method used to increase breast milk production during pregnancy and childbirth. Breast care is care carried out after pregnancy and childbirth with the aim of increasing breast milk production, maintaining breast cleanliness and caring for the nipple area (Wahyuni, et al., 2022). Success in breast milk production can be seen from the baby's weight which always increases every week, so it can be seen that the baby is getting enough breast milk. Increased milk production after childbirth is something that needs to be considered especially to support the success of the breastfeeding process, because the first day after giving birth is an important time that can affect the sustainability of breastfeeding. Efforts made to increase milk production in addition to squeezing breast milk can also be by providing pharmacological therapy such as drugs or breastfeeding pills while non-pharmacological therapy such as oxytocin massage, Woolwich massage, and breast care.

Breast care that is still mostly done today uses hands, besides that it requires an impractical duration, even though in the current era it should be able to use the latest technology in accordance with technological advances. In line with research conducted by Nasution, Erniyati, & Aizar, (2018), about vibration stimulus in the form of a bra that is used to overcome the smoothness of breast milk. Where the results indicate the influence of the vibrating bra for the smooth running of milk, but the vibrating bra has a weakness that is the size of the bra can

be different for each mother. Therefore the innovation that will be done by the author is to develop an electric massage tool for the breast with a vibrating technique in it. The benefit is that the breasts experience stimulation with massage tools used and facilitate milk production. DC Vibratory Motor which provides a vibrational effect to stimulate the hormone prolactin and oxytocin. DC Vibratory Motor Method is a vibrational spreading method that vibrates ions in the breast which is directed at the activation of the hormone prolactin and oxytocin which results in alveoli stimulation in flowing milk to the lacterus duct and entering the milk sin and the nipple hole (Nasution, Erniyati, & Aizar, 2018). Based on the background above the author is interested in conducting research related to vibration-based breast massage tools as breast care for milk production in postpartum mothers. Produce innovations in the development of viable vibration-based electric massage devices and analyze their effectiveness on breast milk production in postpartum mothers.

2. RESEARCH METHOD

This type of research this research method uses quantitative research with a Quasy Experiment design approach which is divided into two groups, namely the intervention group and the control group. In creating a product or model, researchers use descriptive methods to collect data, while to test the effectiveness of the product, they use the analytic method.

The research and development process involves five main steps, namely: 1) information gathering, 2) product design, 3) validation by experts and revisions, 4) product or model testing, and 5) the final results of the product or model.

The reference population in this study is a mother who has given birth and is on the 14th day after delivery and provides exclusive breastfeeding to her baby. The study population is the puerperium mother who breastfed her baby in the period December 2023 to February 2024 in the working area of the Secang 2 Puskesmas which includes the fulfillment of the inclusion and exclusion criteria that have been set. The sampling technique uses random sampling. The sample of this study for each group was 23 people with a total number of samples of 46 people.

The independent variable in this study is breast care with vibration-based electric massage tools. The dependent variable in this study is milk production with baby weight indicators. Confounding variables in this study are energy intake, protein intake, fluid intake, breastfeeding frequency, and maternal psychology.

Univariate analysis aims to explain or describe the characteristics of each research variable (Notoadmodjo, 2018). Univariate analysis is carried out using a computer program to produce presentation values, minimal, maximal, and standard deviation that is presented in the frequency distribution of respondents' characteristics presented in the form of tables consisting of age, parity, work, breastfeeding frequency, and nutritional intake. Bivariate analysis aims to consider the interaction between two variables, namely the dependent variable and free variables with a degree of significance of 95% or α 0.05.

Normality testing is used to evaluate whether the data follows the normal distribution or not. In this study, the data normality test used the Shapiro-Wilk test because the number of respondents samples was less than 50. Data was normally distributed if $p > \alpha$ ($\alpha = 0.05$), while the data was not normal if $p < \alpha$ ($\alpha = 0.05$).

This research hypothesis test, to analyze the impact of breast care using tools on milk production by observing changes in the baby's weight before and after treatment in each group, the data that is normally distributed it uses the Repeated Measure Anova test followed by the HOC Pairwise Comparates Post Test. For the average difference test that is used, the t-test is not paired (independent t-test).

Linear multivariate regression analysis is carried out to determine the presence or absence of confounding variables (energy intake, protein, fluid, breastfeeding frequency, and

psychological status) simultaneously and each of the milk production based on indicators of the baby's weight. Furthermore, the researcher tested the magnitude of the effect (effect size) using Cohen's test. This study has obtained research ethics permission number 1293/EA/KEPK/2023.

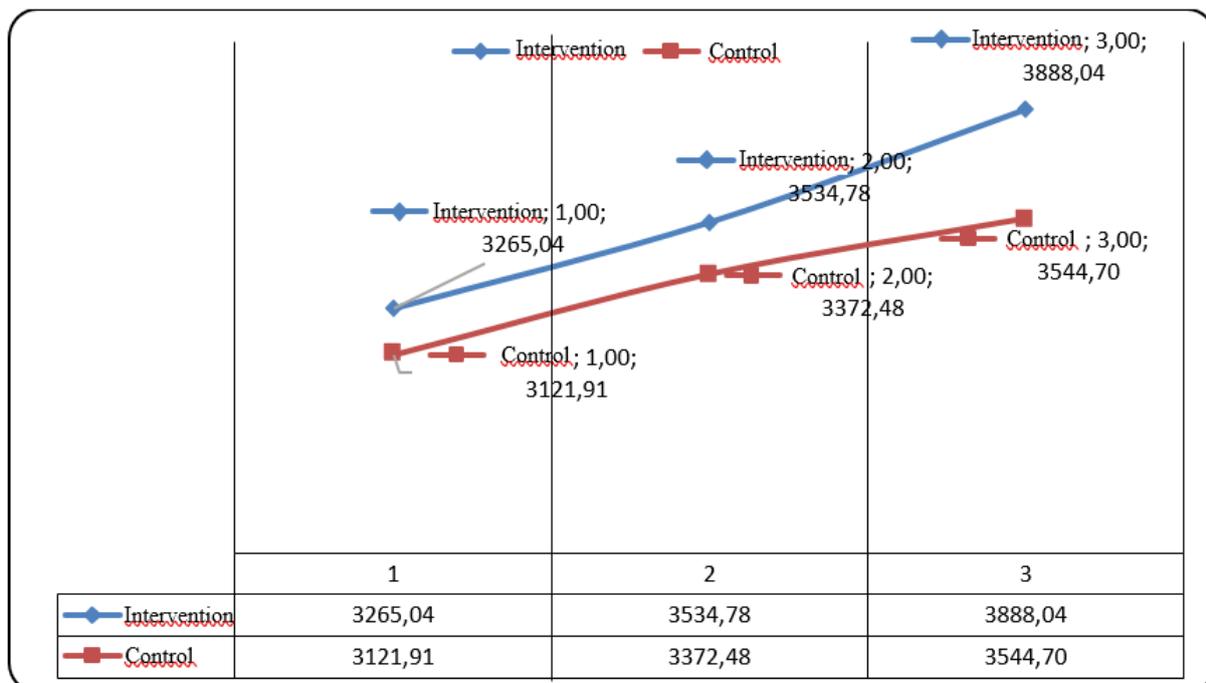
3. RESULTS AND DISCUSSION

Table 1. Results of Measurement of ASI Production Indicators of Infant Body Weight (gram) in The Intervention Group And The Control Group

Group	Measurement	n	Mean±SD	Min	Max
Intervention	Pretest (baby age 14 days)	23	3265.04±257.365	2830	3920
	Posttest 1 (baby age 21 days)	23	3534.78±283.451	3075	4210
	Posttest 2 (baby age 28 days)	23	3888.04±293.652	3340	4510
	Δ Pretest – Posttest 2		623±36,287	510	590
Control	Pretest (baby age 14 days)	23	3121.91±211.470	2710	3490
	Posttest 1 (baby age 21 days)	23	3372.48±169.031	2990	3690
	Posttest 2 (baby age 28 days)	23	3544.70±166.803	3308	3920
	Δ Pretest – Posttest 2		422,79±44.667	598	430

Table 1 In the first week the treatment of the average intervention group of the baby's body weight increased by 269.74 grams, while in the control group the average increase of 250.57gram. In the second week the treatment of the average intervention group of the baby's body weight increased 353.26 grams, while in the control group of 172.22 grams. It can be concluded that the average BB baby intervention group is higher than the baby's body weight in the control group.

To see the difference in increase that occurs in the baby's weight intervention and control groups by looking at the average weight of the baby at the first weighing of the baby's age 14 days, weighing the 2 ages of the baby 21 days, and weighing the 3 ages of the baby 28 days can be seen from the diagram below :



Based on the graph above, it is known that there is an increase in the average weight of the baby's weight in the intervention group and the control group, but in the intervention group

given breast care with vibration-based electric massage devices experienced more increases than the control group given ordinary breast care for 14 days. Therefore, the treatment of breast care with vibration-based electric massage devices affects the effect of increasing milk production with baby body weight (BW) indicators in postpartum mothers.

Table 2. Test The Baby's Weight Difference Before and After The Treatment of The Intervention Group and The Control Group (Gram)

Variable	Data	Group	
		Intervention	Control
Baby Weight	Pretest (baby age 14 days)	3265.04±257.365	3121.91±211.470
	Posttest 1 (baby age 21 days)	3534.78±283.451	3372.48±169.031
	Posttest 2 (baby age 28 days)	3888.04±293.652	3544.70±166.803
	p-value	0.000*	0.000*

Table 2 concerning the Analysis of the Effect of Breast Care with Vibration-Based Electric Massage Devices on ASI Production in Baby's Weight Obtained an average baby's BB in the intervention group with the value of day 1 of 3265.04 grams, on the 8th day 3534.78 grams, on The 15th day is 3888.04 grams. Whereas in the control group the average baby's BB value before the treatment of Ahri 1 was 3121.91 grams, the 8th day 3372.48 grams, the 15th day was 3544.70 grams. Then seen from the BB for the two groups of groups in each group of intervention and controls on day 1, day 8 and day 15 using the Repeated Measure Anovan test $p = 0,000$ so that H_0 is rejected, meaning there is a significant difference in the average weight of the baby day 1 day 1 , 8, and 15 in intervention groups and control groups. To find out the results of the measurement of time which most influences the baby's body weight, it will be continued by the Post Hoc Pairwise Comparisons test.

Table 3. Test of Measurement Time Differences Before and After Treatment of Intervention And Control Groups

Data	Group			
	Intervention	p-value	Control	p-value
Pretest- Posttest 1 (baby age 14 -21 days)	269,739	0,000	250,56	0,000*
Posttest 1- Posttest 2 (baby age 21 -28 days)	353,261	0,000	172,22	0,000*
Pretest- Posttest 2 (baby age 14 -28 days)	623	0,000	422,78	0,000*

Based on table 3 shows that the Post Hoc Statistics Test results Table 3 There is a significant difference between day 1 to day 8, and days 8-15 in the intervention group and the control group with $P = 0,000$. When viewed from the best difference in intervention the 8th - day 15, while for the control group is on days 1 - day 8.

Table 4. Test The Baby's Weight Difference Before and After Treatment Between Intervention and Control Groups (Grams)

Variable	Data	Group		p-value
		Intervention Mean±SD	Control Mean±SD	
Baby Weight	Pretest (baby age 14 days)	3265.04±257.365	3121.91±211.470	0,045*
	Posttest 1 (baby age 21 days)	3534.78±283.451	3372.48±169.031	0,023*
	Posttest 2 (baby age 28 days)	3888.04±293.652	3544.70±166.803	0,000*
	Δ Pretest – Posttest 2	623±36,287	422,79±44.667	0,000*

Table 4 in the Independent Sample T-Test test shows that in both groups 1, a 0.045 p-value means that there is a difference between the intervention group and the control group. In the two groups, 8 obtained a p-value 0.023 means there is a difference between the intervention group and the control group. In both groups, 15 obtained a p-value 0,000 means there is a difference between the intervention group and the control group. In addition, there is a difference in the average difference in the intervention group of 623 grams and the control group of 422.79 grams with a p-value of 0,000. This shows that there is a difference in the increase in infants between the intervention group and the control group. In general and the average difference of infant intervention and control of the difference is greater than 200.21 grams of the control group, it can be concluded that breast care using electric massage-based vibration-based devices is more effective in increasing milk production in postpartum mothers with a p-value of 0,000.

Table 5. Effect Size Providing Breast Care with a Vibration-Based Electric Massage Device

Group	n	Breast Milk Production Based on The Baby's Weight Indicator After Treatment	Effect size
		Mean±SD	
Intervention	23	3888.04±293.652	1,43*
Control	23	3544.70±166.803	

Table 5 shows the results of the effect size using the cohens'd formula that the provision of breast care with vibration-based electric massage tools for 14 days compared to manual breast care obtained a value of 1.43 with the range of criteria in the coohen's d formula which is 0.8-2.0 Included in the high category based on the Effect Size test classification, meaning that the provision of breast care with massage tools to milk production seen from the baby's body weight can have a large influence.

Table 6. The Results of The Analysis of Confounding Variables (Energy Intake, Protein, Fluid, Breastfeeding Frequency, And Psychological) on Milk Production Based on Baby's Weight

Variable	R	R Square	Baby Weight	
			Sig.	p-value
Energy Intake	0,298	0,089	0,785	0,568
Protein Intake			0,948	
Fluid Intake			0,153	
Breastfeeding Frequency			0,216	
Psychological			0,569	

Table 6 shows that the statistical test of the confounding variable uses multiple linear regression tests that the confounding variable of energy intake, protein, fluid, breastfeeding frequency, and psychological status simultaneously or each does not contribute to the production of breast milk based on the baby's weight indicator, this is indicated by The value of $P > 0.05$, then H_0 is accepted which has the meaning of an increase in milk production based on the baby's BB indicator in nursing mothers is not influenced by confounding variables. And based on the R Square value of 0.089, this illustrates that the effect of the confounding variable simultaneously on ASI production is 8.9%.

DISCUSSION

Analysis of Breast Care with Vibration-Based Electric Massage Tools on Breast Milk Production With Baby Weight Indicators

The provision of breast care with vibration-based electric massage tools given for 14 days to 23 respondents can affect the production of milk assessed from the baby's weight indicator. Assessment of milk production can refer to the amount of breast milk produced by the mother's breasts. ASI that is produced and stored in the storage of milk is then estimated to be the number by looking at how much breast milk is drunk by the baby. The baby's weight is influenced by lactose in digestion which is converted into glucose and galactose, which is then used as a source of energy and calories. Therefore, one way to assess milk production is through observation of the baby's weight.

The results of the statistical test in the intervention group by giving breast care with vibration-based electric massage tools are very significant to the baby's body weight starting on the 1st to the 15th day where the value of $p=0.000$. This means that there is an influence of breast care provision with vibration-based electric massage tools on milk production. Whereas standard breast care also has an influence on breast milk production where the value of $p=0,000$.

This study was conducted on puerperal mothers who breastfed the baby on the 14th day or 2 weeks of the puerperium on their babies were weighted before giving interventions, namely the day-1 intervention, the 7th day of the intervention and after the administration was on the 15th day. Selection at the age of 2 weeks in the first days after giving birth, newborns often lose about 10% of the initial body weight. Therefore research avoids bias that is likely to make this research there are other factors of the baby's weight. This weight loss is common and is caused by things like urine excretion and feces. However, the child's weight will return to his birth weight after approximately 2 weeks (Wulandari, Wijayanti, & Sunarjo, 2022).

Increased baby's weight will increase by about 125 grams every week (Wulandari, Wijayanti, & Sunarjo, 2022). In this study, there was an increase every week of more than 125 grams. Can be seen from the average obtained in the baby's body weight in the intervention group before treatment of 3265.04gram to 3888.04 grams with an average difference in the baby's weight gain of 623 grams while in the average control group of the baby's body weight of 3121, 91 grams to 3544.70 grams and has an average difference in an increase of 422.79 grams. From the results of the statistical test the average difference in difference in the results of p -value 0,000 can be interpreted that breast care with vibration-based electric massage devices is more effective for increasing milk production, generally visible and the difference between infant intervention and the difference in the difference is greater 200.21 Gram where. The intervention group is higher than the control group.

Breast care is one of the appropriate non-pharmacological efforts to carry out because it provides stimulation to the breasts to increase breast milk production. This breast care is achieved by sending signals to the pituitary to release the hormones oxytocin and prolactin (Anshari, 2023). In line with Rohani Siregar's research that there is an influence of breast care on breast milk production in postpartum mothers with a p value of 0.0001 (Siregar, 2023).

Breast massage tools are breast care that is developed using electronic components that are put together in the form of displaying which is flowed by an electric current. The breast care process is certainly inseparable from the development of technology in the health sector so there will be the latest innovation in the development of breast care. So that every mother postpartum is able to do breast care independently. Breast care done using tools one of them using a device based on vibration can increase milk production.

The mechanism of vibration -based electric massage tools that provide a vibrational effect to stimulate the hormone prolactin and oxytocin (Nasution et al., 2018). In the prolactin hormone when the vibration attaches to the breast around the nipple and mammae areola will cause stimulation in the sympathetic nerves in that place. Stimulation will be forwarded to the brain through anterior pituitary to remove the hormone prolactin which functions to produce

breast milk. In addition, stimuli will also be forwarded to the brain through posterior pituitary to remove the hormone oxytocin into the mother's bloodstream. The hormone oxytocin functions to encourage small muscles around the milk-producing cells (alveoli) to contract. Alveoli is a part that contains cells that secrete milk. Each alveoli is coated with cells that secrete milk called ACINI. ACINI secretes the factors of the blood that are important for the formation of milk. Around each alveoli there are myoepithelial cells that are sometimes called basket cells (basket cells) or spider cells (spider cells). If this cell is stimulated by oxytocin, it will contract. This contraction will push milk out of the alveoli through the lactiferous duct to the Lactiferous Sinus where the ASI is stored (Estiwidani, 2018).

The Effect of Confounding Variable on Milk Production with Weight Indicators Food Intake (Energy, Protein, and Fluid)

In this study, food intake /food intake in both groups both from energy, protein and fluid intake has a homogeneity where $p\text{-value} > 0.05$, where energy intake $p = 0.223$, protein intake $p = 0.088$, and fluid $p = 0.221$ so it is known that from energy intake, protein and fluid the same or homogeneous. Further analysis shows that there is no relationship between energy, protein, and fluid intake and milk production based on the baby's weight indicator with $p\text{-value} > 0.05$ for energy intake $p = 0.785$, protein intake $p = 0.948$, and fluid intake $p = 0.153$. So it can be seen that the increase in milk production based on the baby's weight indicator is not affected by energy, protein and fluid intake. This is in line with Nurul Asikin's research that there is no relationship between food intake and mother's milk production (Asikin, Agrina, & Rismadefi Woferst, 2023).

Besides that, in line with other studies that there is no correlation between energy intake, protein against milk production (Butts et al., 2018). But it is different from the research conducted by Lien Meilya that there is a significant relationship between the intake of protein eaten by the mother to the production of mother's milk, where the protein contained in the mother's milk is used for child growth (Prastiyaning & Nuryanto, 2019). In line with research conducted by Niar, a mother with good fluid intake has the opportunity to be 4.219 times to have good milk production (Niar, Dinengsih, & Siauta, 2021).

Breastfeeding Frequency

Further analysis of this study does not indicate the relationship between breastfeeding frequency and milk production based on baby weight indicators in all mothers breastfeeding $0.216 > 0.05$ so it can be known to increase milk production based on the baby's weight indicator in nursing mothers not affected by frequency breastfed. This explains that although mothers breastfeed frequently, they do not have a significant impact on milk production because milk production is influenced by many factors besides breastfeeding frequency.

In contrast research conducted in the outpatient polyclinic of the Kediri Baptist Hospital, it shows that the frequency of breastfeeding affects the production of breast milk in postpartum mothers (Syari, Arma, & Mardhiah, 2022) Supported research conducted by Ariani, et al., (2021), explained that there is a relationship between the frequency of breastfeeding and milk production in breastfeeding mothers (Ariani, et al., 2021). In addition, based on research Maharlika & Yuliana, (2023) show that there is a relationship between breastfeeding frequencies with breast milk production with the average frequency of breastfeeding 10-12 times a day (Maharlika & Yuliana, 2023).

Psychological

Further analysis in this study produced assumptions that there was no relationship between psychological status and milk production based on baby weight indicators with a value of $0.569 > 0.05$ so it could be said to be an increase in milk production based on baby weight

indicators not influenced by psychological status. The results of the research are in line with Dewia, Kusumastuti, & Astutic, (2023), research that psychological status does not affect breastfeeding to infants by postpartum mothers (Dewia, Kusumastuti, & Astutic, 2023). However, this is the opposite of the results of sustainable research that psychological factors are associated with milk production in postpartum mothers. Psychological factors are one that can affect milk production. When the mother's psychological condition is in good condition, the smooth muscle around the breast glands will stimulate the release of the hormone oxytocin and prolactin to launch the expenditure of milk and increase milk production (Lestari et al, 2022).

This research is not free from several limitations and weaknesses, including This study did not use biomarkers that support breast milk production. No temperature sensor regulates heat to monitor the existing heat temperature. The battery life is still low, so it requires recharging the battery several times.

4. CONCLUSION

Development of vibration-based electric massage devices has been created and can increase the production of breast milk seen from the baby's body weight by doing a pretest and posttest where there are significant differences in the average weight of the baby on day 1, 8, and 15 in the intervention group and the control group by getting p-value = 0,000. Electric massage-based vibrational massage tools are effective for increasing milk production seen from the baby's body weight by getting p-value = 0,000. Future researchers can expand the scope of the research by incorporating additional variables and expanding the population and sample coverage so that the research results become more diverse.

REFERENCES

- Anshari, Z. (2023). Analysis of Breast Care Factors for Increasing Breast Milk Production In Pagar Merbau Public Health Community Center, Medan. *Buletin Farmatera*, 8(1), 9-17. <https://doi.org/10.30596/bf.v8i1.5522>
- Ariani, P., Ariescha, P. A. Y., Sari, N. M., & Terulin, A. (2021). Hubungan Umur, Paritas Dan Frekuensi Menyusui Dengan Produksi Air Susu Ibu (ASI). 5(2), 26–30. Retrieved from <https://journal.universitaspahlawan.ac.id/index.php/doppler/article/view/2295>
- Asikin, N., Agrina, A., & Woferst, R. (2023). Hubungan Pola Makan Dengan Produksi ASI Pada Ibu Menyusui. *Jurnal Ilmu Kedokteran dan Kesehatan Indonesia*, 3(1), 13-27. <https://doi.org/10.55606/jikki.v3i1.1010>
- BPS. (2022). *Profil Kesehatan Ibu dan Anak 2022*. Jakarta: Badan Pusat Statistik.
- Butts, C. A., Hedderley, D. I., Herath, T. D., Paturi, G., Glyn-Jones, S., Wiens, F., Gopal, P. (2018). Human Milk Composition And Dietary Intakes Of Breastfeeding Women Of Different Ethnicity From The Manawatu-Wanganui Region Of New Zealand. *Nutrients*, 10(9), 1231. <https://doi.org/10.3390/nu10091231>.
- Chomaria, N. (2020). *Filosofi Payudara dan ASI*. Jakarta: Elex Media Komputindo.
- Cynthia, C., Suryawan, I. W. B., & Widiassa, A. M. (2019). Hubungan asi eksklusif dengan kejadian stunting pada anak usia 12-59 bulan di rsud wangaya kota denpasar. *Jurnal kedokteran meditek*, 25(1), 29-35.
- Dewia, A. P. S., Kusumastuti, & Astutic, D. P. (2023). Hubungan Perilaku Menyusui, Pola Hidup Sehat Dan Kondisi Kesehatan Dengan Pemberian ASI. 4(1), 154–160. <https://doi.org/10.26751/jikk.v14i1.1629>
- Emilda, S. (2022). Pengaruh Perawatan Payudara Terhadap Kelancaran Pengeluaran Asi Pada Ibu Postpartum Di Pmb Misni Herawati Palembang Tahun 2022. *Jurnal Kesehatan Dan Pembangunan*, 12(23), 100–107.

- Estiwidani, D. (2018). Aplikasi Mangkok Getar Sebagai Upaya Pencegahan Pembengkakan Payudara Ibu Menyusui. Yogyakarta: Poltekkes Kemenkes Yogyakarta.
- Handayani, E., & Pujiastuti, W. (2016). *Asuhan Holistik Masa Nifas dan Menyusui*. Yogyakarta: Trans Medika.
- Hasan, M., & Saputra, S. D. (2023). Edukasi Pentingnya Pemberian ASI Eksklusif pada Bayi di Puskesmas Gambesi. *Jurnal Surya Masyarakat*, 5(2), 208-213. <https://doi.org/10.26714/jsm.5.2.2023.208-213>
- Hipson, M., Handayani, S., & Erwanda, E. (2023). Hubungan Perawatan Payudara Pada Masa Kehamilan Dengan Kelancaran Pengeluaran Asi Pada Ibu Nifas. *Jurnal'Aisyiyah Medika*, 8(2), 274-282
- Katuuk, M., & Kundre, R. (2018). Hubungan pengetahuan Perawatan Payudara Dengan Kelancaran Produksi ASI Pada Ibu Post Partum Di Ruang Dahlia RSD Liun Kendaghe Tahuna Kabupaten Kepulauan sangihe. *Jurnal Keperawatan*, 6(1), 1-8. <https://ejournal.unsrat.ac.id/index.php/jkp/article/view/25180>
- Lestari, P., Fatimah, F., Ayuningrum, L., Herawati, H. D., & Afifaturrohmah, N. (2022). Influence Oxytocin Massage on Reduce Lactation Problems and Support Infants Growth. *Open Access Macedonian Journal of Medical Sciences*, 10(T8), 81-85. <https://doi.org/10.3889/oamjms.2022.9487>
- Linda, E. (2019). *ASI EKSKLUSIF*. Cilacap: Yayasan Jamiul Fawaid.
- Louis, S. L., Mirania, A. N., & Yuniarti, E. (2022). Hubungan Pemberian ASI Eksklusif dengan Kejadian Stunting pada Anak Balita. *Maternal & Neonatal Health Journal*, 3(1), 7–11. <https://doi.org/10.37010/mnhj.v3i1.498>.
- Maharlika, R., & Yuliana, D. (2023). Hubungan Teknik Dan Frekuensi Menyusui Dengan Kecukupan Asi Pada Bayi Di Ruang Delima RSUD Dr. H. Abdul Moeloek Provinsi Lampung Tahun 2023. *Nusantara Journal of Multidisciplinary Science*, 1(3), 399-411.
- Mukarramah, S., Nurdin, S. S. I., & Ahmad, Z. F. (2021). Pengaruh Perawatan Payudara Terhadap Kelancaran Produksi Asi Pada Ibu Postpartum Di Puskesmas Kassi-Kassi, Makassar. *Media Keperawatan: Politeknik Kesehatan Makassar*, 12(1), 11-16.. <https://doi.org/10.32382/jmk.v12i1.2143>
- Nasution, S. S., Erniyati, E., & Aizar, E. (2018). The effectiveness of DC Motor Vibrilatory Stimulus (DMV) among Postpartum Women on Giving Breast Milk. *Open access Macedonian journal of medical sciences*, 6(12), 2306–2309. <https://doi.org/10.3889/oamjms.2018.436>
- Niar, A., Dinengsih, S., & Siauta, J. (2021). Factors Affecting the Production of Breast Milk Breastfeeding Mother at Harifa RSB, Kolaka District Southeast Sulawesi Province. *Jurnal Kebidanan Midwiferia*, 7(2), 10–19. <https://doi.org/10.21070/midwiferia.v7i2.1288>
- Notoadmodjo, S. (2018). *Metodologi Penelitian Kesehatan*. Jakarta: Rineka Cipta
- Prastiyani, L. M. M., & Nuryanto, N. (2019). Hubungan Antara Asupan Protein Dan Kadar Protein Air Susu Ibu. *Journal of Nutrition College*, 8(4), 246–253.
- Rahayuningsih, T. (2020). Perawatan Payudara Dan Pijat Oksitosin. *Yogyakarta: Gosyen*.
- Pratama, E. R., Pitaloka, M. F., Wulandari, M., Annisa, N., Pratiwi, S. A., Suherni, W., & Indri, Y. (2023). Perawatan Payudara (Breast Care) di Ruang Rawat Kebidanan Rumah Sakit Islam Yarsi Bukittinggi. *Altafani: Jurnal Abdimas*, 1(1), 12-15.
- Siregar, R. (2023). Pengaruh Breast Care Terhadap Peningkatan Produksi ASI Pada Ibu Post Partum. *JURNAL ILMIAH OBGIN: Jurnal Ilmiah Ilmu Kebidanan & Kandungan*, 15(3), 473–479.
- Syari, M., Arma, N. ., & Mardhiah, A. . (2022). Faktor yang Mempengaruhi Produksi ASI Pada Ibu Menyusui. *Maternity and Neonatal : Jurnal Kebidanan*, 10(01), 1–9. <https://doi.org/10.30606/jmn.v10i01.1306>
- Wahyuni, E., Andriani, L., Yanniarti, S., & Yorita, E. (2022). *Perawatan Payudara (Breast*

Care) untuk Mengatasi Masalah Puting Susu. Penerbit NEM

WHO. (2022). *World health statistics 2022: monitoring health for the SDGs, sustainable development goals*. World Health Organization

Wulandari, S. S., Wijayanti, K., & Sunarjo, L. (2022). *Pengaruh Pemberian Dendeng Daun Singkong Terhadap Kadar Hormon Prolaktin Dan Produksi ASI Pada Ibu Menyusui*. Magelang: Pustaka Rumah Cinta.

Jurnal Info Kesehatan

Vol. 22, No. 2, June 2024, pp. 347-356

P-ISSN 0216-504X, E-ISSN 2620-536X

DOI: [10.31965/infokes.Vol22.Iss2.1585](https://doi.org/10.31965/infokes.Vol22.Iss2.1585)Journal homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>**RESEARCH****Open Access****Evaluation of Hematotoxicity in Female Wistar Rats Following Sub-Acute Inhalation Exposure to Polyethylene Microplastic****Hikmawan Wahyu Sulistomo^{1a*}, Anisa Setyowati^{2b}, Melani Chysti Situmorang^{2c}, Ita Sulistiani^{2d}, Dewi Azar Nuria Wardani^{2e}, Kharisma Ciptaning Gusti^{2f}, Nurdiana^{1g}, Ihda Dian Kusuma^{3h}, Bambang Rahardjo⁴ⁱ, Subandi Reksohusodo^{4j}**¹ Department of Pharmacology, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia² Master Program of Midwifery, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia³ Department of Anatomical Pathology, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia⁴ Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia^a Email address: hikmawan_ws@ub.ac.id^b Email address: anisacik87@gmail.com^c Email address: melanichristysitumorang@gmail.com^d Email address: itabeny171@student.ub.ac.id^e Email address: dewiazar20@student.ub.ac.id^f Email address: kharismacipta@student.ub.ac.id^g Email address: nurdianafarmako.fk@ub.ac.id^h Email address: ihdadk.pa.fkub@ub.ac.idⁱ Email address: bar_feto@yahoo.com^j Email address: desobg@gmail.com

Received: 7 June 2024

Revised: 29 June 2024

Accepted: 30 June 2024

Abstract

Polyethylene (PE) becomes a source of microplastics that can be widely distributed through the digestive and respiratory systems. However, its effects on blood cells are still being investigated. This study aims to analyze the impact of Polyethylene Microplastic (PE-MPs) exposure on the blood of female rats, including erythrocytes, leukocytes, and platelets. This study used female Wistar rats, which were divided into control and PE-MP groups. PE-MP was administered via whole-body inhalation at a concentration of 15 mg/m³ for 4 hours daily for 28 days. The absorption of plastic particles detected in the human bloodstream is likely to occur through mucosal contact (either through ingestion or inhalation). After the exposure period, the rats were euthanized to collect blood samples through the heart. A complete blood count was performed using an automatic hematology analyzer, and blood morphology was analyzed using thin blood smears. This study used the Mann-Whitney test. PE-MP exposure increased erythrocyte and platelet counts without a corresponding rise in leukocytes. Erythrocytes showed abnormal morphology (12.73% with ovalocytes and tear-shaped cells). Erythrocyte indices (MCV, MCH, MCHC) showed no significant differences. Platelet count rose by 1.7% (p-value= 0.017). Leukocyte and neutrophil counts were lower (0.84 and 0.94 times lower, respectively), while lymphocytes and monocytes were higher (1.03 and 1.61 times higher, respectively) in the PE-MP group compared to controls. The neutrophil-to-lymphocyte ratio did not differ significantly. PE-MP exposure in rats disrupts blood parameters, altering erythrocyte morphology and increasing platelet counts. Potential causes include oxidative stress, immune responses, and compensatory mechanisms. Study limitations include a small sample size and exclusive focus on inhalation exposure. Integrating multiple exposure routes (inhalation, ingestion, dermal) could offer a broader view of microplastic impacts. Future research with larger samples, diverse doses and durations, and exploration of additional markers or organ-specific effects is crucial for understanding PE-MP toxicity in real-world scenarios.

Keywords: Microplastic, Polyethylene, Inhalation, Blood, Toxicity.***Corresponding Author:**

Hikmawan Wahyu Sulistomo

Department of Pharmacology, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia

Email: hikmawan_ws@ub.ac.id

©The Author(s) 2024. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

1. INTRODUCTION

According to UN Comtrade data in 2018, the five major countries exporting plastic waste to Indonesia are the United States, Germany, the Marshall Islands, the Netherlands, and Australia. Approximately 9 million tons of plastic waste are produced annually in Indonesia, accounting for 15% of the national total. Plastic waste often ends up in rivers, comprising 20% to 38% of debris collected from water bodies in cities, with recycling efforts predominantly informal (15%). Plastic waste is frequently purchased by brokers or small traders. In East Java, Bangun and Tropodo villages in Sidoarjo Regency receive over 50 tons of plastic waste daily. In Tropodo, Sidoarjo Regency, East Java, about 50 tofu factories use plastic waste as fuel for their furnaces (Petrlik et al., 2019). Overall, this issue highlights serious challenges in plastic waste management in Indonesia, with significant environmental and public health impacts. Plastic waste inevitably releases into the environment, degrading mechanical and physicochemical characteristics, leading to the formation of plastic fragments. These fragments are termed microplastics (MPs) if their size is below 5 mm (Stock et al., 2021). MPs spread through air, water, and soil and can enter human bodies through ingestion and respiration. Exposure to MPs can cause toxicity in humans through inflammation and the accumulation of reactive oxygen species (ROS) (Enyoh et al., 2019).

Free radicals from MP exposure can cause damage to red blood cells and a decrease in erythrocytes (Franco et al., 2019). The high concentration of transition metal ions, oxyhemoglobin, and molecular oxygen in erythrocytes makes these cells very susceptible to oxidative damage (Qasim & Mahmood, 2015). The toxic effects of MP exposure also cause immune responses and reproductive and developmental toxicity (Li et al., 2023). Additionally, studies on MP exposure in fish cells in vitro reveal that MPs alter leukocyte and B cell activity (Zwollo et al., 2021; Espinosa et al., 2018). A recent study (Wu et al., 2023) showed that microplastics were found in thrombi and could increase the risk of hemostasis disorders. The impact of MP use on female mammals, whose blood is predominantly affected, remains uncertain. Microplastics can enter the fetus through the placenta, and babies can also ingest particles through breast milk.

In this study, we investigated the impact of polyethylene MP in vivo. Polyethylene Microplastics (PE-MPs) were used as a representative plastic test material because it is the most common type of plastic in the world (Rodrigues et al., 2019). Polyethylene plastic is often used as single-use plastics (SUP), such as plastic bags, plastic bottles, houseware, and other items (Mentes et al., 2023). PE has the ability to change easily during processing, providing relatively longer chain length, density, and crystallinity, allowing PE products to have properties tailored for various applications. High-density polyethylene (HDPE) and low-density polyethylene (LDPE) are some types of PE plastics. HDPE is lightweight and has good tensile strength, while LDPE has good chemical resistance (Kumar et al., 2022). Research shows that PE MPs are more easily absorbed and accumulated in the body (Yang et al., 2022).

In this study, PE-MPs were administered via whole-body inhalation to female rats. Female rats were chosen because they have a distinctive estrous cycle, making them ideal for studying the impact of environmental toxins on reproductive and endocrine systems. Additionally, this choice helps avoid bias in the study. Microplastics were administered at multiple doses for 28 days to assess their toxicity in the blood, particularly on red blood cells/erythrocytes, white blood cells/leukocytes, and platelet/thrombocyte profiles. The broader implications of our research findings highlight significant public health concerns. Since polyethylene microplastics are prevalent in the environment and commonly used in single-use plastics, our study suggests that chronic exposure to these particles could adversely affect blood health. This raises concerns about similar impacts on human health, especially for populations with high exposure to

airborne microplastics. Understanding the toxicological effects of PE-MPs can inform regulatory policies and promote the development of safer plastic alternatives, ultimately contributing to improved public health outcomes by mitigating the risks associated with microplastic exposure.

2. RESEARCH METHOD

This study is a true experimental research with a post-test-only control group design conducted in 2023 at the Pharmacology and Biochemistry Laboratory of the Faculty of Medicine, Brawijaya University. The experimental animals used in this study were female Wistar rats (*Rattus norvegicus*) aged 12-15 weeks. The rats were housed and fed standard food and water. They were divided into Control and Polyethylene Microplastics (PE-MPs) groups, consisting of 5 rats in the Control group and 6 rats in the PE-MP group, with the difference in sample size due to dropouts. The determination of sample size in this study followed the "Resource Equation Approach" described in the journal (Arifin and Zahiruddin, 2017). All rats were housed in standard animal facilities at Brawijaya University, and every effort was made to minimize the number of animals used and their suffering. Only rats with regular estrous cycles were included in the study. These animals were synchronized to the same estrous cycle phase. The estrous cycle influences various physiological processes, including immune function and metabolism. By selecting rats with regular cycles and exposing them at the same phase, this study controlled hormonal fluctuations, ensuring that any observed effects on blood toxicity could be directly attributed to PE-MP exposure rather than hormonal variations, thus reducing potential confounding variables related to hormonal changes. The entire experimental protocol was approved by the Animal Care and Use Committee of Brawijaya University (Approval No. 254/EC/KEPK/08/2023).

Polyethylene Microplastics (PE-MP) exposure was conducted on rats in the estrous phase. PE-MP exposure was done using a blower containing PE powder. The average size of PE-MPs in this study was 252.76 ± 4.401 micrometers, obtained from a fine powder form of PE plastic purchased from CV. Subur Kimia Jaya (lot number 2110052) and analyzed at the Ecoton Laboratory in Gresik. The exposure was conducted via whole-body inhalation for 4 hours daily for 28 days with a PE-MP concentration of 15 mg/m³ in a 60x60x60 cm inhalation chamber. The exposure dose and duration followed the limits allowed by the Occupational Safety and Health Administration (OSHA) (Cary, et al., 2022).

Blood Collection and Hematology Analysis. Blood collection was done after 28 days of PE-MP exposure by extracting 0.5 ml of blood from the rat's heart. The rats were euthanized via deep anesthesia using diethyl ether for 1 minute to help minimize the stress response in the animals. Stress can significantly alter blood parameters, such as increasing cortisol levels and affecting white blood cell counts. By reducing stress, the anesthetic approach helps maintain the validity of the measured blood parameters. Blood was then collected in vacutainer tubes containing EDTA (Ethylene Diamine Tetraacetic Acid). The following parameters were analyzed using an automatic hematology analyzer (ABX Micros 60 Hematology Analyzer): total red blood cell/erythrocyte count, erythrocyte indices, platelet count, and leukocyte count.

Blood was thinly smeared on glass slides, and its morphology was analyzed to identify changes in erythrocyte shape. To determine the impact of PE-MP exposure as a xenobiotic agent triggering the immune system, leukocyte analysis was performed using an Olympus® CX-21 microscope with 400x magnification for each group.

Data obtained in this study were statistically analyzed using the Mann-Whitney test with GraphPad Prism® 5 software at a significance level of 0.05 ($p < 0.05$). Data are presented as mean \pm S.E.

3. RESULTS AND DISCUSSION

Polyethylene Microplastic (PE-MP) Exposure may increase the number of erythrocytes. We analyzed erythrocyte count, hematocrit, and hemoglobin levels to determine the effect of PE-MP exposure on the number and oxygen-binding ability of erythrocytes. As shown in Figure 1, the number of erythrocytes in the PE-MPs group is 1.07 times higher than control. Hematocrit and hemoglobin levels in the PE-MP group also show 1.08 and 1.09 times higher than the control, respectively, even though not statistically different. Subsequently, we were interested in learning the size and volume of the variance of erythrocytes. Then, we performed The Red Cell Distribution Width (RDW) test. In this study, the PE-MP group shows no different variations compared to the control group.

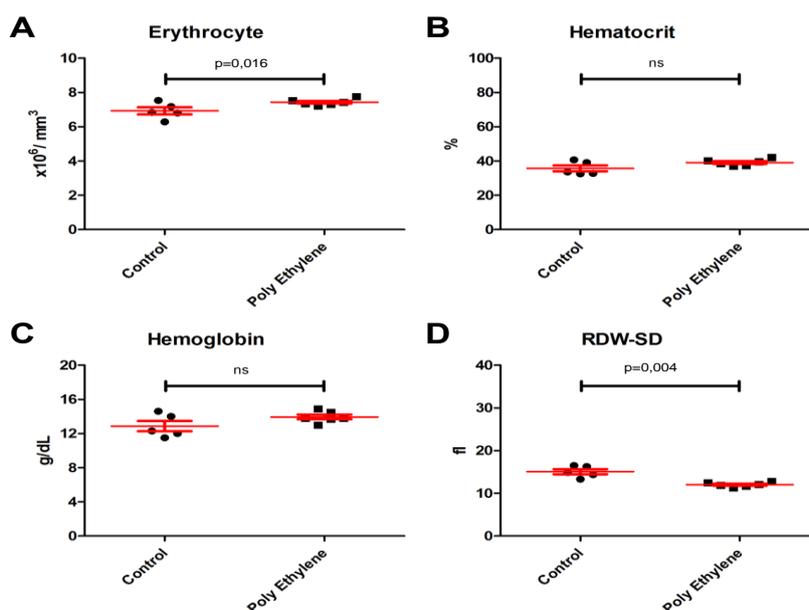


Figure 1. Polyethylene microplastic (PE-MP) exposure may increase erythrocyte counts, hematocrit, and hemoglobin level but not red cell distribution width. (A) Erythrocyte count (B) Hematocrit test (C) Hemoglobin level (D) Red cell distribution width- standard deviation. *ns*, insignificant.

Polyethylene Microplastic (PE-MP) Exposure alters the morphology of erythrocytes. To identify alterations in the form of erythrocytes, we underwent a blood smear. The control group displays erythrocytes with a round form shaped like a disc. Conversely, PE-MP group displays 12.73% of abnormal erythrocyte such as ovalocytes and teardrop-like cells (Figure 2).

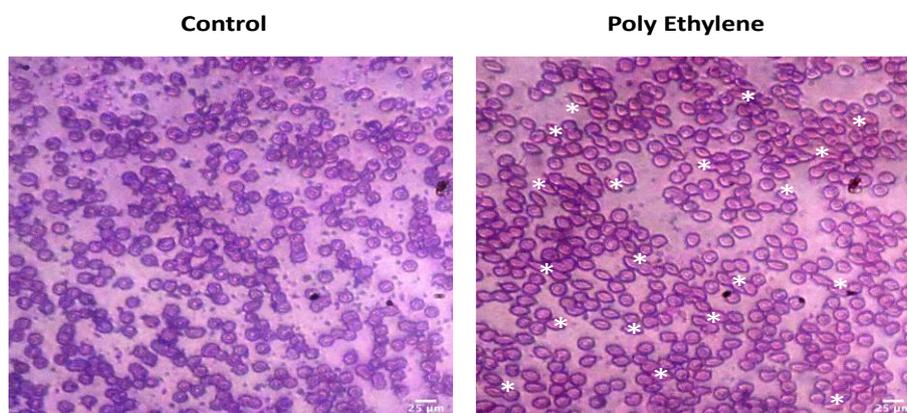


Figure 2. A blood smear of the PE-MPs group reveals a large number of erythrocytes with abnormal morphology. Asterisk (*) means cells with abnormal morphology.

Polyethylene Microplastic (PE-MP) Exposure does not affect the measurement of the erythrocyte index. To re-check the volume and concentration amount of hemoglobin in erythrocytes, we performed an erythrocyte index analysis consisting of Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin, and Mean Corpuscular Hemoglobin Concentration (MCHC). There are no significant differences between groups (Figure 3).

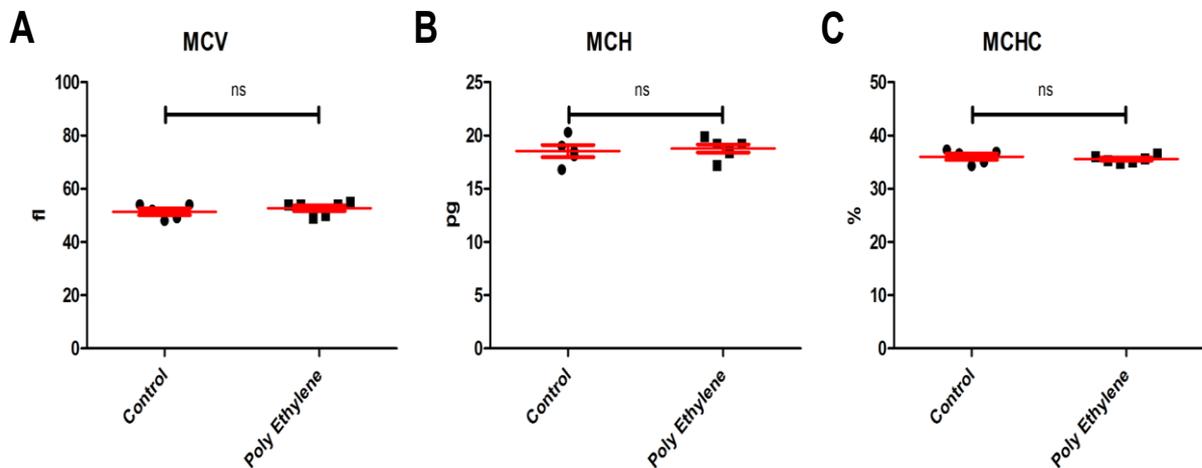


Figure 3 Polyethylene Microplastic Exposure may result in a slight increment of erythrocyte index. (A) Mean Corpuscular Volume (B) Mean Corpuscular Hemoglobin (C) Mean Corpuscular Hemoglobin Concentration. *ns*, insignificant.

Polyethylene Microplastic (PE-MP) Exposure enhances the number of thrombocytes. To identify the blood clotting risk, we examine the thrombocyte count. As shown in Figure 4, the number of thrombocytes increases by 1.7% after exposure to PE-MPs (p -value= 0.017).

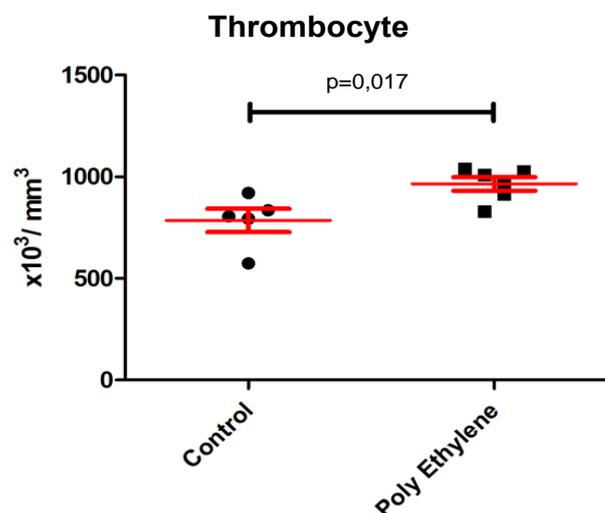


Figure 4. Polyethylene microplastics increase thrombocyte count.

Polyethylene Microplastic (PE-MP) Exposure does not affect the leukocytes. To know about the effect of PE-MP exposure as a xenobiotic agent triggers the immune system, we underwent leukocyte analysis (Figure 5). Leukocyte and Polymorphonuclear leukocyte/neutrophil counts in the PE-MP group tend to be lower than in control groups (0.84 and 0.94 times lower, respectively). In contrast, mononuclear leukocyte count, such as lymphocyte and monocyte in the PE-MP group, show a higher amount than the control. More specifically, the mononuclear leukocyte count of the PE-MP group displays 1.03 and 1.61 times greater than the control. The neutrophil-lymphocyte ratio is conducted to evaluate inflammatory responses. We found that the ratio between groups was quite similar.

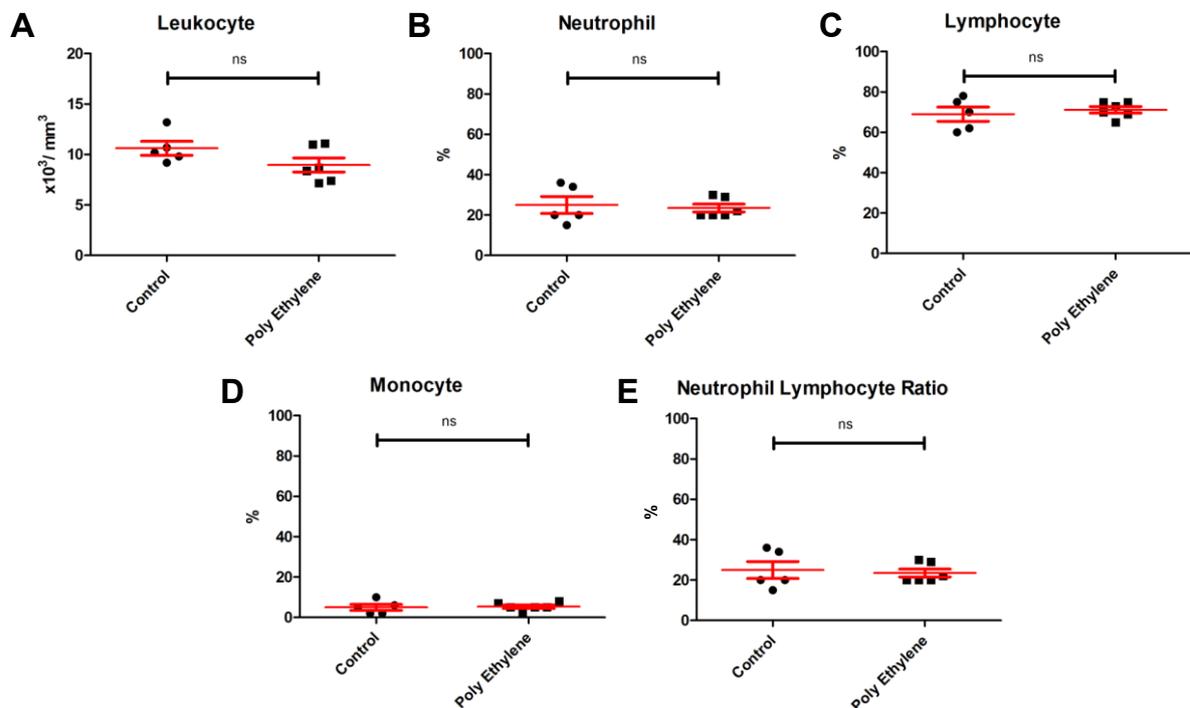


Figure 5. Leukocytes counts are unaffected by exposure to Poly Ethylene microplastics. (A) Leukocyte count (B) Neutrophil count (C) Lymphocyte count (D) Monocyte count (E) Monocyte count (F) Neutrophyl/ Lymphocyte ratio. *ns*: not significant.

The studies on how microplastics, especially PE-MPs, affect the human body are still in the exploratory stages. Plastic particles can enter the human body through the consumption of contaminated food and water supplies or by inhaling plastic particles in the air originating from synthetic textiles and polluted outdoor air. Although the skin membrane is too fine to be penetrated by plastic particles, nanoplastics can penetrate wounds and weaken the skin barrier, either directly or indirectly (Yee et al., 2021). This research demonstrates PE-MP exposure to blood cell parameters using a female rat model. The initial analysis found a significant increase in erythrocytes, commonly referred to as red blood cell count, after exposure to microplastics. Additionally, the results indicate that although hematocrit and hemoglobin levels did not significantly differ between groups, it is noteworthy that PE-MP treatment resulted in higher hematocrit and hemoglobin concentrations compared to the control. The high erythrocyte count in this study may be related to the model's compensatory ability to exposure. Microplastics can impact erythrocyte deformability and oxygenation, leading to low oxygen status in the body (Nader et al., 2019). Perceived oxygen deficiency can trigger the release of erythropoietin from the kidneys, stimulating the bone marrow to produce more erythrocytes for adequate reoxygenation processes (Arias et al., 2024; Lee et al., 2019). On the other hand, the high hemoglobin concentration observed may be due to microplastic-mediated ROS-induced erythrocyte hemolysis.

Polyethylene Microplastic (PE-MP) Exposure may increase the number of erythrocytes, the morphology of erythrocytes, the measurement of the erythrocyte index, and the number of thrombocytes, affect the leukocytes. Intoxicated erythrocytes showing abnormal morphology may reflect pre-apoptotic erythrocyte death, as seen in fish experiments (Hamed et al., 2021). These findings are partially confounded by previous studies stating that microplastic exposure

for over a month can inhibit hematopoiesis, causing erythrocyte morphological changes (poikilocytosis) and inducing eryptosis through cellular reactive oxidative stress and inflammation, which, through these mechanisms, are expected to decrease erythrocyte lifespan and count (Abdel-Zaher et al., 2023; Hamed et al., 2021; Rajendran & Chandrasekaran, 2023).

This study also shows that platelets experienced a significant increase in PE-MP exposure compared to the control. This somewhat aligns with other research where microplastics increase the risk of thrombosis, which cannot occur without an increase in platelet count (Lett et al., 2021). Inflammation and response to chemicals can trigger thrombocytosis (Schafer, 2001).

Plastic particles have various excretion pathways from the human body, including elimination through the kidneys, bile, or accumulation in organs like the liver, spleen, or other organs. Various factors such as particle size, shape, surface, chemistry, and charge influence their interaction with biological systems, including the formation of a protein layer on the particle surface. Blood, as the primary transport medium in the body, allows direct sampling without contact with plastic materials, making it an ideal matrix for monitoring plastic particles in the human body (Leslie et al., 2022).

No significant statistical differences between groups were found in leukocyte analysis. Interestingly, polymorphonuclear leukocytes (PMNs) showed a decreasing trend while mononuclear leukocytes (MNs) showed the opposite trend. Lead poisoning shows a similar “right shift” leukocyte analysis (Chwalba et al., 2018). One indicator of inflammation is the neutrophil-to-lymphocyte ratio. However, the overall estimated group ratio is still less than 1, indicating a decrease in neutrophil count in the blood, thus reducing the ability for an appropriate immune response (Buonacera et al., 2022).

The potential internalization and clearance mechanisms of microplastics in the lungs involve lung lining fluid (surfactant and mucus) reducing the likelihood of microplastic transfer. Particles <10 µm are cleared by mucociliary action, while particles <1 µm are absorbed through the epithelium and can also penetrate the thin lung lining fluid and contact the epithelium, circulating and metastasizing via diffusion or active cellular uptake. Surface charge and molecular surface interactions of different microplastics affect immune cell clearance. For macrophages, positively charged MPs and coated MPs (combined with proteins or other substances) are more likely to interact with cell membranes. Other immune cells are also involved in defense against MPs, such as antigen presentation by dendritic cells and trapping and phagocytosis of MPs through NET release by neutrophils, leading to immune activation (Yang et al., 2022).

The differences in results compared to previous studies are thought to be due to the relatively low microplastic exposure concentration, which is 15 mg/m³. As suggested by many studies, differences in size, dose, and exposure time to MPs can produce different defense mechanisms and toxicity effects for different organs and systems (Frag et al., 2023; Iheanacho & Odo, 2020; Wang et al., 2022). Subacute inhalation toxicity studies often aim to determine concentration limits to assess risks for workers in occupational environments. This study was conducted to assess the impact of repeated daily inhalation exposure to chemicals over a 28-day period (OECD, 2018). Further research needs to address these issues using higher concentrations and longer microplastic exposure times to obtain more conclusive results.

4. CONCLUSION

This research shows that exposure to PE-MPs increases the number of erythrocytes and thrombocytes without increasing leukocyte response. Potential causes include oxidative stress, immune system reactions, and compensatory mechanisms. Limitations of this study include a relatively small sample size of rats and focusing only on inhalation exposure. A combination of exposure routes (inhalation, ingestion, dermal) may provide a more comprehensive

understanding of the overall impacts of microplastics. The study primarily focused on hematological parameters. Other biological markers or specific organ effects were not assessed, which could provide additional insights into PE-MP toxicity. Further research is needed with larger sample sizes, varying concentrations and durations of exposure, and combining different exposure routes (inhalation, ingestion, dermal) to better mimic real-world scenarios and gain a holistic understanding of microplastic effects. Recommendations for tofu factory workers, scavengers, and sanitation workers include using personal protective equipment such as gloves, masks, and coveralls to reduce direct contact with plastic waste. Supporting policies that promote safer working conditions, reduce environmental pollution from plastic waste, and enhance waste management practices and recycling initiatives are also crucial.

REFERENCES

- Abdel-Zaher, S., Mohamed, M. S., & Sayed, A. E. D. H. (2023). Hemotoxic effects of polyethylene microplastics on mice. *Frontiers in Physiology*, 14, 1072797. <https://doi.org/10.3389/fphys.2023.1072797>
- Arias, C. F., Valente-Leal, N., Bertocchini, F., Marques, S., Acosta, F. J., & Fernandez-Arias, C. (2024). A new role for erythropoietin in the homeostasis of red blood cells. *Communications Biology*, 7(1), 58. <https://doi.org/10.1038/s42003-023-05758-2>
- Arifin, W.N., Zahiruddin, W.M. (2017). Sample size calculation in animal studies using resource equation approach. *Malays. J. Med. Sci.* 24, 101–105. <https://doi.org/10.21315/mjms2017.24.5.11>
- Buonacera, A., Stancanelli, B., Colaci, M., & Malatino, L. (2022). Neutrophil to Lymphocyte Ratio: An Emerging Marker of the Relationships between the Immune System and Diseases. *International Journal of Molecular Sciences*, 23(7), 3636. <https://doi.org/10.3390/ijms23073636>
- Cary, C. M., Seymore, T. N., Singh, D., Vayas, K. N., Goedken, M. J., Adams, S., Polunas, M., Sunil, V. R., Laskin, D. L., Demokritou, P., & Stapleton, P. A. (2023). Single inhalation exposure to polyamide micro and nanoplastic particles impairs vascular dilation without generating pulmonary inflammation in virgin female Sprague Dawley rats. *Particle and fibre toxicology*, 20(1), 16. <https://doi.org/10.1186/s12989-023-00525-x>
- Chwalba, A., Maksym, B., Dobrakowski, M., Kasperczyk, S., Pawlas, N., Birkner, E., & Kasperczyk, A. (2018). The effect of occupational chronic lead exposure on the complete blood count and the levels of selected hematopoietic cytokines. *Toxicology and applied pharmacology*, 355, 174–179. <https://doi.org/10.1016/j.taap.2018.05.034>
- Enyoh, C. E., Verla, A. W., Verla, E. N., Ibe, F. C., & Amaobi, C. E. (2019). Airborne microplastics: A review study on method for analysis, occurrence, movement and risks. *Environmental Monitoring and Assessment*, 191(11), 668. <https://doi.org/10.1007/s10661-019-7842-0>
- Espinosa, C., Beltrán, J. M. G., Esteban, M. A., & Cuesta, A. (2018). In vitro effects of virgin microplastics on fish head-kidney leucocyte activities. *Environmental Pollution*, 235, 30–38. <https://doi.org/10.1016/j.envpol.2017.12.054>
- Farag, A. A., Youssef, H. S., Sliem, R. E., El Gazzar, W. B., Nabil, N., Mokhtar, M. M., Marei, Y. M., Ismail, N. S., Radwaan, S. E., Badr, A. M., & Sayed, A. E.-D. H. (2023). Hematological consequences of polyethylene microplastics toxicity in male rats: Oxidative stress, genetic, and epigenetic links. *Toxicology*, 492, 153545. <https://doi.org/10.1016/j.tox.2023.153545>
- Franco, R., Navarro, G., & Martínez-Pinilla, E. (2019). Antioxidant defense mechanisms in erythrocytes and in the central nervous system. *Antioxidants*, 8(2), 46.

- <https://doi.org/10.3390/antiox8020046>
- Hamed, M., Osman, A. G. M., Badrey, A. E. A., Soliman, H. A. M., & Sayed, A. E.-D. H. (2021). Microplastics-Induced Eryptosis and Poikilocytosis in Early-Juvenile Nile Tilapia (*Oreochromis niloticus*). *Frontiers in Physiology*, 12, 742922. <https://doi.org/10.3389/fphys.2021.742922>
- Iheanacho, S. C., & Odo, G. E. (2020). Neurotoxicity, oxidative stress biomarkers and haematological responses in African catfish (*Clarias gariepinus*) exposed to polyvinyl chloride microparticles. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 232, 108741. <https://doi.org/10.1016/j.cbpc.2020.108741>
- Kumar, N., Ukey, P. D., Francis, V., Singh, R. P., & Sahu, S. (2022). Plastic pellets. In *Polymers for 3D Printing* (pp. 307-323). William Andrew Publishing. <https://doi.org/10.1016/B978-0-12-818311-3.00019-7>
- Lee, C.-J., Smith, D. W., Gardiner, B. S., & Evans, R. G. (2019). Stimulation of erythropoietin release by hypoxia and hypoxemia: Similar but different. *Kidney International*, 95(1), 23–25. <https://doi.org/10.1016/j.kint.2018.09.025>
- Leslie, H.A., van Velzen, M.J.M., Brandsma, S.H., Vethaak, A.D., Garcia-Vallejo, J.J., Lamoree, M.H. (2022). Discovery and quantification of plastic particle pollution in human blood. *Environ. Int.* 163, 107199. <https://doi.org/10.1016/j.envint.2022.107199>
- Lett, Z., Hall, A., Skidmore, S., & Alves, N. J. (2021). Environmental microplastic and nanoplastic: Exposure routes and effects on coagulation and the cardiovascular system. *Environmental Pollution*, 291, 118190. <https://doi.org/10.1016/j.envpol.2021.118190>
- Li, Y., Tao, L., Wang, Q., Wang, F., Li, G., & Song, M. (2023). Potential Health Impact of Microplastics: A Review of Environmental Distribution, Human Exposure, and Toxic Effects. *Environment & Health*, 1(4), 249–257. <https://doi.org/10.1021/envhealth.3c00052>
- Mentes, D., Nagy, G., Szabó, T. J., Hornyák-Mester, E., Fiser, B., Viskolcz, B., & Póliska, C. (2023). Combustion behaviour of plastic waste – A case study of PP, HDPE, PET, and mixed PES-EL. *Journal of Cleaner Production*, 402, 136850. <https://doi.org/10.1016/j.jclepro.2023.136850>
- Nader, E., Skinner, S., Romana, M., Fort, R., Lemonne, N., Guillot, N., Gauthier, A., Antoine-Jonville, S., Renoux, C., Hardy-Dessources, M.-D., Stauffer, E., Joly, P., Bertrand, Y., & Connes, P. (2019). Blood Rheology: Key Parameters, Impact on Blood Flow, Role in Sickle Cell Disease and Effects of Exercise. *Frontiers in Physiology*, 10, 1329. <https://doi.org/10.3389/fphys.2019.01329>
- OECD. (2018). *Test No. 412: Subacute Inhalation Toxicity: 28-Day Study*. OECD guidelines for the testing of chemicals, section, 4, 263-282.
- Petrlík, J., Ismawati, Y., DiGangi, J., Arisandi, P., Bell, L., & Beeler, B. (2019). *Sampah plastik meracuni rantai makanan Indonesia*. IPEN
- Qasim, N., & Mahmood, R. (2015). Diminution of Oxidative Damage to Human Erythrocytes and Lymphocytes by Creatine: Possible Role of Creatine in Blood. *PLOS ONE*, 10(11), e0141975. <https://doi.org/10.1371/journal.pone.0141975>
- Rajendran, D., & Chandrasekaran, N. (2023). Journey of micronanoplastics with blood components. *RSC Advances*, 13(45), 31435–31459. <https://doi.org/10.1039/D3RA05620A>
- Rodrigues, M. O., Abrantes, N., Gonçalves, F. J. M., Nogueira, H., Marques, J. C., & Gonçalves, A. M. M. (2019). Impacts of plastic products used in daily life on the environment and human health: What is known? *Environmental Toxicology and Pharmacology*, 72, 103239. <https://doi.org/10.1016/j.etap.2019.103239>
- Schafer A. I. (2001). Thrombocytosis and thrombocythemia. *Blood reviews*, 15(4), 159–166. <https://doi.org/10.1054/blre.2001.0162>

- Stock, V., Laurisch, C., Franke, J., Dönmez, M. H., Voss, L., Böhmert, L., Braeuning, A., & Sieg, H. (2021). Uptake and cellular effects of PE, PP, PET and PVC microplastic particles. *Toxicology in Vitro*, 70, 105021. <https://doi.org/10.1016/j.tiv.2020.105021>
- Wang, L., Xu, M., Chen, J., Zhang, X., Wang, Q., Wang, Y., Cui, J., & Zhang, S. (2022). Distinct adverse outcomes and lipid profiles of erythrocytes upon single and combined exposure to cadmium and microplastics. *Chemosphere*, 307, 135942. <https://doi.org/10.1016/j.chemosphere.2022.135942>
- Wu, D., Feng, Y., Wang, R., Jiang, J., Guan, Q., Yang, X., Wei, H., Xia, Y., & Luo, Y. (2023). Pigment microparticles and microplastics found in human thrombi based on Raman spectral evidence. *Journal of advanced research*, 49, 141–150. <https://doi.org/10.1016/j.jare.2022.09.004>
- Yang, W., Jannatun, N., Zeng, Y., Liu, T., Zhang, G., Chen, C., Li, Y. (2022). Impacts of microplastics on immunity. *Front. Toxicol.* 4, 956885. <https://doi.org/10.3389/ftox.2022.956885>
- Yee, M. S. L., Hii, L. W., Looi, C. K., Lim, W. M., Wong, S. F., Kok, Y. Y., ... & Leong, C. O. (2021). Impact of microplastics and nanoplastics on human health. *Nanomaterials*, 11(2), 496. <https://doi.org/10.3390/nano11020496>
- Zwollo, P., Quddos, F., Bagdassarian, C., Seeley, M. E., Hale, R. C., & Abderhalden, L. (2021). Polystyrene microplastics reduce abundance of developing B cells in rainbow trout (*Oncorhynchus mykiss*) primary cultures. *Fish & shellfish immunology*, 114, 102–111. <https://doi.org/10.1016/j.fsi.2021.04.014>

Jurnal Info Kesehatan

Vol. 22, No. 2, June 2024, pp. 357-368

P-ISSN 0216-504X, E-ISSN 2620-536X

DOI: [10.31965/infokes.Vol22.Iss2.1538](https://doi.org/10.31965/infokes.Vol22.Iss2.1538)Journal homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>**RESEARCH****Open Access****The Effect of Edamame Jelly on Lowering Blood Pressure in Primary Hypertension Patients****Yualeny Valensia^{1,2a*}, Budiyantri Wiboworini^{1b}, Nur Hafidha Hikmayani^{3c}**¹ Postgraduate in Nutrition Sciences, Sebelas Maret University, Surakarta, Central Java, Indonesia² Department of Nutrition, Poltekkes Kemenkes Kupang, Kupang, East Nusa Tenggara, Indonesia³ Faculty of Medicine, Sebelas Maret University, Surakarta, Central Java, Indonesia^a Email address: valensia.yv@gmail.com^b Email address: budiyantri_w@staff.uns.ac.id^c Email address: hafidha@staff.uns.ac.id

Received: 27 May 2024

Revised: 29 June 2024

Accepted: 30 June 2024

Abstract

Hypertension is the cause of death in Indonesia, with 23.8% of the 1.7 million population. Management of primary hypertension is non-pharmacological therapy carried out by modifying lifestyle, namely increasing consumption of nuts that contain bioactive components that are beneficial for sufferers of primary hypertension, such as edamame (*Glycine max* (L.) Merrill). This study aims to determine how giving edamame jelly reduces blood pressure in primary hypertension patients. This research was quasi-experimental and used a pre-post-test control group design. The study population consisted of outpatients at the Summersari Jember Community Health Center who met the inclusion and exclusion criteria, totaling 42 subjects. Subjects were randomly divided into treatment and control groups. The intervention group was given amlodipine 5 mg/day and edamame jelly 150 grams/day. The control group was only assigned 5 mg of amlodipine. Statistical tests use the Paired t-test, Wilcoxon signed-rank test, and Mann-Whitney U-tests. The test results showed a significant difference in systolic and diastolic blood pressure between the control and treatment groups with a p-value <0.001. The median systolic blood pressure of the control group was 147.5 mmHg, and that of the treatment group was 130 mmHg. The median diastolic blood pressure in the control group was 90 mmHg, and the treatment group's was 80 mmHg. The reduction in systolic blood pressure in the treatment group was 17.5 mmHg greater than in the control group. The decrease in diastolic blood pressure in the treatment group was 10 mmHg greater than in the control group. Giving 150 grams of edamame jelly/day for 30 days effectively reduces blood pressure in sufferers of primary hypertension.

Keywords: Blood Pressure, Edamame, Primary Hypertension.***Corresponding Author:**

Yualeny Valensia

Postgraduate in Nutrition Sciences, Sebelas Maret University, Surakarta, Central Java, Indonesia

Email: valensia.yv@gmail.com

©The Author(s) 2024. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

1. INTRODUCTION

Hypertension is a trigger for cardiovascular disease, which causes death throughout the world. Hypertension is called the silent killer because it often occurs without symptoms (Lazdia et al., 2020). Hypertension attacks 22% of the world's population, and the incidence rate in Southeast Asia has reached 36%. The death rate due to hypertension in Indonesia was 23.8% of the 1.7 million population in 2019 (Abbafati et al., 2020). Based on the 2023 Indonesian Health Survey (SKI) report, the national prevalence of hypertension is 29.2%, and East Java Province exceeds the national average of 32.8% (Kementerian Kesehatan RI, 2023). The number of hypertension sufferers aged ≥ 15 years in Jember Regency is 741,735 people, with the most significant number of hypertension sufferers being at the Summersari Community Health Center, with 26,736 people (Dinas Kesehatan Kabupaten Jember, 2020)

Hypertension occurs due to the thickening of the blood vessel walls and loss of elasticity of the artery walls. The heart will pump blood faster, and peripheral resistance increases, increasing blood pressure (Yuni, 2016). Consuming foods containing potassium, calcium, and magnesium can lower blood pressure in people with hypertension. Potassium lowers blood pressure by reducing sodium in the urine and extracellular fluid, while calcium relaxes blood vessel muscles to stabilize blood pressure (Rohatin & Prayuda, 2020). According to Nurmayanti & Kaswari (2020), if the magnesium concentration in the blood increases, the heart muscle can work optimally and reduce blood pressure.

Hypertension is divided into two, namely primary and secondary hypertension. Primary hypertension is suffered by $>90\%$ of patients. Management of primary hypertension is divided into pharmacological and non-pharmacological therapy. One of the non-pharmacological therapies can be done by modifying lifestyle (Yulanda & Lisiswanti, 2017). One healthy lifestyle that you can adopt is increasing your consumption of nuts. Nuts that contain bioactive components that are beneficial for sufferers of primary hypertension are vegetable soybeans or edamame (*Glycine max* (L.) Merrill). Edamame is a source of protein, carbohydrates, fibre, amino acids, potassium, calcium, and magnesium. Phytochemical components in edamame, such as isoflavones, sterols, and saponins, can reduce the risk of hypertension (Samruan et al., 2014).

The vitamin C in edamame helps reduce systolic and diastolic blood pressure because an increase in nitric oxide can counteract oxidative stress in the endothelium (Bhagwat & Haytowitz, 2015). Isoflavones, free radical-fighting antioxidants found in edamame, can improve the immune system, reduce the risk of arteriosclerosis (hardening of the arteries), and lower blood pressure (Ningsih et al., 2018). Research using 25 grams of edamame with the drug amlodipine 5 grams/day for 30 days by Sumarni et al. (2020) Showed results in reduced blood pressure, both systolic and diastolic, in WUS suffering from hypertension.

Edamame is one of the mainstay commodities that is characteristic of Jember Regency. The largest and most superior edamame production center in Indonesia is also in Jember Regency because the edamame produced has succeeded in penetrating the international market or being exported abroad. Edamame has been developed into a functional food by processing it into jelly as an intervention for primary hypertension sufferers. The choice of jelly was because, in previous research, the edamame was given in frozen form with a hard texture. In contrast, jelly is a semi-solid food ingredient popular with all groups because of its dense and chewy texture (Prakarsa, 2017). Making edamame jelly involves mixing edamame with carrageenan, a seaweed ingredient that functions as a gel former. Carrageenan has a high fibre and potassium content, which can help reduce the risk of hypertension if consumed (Kusumaningrum & Rahayu, 2018). This study aims to determine how giving edamame jelly reduces blood pressure in primary hypertension patients at the Summersari Community Health Center, Jember Regency.

2. RESEARCH METHOD

This research was quasi-experimental and used a pre-test and post-test with a control group design. This design uses two groups: a control group and a treatment group. The treatment group was given standard medication from the community health center, namely 5 mg amlodipine and edamame jelly, for 30 days, while the control group was only given 5 mg amlodipine obtained from the community health center.

Making edamame jelly refers to previous research with several modifications to the additional ingredients (Afiska et al., 2021). The ingredients used are 25 grams of cooked edamame, 16.7 grams of granulated sugar, 2.3 grams of carrageenan, 0.7 grams of vanilla powder, and 450 ml of water. The total weight of cooked edamame used refers to previous research (Sumarni, 2020). By modifying the recipe, it produces 150 grams of edamame jelly per serving. The process for making edamame jelly is to boil raw edamame for ± 5 minutes, peel the edamame and weigh it according to the weight listed, blend the edamame with 100 ml of boiled water until smooth, then pour it into a container, dissolve the carrageenan with 50 ml of boiled water in a separate container then stir well. Until there are no lumps, add granulated sugar to the bowl of fine edamame and stir well; put the dissolved carrageenan into the edamame container, then stir until evenly mixed; heat and cook the mixture over low heat while continuing to stir, add vanilla powder if the mixture pops and stir evenly, remove the mixture, pour it into serving cups, then refrigerate for at least 2 hours. The edamame jelly is ready to serve.

The research population was outpatients at the Summersari Community Health Center, Jember Regency, in March – April 2023 who had been diagnosed with primary hypertension with systolic blood pressure >140 mmHg and diastolic >90 mmHg based on the results of examinations carried out by the doctor in charge of the community health centre and patient register data totaling 139 people. This data was matched with patient medical records, and it was discovered that 72 patients had complications, 11 people were taking amlodipine 10 mg, and three people were pregnant. Fifty-three research subjects met the inclusion criteria, including outpatients who had been diagnosed with primary hypertension with systolic blood pressure >140 mmHg and diastolic >90 mmHg based on the results of examinations carried out by the doctor in charge of the health center, taking amlodipine 5 mg, and able to communicate. The subject has no memory problems, is 31 – 59 years old, is not pregnant, is not breastfeeding, and is willing to sign an informed consent form. Subjects were excluded if they were diagnosed with diabetes mellitus, coronary heart disease, kidney dysfunction, stroke, and cancer, which was known from the diagnosis of the doctor in charge of the community health center and based on medical record data from the community health center, had experienced allergies or intolerance to soybeans or edamame, smoked and consumed alcohol, the subject is not willing to be a respondent and the subject's address is not found. The sample size is calculated using the following formula (Sastroasmoro & Ismael, 2014):

$$n1 = n2 = 2 \left[\frac{(Z\alpha + Z\beta) \times SD}{X1 - X2} \right]^2$$

Information :

n = Sample size

Z α = Type I error (1.96) 5%

Z β = Type II error (0.84) 80%

SD = Standard deviation based on previous research

X1 – X2 = Average difference based on previous research

Based on previous research by Sumarni et al (2020), the standard deviation was 20.575, and the mean difference was 20.7.

$$\begin{aligned}
 n &= 2 \left[\frac{(Z\alpha + Z\beta) \times SD}{X_1 - X_2} \right]^2 \\
 &= 2 \left[\frac{(1,96 + 0,84) \times 20,575}{20,7} \right]^2 \\
 &= 2 (2,78)^2 \\
 &= 15,4 = 15
 \end{aligned}$$

The number of research samples was calculated by adding the previous values into the formula; the result was that $n_1 = n_2$ was 15 to anticipate dropout, then 10% was added so that $n_1 = n_2$ was 17 samples and a total of 34 samples. The research sample criteria were as described previously by looking at patient visit data in March and April 2023, then selected according to consecutive sampling to obtain a minimum number of subjects. The subjects of this research were 42 people who met the inclusion and exclusion criteria. Two people dropped out because they traveled out of town and did not want to continue, so the number of subjects who took part in this research until completion was 40, with details of 20 people in the treatment group and 20 people in the control group.

This research was conducted for 30 days, from May to June 2023. The treatment group received 5 mg amlodipine and 150 grams of edamame jelly, which they consumed every day. Edamame jelly is made from 25 grams of cooked edamame, 16.7 grams of sugar, 2.3 grams of carrageenan, 0.7 grams of vanilla powder, and 150 ml of water. Edamame jelly is consumed every day as an afternoon snack. The control group received 5 mg amlodipine, which was consumed once every day for 30 days. Blood pressure measurements on all research subjects were carried out before entering the treatment day. Blood pressure measurements were performed again on the sixteenth day of treatment and after treatment, namely on the thirty-first day.

Test normality of blood pressure data using the Shapiro-Wilk method (<50 respondents). A comparative test of differences in mean blood pressure before and after treatment in two paired groups using the paired t-test for normally distributed data and the Wilcoxon signed rank test for data that is not normally distributed. The comparative test for differences in mean blood pressure between the control and treatment groups used the Mann-Whitney U Test because there was data that was not normally distributed. Statistical data analysis using the STATA application version 15 and Flourish for data visualization and storytelling. This research has received approval from the Research Ethics Commission of the Faculty of Medicine, Sebelas Maret University, with letter number 92/UN27.06.11/KEP/EC/2023 on May 10, 2023.

3. RESULTS AND DISCUSSION

Subject characteristics in this study included gender, age, BMI, education, and occupation. Table 1 shows the distribution of research subject characteristics.

Table 1. Frequency Distribution Based on Research Subject Characteristics

Subject characteristics	Control		Edamame Jelly		p-value
	n	(%)	n	(%)	
Gender					
Male	5	(25.0)	5	(25.0)	0.642
Female	15	(75.0)	15	(75.0)	
Education					
No school	1	(5.0)	3	(15.0)	

Elementary school	4 (20.0)	5 (25.0)	
Junior high school	5 (25.0)	4 (20.0)	0.825
Senior high school	8 (40.0)	6 (30.0)	
College	2 (10.0)	2 (10.0)	
Work			
Housewife	13 (65.0)	14 (70.0)	
Government employees	1 (5.0)	0 (0.0)	
Private sector employee	4 (20.0)	3 (15.0)	0.703
Businessman	2 (10.0)	2 (10.0)	
Retired	0 (0.0)	1 (5.0)	
Age (years)			
25 – 34	0 (0.0)	1 (5.0)	
35 – 44	3 (15.0)	4 (20.0)	
45 – 54	11 (55.0)	6 (30.0)	0.619
55 – 64	6 (30.0)	9 (45.0)	
Body mass index			
Normal	12 (60.0)	9 (45.0)	
Fat	3 (15.0)	2 (10.0)	0.412
Obesity	5 (25.0)	9 (45.0)	

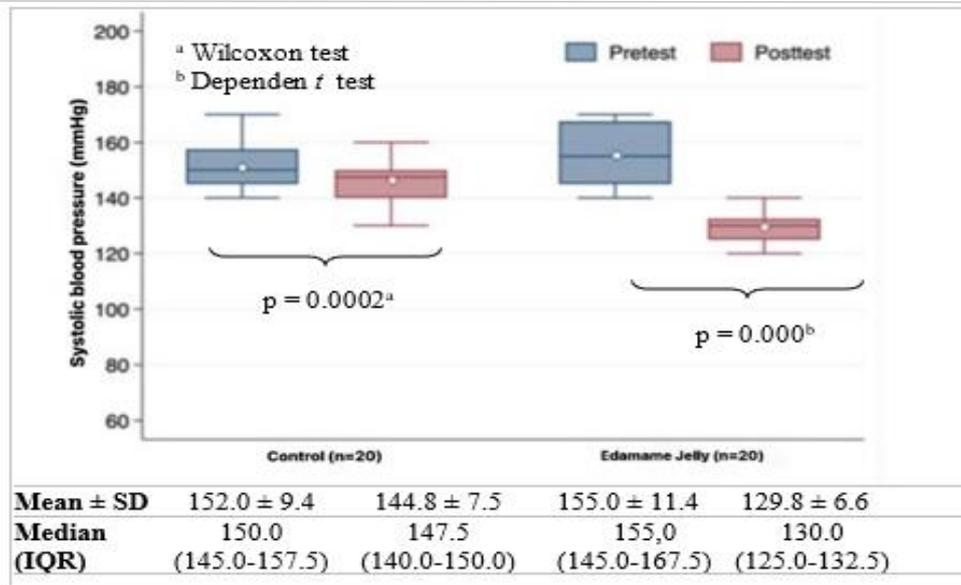
^a χ^2 Test

Source: Primary data (2023)

Based on Table 1, the number of subjects is more female than male. Most respondents in the control and treatment groups were educated in high school. The most common occupation in the control and treatment groups was housewife. Both groups' most extensive age range was 45–54, and the highest BMI was normal. The p -value > 0.05 for each characteristic indicates that gender, education, occupation, and history of hypertension from fathers and mothers from both groups are homogeneous, and there are no significant differences.

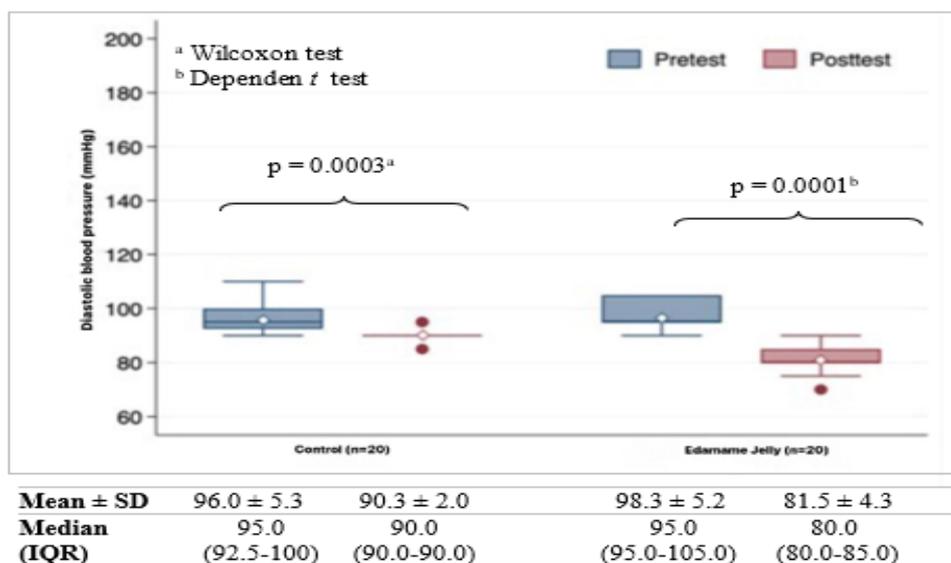
The data obtained in this research was subjected to a normality test to determine whether the data was normally distributed or not. The data normality test in this study used the Shapiro-Wilk test because the research sample was < 50 . In the pretest data normality test, both groups' systolic and diastolic blood pressure was usually distributed. Posttest data on systolic and diastolic blood pressure in the control group showed a p -value < 0.05 , which means the data was not normally distributed, whereas, in the treatment group, the data showed a normal distribution so that the correct test for differences in posttest data between the control and treatment groups was the Mann-Whitney test. The normality test of pretest and post-test data for systolic and diastolic blood pressure in the treatment group showed that the data were normally distributed, so the difference test used the paired t -test. The control group's systolic and diastolic blood pressure contained abnormally distributed data, so the pretest-posttest difference test used the Wilcoxon signed rank test. The next stage of statistical testing was conducted to determine the difference in blood pressure before and after giving edamame jelly to each group and the difference in blood pressure between the treatment and control groups after giving edamame jelly.

The statistical test to determine the difference in blood pressure between the pretest and posttest on a numerical scale was the Wilcoxon signed rank test in the control group and the paired t -test in the edamame jelly treatment group. The statistical test results are shown in Figures 1 and 2. The mean is marked with a white diamond symbol in the middle of the box, and the median is marked with a red horizontal line in the middle.



SD: standard deviation; IQR: interquartile range

Figure 1. The difference in systolic blood pressure before and after giving edamame jelly to the control and treatment groups.



SD: standard deviation; IQR: interquartile range

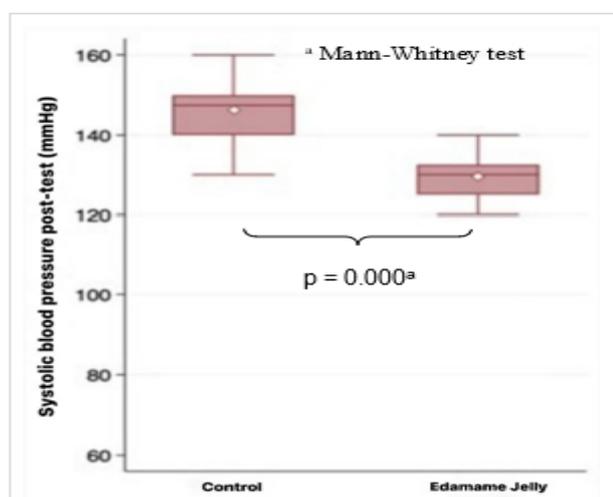
Figure 2. Difference in diastolic blood pressure before and after giving edamame jelly to the control and treatment groups.

In Figure 1, the mean systolic blood pressure in the control group before treatment was 152 mmHg, and after treatment was 144.8 mmHg. The mean systolic blood pressure before giving edamame jelly in the treatment group was 155 mmHg, and after giving edamame jelly was 129.8 mmHg. The reduction in systolic blood pressure in the control group was 7.2 mmHg, and in the treatment group was 25.2 mmHg. In Figure 2, the mean diastolic blood pressure in the control group before treatment was 96 mmHg, and after treatment was 90.3 mmHg. The average diastolic blood pressure before giving edamame jelly in the treatment group was 98.3

mmHg, and after giving edamame jelly was 81.5 mmHg. The reduction in diastolic blood pressure in the control group was 5.7 mmHg, and in the treatment group was 16.8 mmHg.

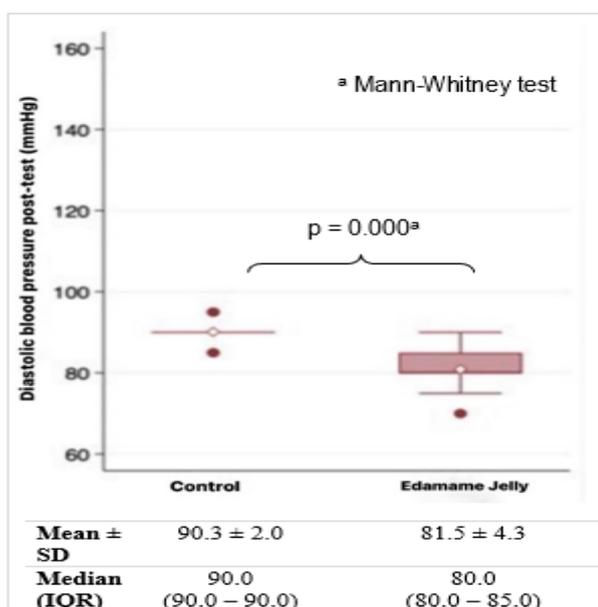
It can be seen that the mean blood pressure, both systolic and diastolic, in the edamame jelly and control groups decreased. Still, the difference in a reduction for systolic blood pressure was significantly more significant in the edamame jelly group (-25.2 mmHg, p-value = 0.000) compared to the control group (-7.2 mmHg, p-value = 0.0002). Similar results were also observed for diastolic blood pressure, where the edamame jelly group had a significantly more significant difference in reduction, namely -16.8 mmHg with a p-value = 0.0001 compared to the control group (-5.7 mmHg, p-value = 0.0003).

Based on the previous normality test results, the Mann-Whitney test was used to analyze the difference in post-test systolic and diastolic blood pressure between the control and edamame jelly groups. The analysis results are shown in Figure 3 for systolic blood pressure and Figure 4 for diastolic blood pressure.



SD: standard deviation; IQR: interquartile range

Figure 3. Differences in systolic blood pressure after administration of edamame jelly



SD: standard deviation; IQR: interquartile range

Figure 4. The difference in diastolic blood pressure after administration of edamame jelly

Systolic and diastolic blood pressure after treatment was significantly lower in the edamame jelly group compared to the control group ($p < 0.001$). The mean systolic blood pressure after treatment in the control group was 144.8 mmHg. In the treatment group, it was 129.8 mmHg, with the difference in mean systolic blood pressure between the control group and edamame jelly being 15 mmHg. The mean diastolic blood pressure in the control group was 90.3 mmHg; in the treatment group, it was 81.5 mmHg, with a mean difference of 8.8 mmHg.

In this study, a multivariate analysis was also carried out to determine the effect of giving edamame jelly on blood pressure after treatment by considering the influence of several characteristics of the respondents at once. The research sample consisted of 40 people, so a maximum of only three confounding variables (covariates) could be added to the multivariate analysis, referring to the rule of thumb $N = 10 \times$ number of covariates. The sociodemographic variables chosen were classic variables relevant to hypertension, namely age, gender, and BMI.

Analysis of covariance (ANCOVA) was used to determine the effect of giving edamame jelly on systolic blood pressure by controlling the variables age, gender, and BMI. The test results are listed in Table 2.

Table 2. Influence of demographic factors on systolic blood pressure after giving edamame jelly

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2417.076 ^a	4	604.269	12.222	.000
Intercept	9520.420	1	9520.420	192.562	.000
Age	2.478	1	2.478	.050	.824
Gender	109.588	1	109.588	2.217	.145
BMI	23.734	1	23.734	.480	.493
Edamame Jelly Feeding	2238.290	1	2238.290	45.272	.000
Error	1730.424	35	49.441		
Total	757650.000	40			
Corrected Total	4147.500	39			
R Squared = .583 (Adjusted R Squared = .535)					

^a ANCOVA; * Statistically meaningful ($p < 0,05$).

Table 2 shows that age, gender, and BMI do not influence systolic blood pressure. Administration of edamame jelly together with sociodemographic factors explained the systolic blood pressure value by 53.5% (adjusted $R^2=0.535$), while the rest was influenced by other factors not studied.

Table 3. Effect of demographic factors on diastolic blood pressure after administration of edamame jelly

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	833.978 ^a	4	208.495	20.248	.000
Intercept	3914.792	1	3914.792	380.186	.000
Age	6.433	1	6.433	.625	.435
Gender	16.342	1	16.342	1.587	.216
BMI	26.766	1	26.766	2.599	.116
Edamame Jelly Feeding	797.686	1	797.686	77.467	.000
Error	360.397	35	10.297		
Total	296175.000	40			
Corrected Total	1194.375	39			
R Squared = .698 (Adjusted R Squared = .664)					

^a ANCOVA; * Statistically meaningful ($p < 0,05$).

Table 3 shows that age, gender, and BMI do not influence diastolic blood pressure. Administration of edamame jelly together with sociodemographic factors explained the systolic blood pressure value by 66.4% (adjusted $R^2=0.664$), while the rest was influenced by other factors not studied.

Based on the results of research in the treatment and control groups, respondents aged >40 years were more likely to suffer from primary hypertension than those aged under 40 years. These results are in line with the research conducted. [Meliana \(2021\)](#), Hypertension is common after a person is 40 years old because the blood vessels narrow and become stiff, so blood

pressure will also increase. Gender in the treatment and control groups, more female respondents suffered from primary hypertension than men. These results are in line with the research conducted. [Ayukhaliza \(2020\)](#), it states that women tend to have higher blood pressure due to hormonal factors, namely a reduction in the hormone estrogen, and psychological factors, such as changes within the woman.

The results of the test analysis show that there are significant differences before and after treatment in both the treatment and control groups. The mean reduction in systolic blood pressure in the treatment group was 25.2 mmHg ($p = 0.000$), and the diastolic blood pressure was 16.8 mmHg ($p = 0.0001$). The mean decrease in systolic blood pressure in the control group was 7.2 mmHg ($p = 0.0002$), and the reduction in diastolic blood pressure was 5.7 mmHg ($p = 0.0003$). This shows a more significant decrease in systolic and diastolic blood pressure in the treatment group than in the control group. A reduction in blood pressure of 20/10 mmHg in hypertensive patients can reduce the risk of cardiovascular events by 50% ([Kandarini, 2017](#)).

Previous research conducted by [Maulina et al., \(2020\)](#), it was stated that giving 25 grams of edamame/day was very effective in reducing blood pressure in women of childbearing age with hypertension, so consuming 25 grams of cooked edamame/day is recommended for hypertension sufferers. The bioactive components in edamame jelly provide a vasodilating effect and lower blood pressure ([Yuni, 2016](#)). Analysis tests showed a significant reduction in blood pressure in the treatment group given edamame jelly and 5 mg of amlodipine. These results are due to how amlodipine works: it reduces calcium ion levels, providing a vasodilation effect on blood vessels. When combined with consuming edamame jelly, this significantly lowers blood pressure ([Maulina et al., 2020](#)).

This research chose to process edamame into jelly because, in previous research, the edamame was given in frozen form with a hard texture, while jelly is a semi-solid food ingredient that is popular with all groups because its texture is dense and chewy ([Prakarsa, 2017](#)). The process of making edamame jelly is by mixing edamame with carrageenan, which is an ingredient from seaweed and functions as a gel former. According to [Kusumaningrum & Rahayu \(2018\)](#), carrageenan has a high fibre and potassium content, which, if consumed, can help reduce the risk of developing hypertension.

The control group that only received amlodipine also showed decreased systolic blood pressure. Still, the reduction in blood pressure in the treatment group was 18 mmHg greater, and diastolic blood pressure was 11 mmHg greater than in the control group. This is in line with a similar study using amlodipine as a control, which was also carried out ([Maulina et al., 2020](#)). The result was a difference in the mean systolic and diastolic blood pressure values before and after treatment with 5 mg amlodipine therapy of 10.75 mmHg and 6.5 mmHg, respectively.

The results of statistical tests of differences in blood pressure between the treatment group and the control group after giving edamame jelly showed a significant difference in reducing systolic and diastolic blood pressure ($p < 0.001$). The treatment group's median posttest systolic blood pressure was 130 mmHg and diastolic 80 mmHg. The median post-test systolic blood pressure in the control group was 147.5 mmHg and diastolic 90 mmHg. The decrease in systolic blood pressure in the treatment group was 17.5 mmHg greater than the control group. In comparison, the reduction in diastolic blood pressure in the treatment group was ten mmHg greater than the control group.

Edamame jelly in this study was given for 30 days to subjects in the treatment group. Giving edamame jelly affected reducing blood pressure in the treatment group. One hundred fifty grams of edamame jelly is consumed daily as an afternoon snack. The amount per serving of edamame jelly is based on the dosage given for edamame in previous research as well as modification of the recipe, which has gone through the organoleptic testing stage and nutritional value testing in the laboratory. Edamame jelly per serving contains 139.5 kcal calories, 9.1

grams of protein, 2 grams of fat, 21.4 grams of carbohydrates, 147 mg potassium, 36 mg calcium, 19.5 mg magnesium, and 8.1 mg isoflavones. Subjects in the treatment group and control group also received amlodipine 5 mg as pharmacological therapy for sufferers of primary hypertension.

Research conducted by [Wei et al. \(2020\)](#), it was stated that the group that consumed ≥ 125 grams/day of soy products had lower systolic and diastolic blood pressure than those that consumed < 125 grams/day. The study also stated that intake of soy products can reduce blood pressure in the long term and can be implicated in preventing primary hypertension.

Another study comparing food sources of vegetable (soybean) and animal (cow's milk) protein sources showed that respondents who consumed 25 grams of soybeans/day had lower systolic and diastolic blood pressure than those who drank cow's milk ([Ghidanac et al., 2023](#)). The vegetable protein in edamame contains essential amino acids that increase the active transport process from the blood to muscle cells and other tissues. This effect on the cardiovascular system increases peripheral blood flow, increasing cardiac output, which decreases blood pressure ([Kusumastuty et al., 2016](#)). The potassium content in edamame jelly provides a diuretic and vasodilator effect. It maintains the balance of extracellular sodium and potassium concentrations to reduce blood pressure in hypertension sufferers ([Yuni, 2016](#)). Respondents also consumed edamame jelly, which increased calcium and magnesium intake and maintained the balance of calcium and magnesium in the blood. Blood calcium levels are essential because calcium can help flex blood vessel muscles, while blood magnesium makes the heart muscle work optimally and decreases blood pressure ([Rohatin & Prayuda, 2020](#)).

Edamame contains isoflavones, which are thought to show antihypertensive activity by increasing NO, reducing angiotensin, and inhibiting the formation of free radical reactions. Isoflavones work as antihypertensive agents by inhibiting the conversion of angiotensin I to angiotensin II, thereby inhibiting the release of aldosterone by ACE. Aldosterone impacts the kidneys in retaining sodium and water; more water is excreted, and blood pressure will decrease if aldosterone production is inhibited. ([Putri & Nofia, 2020](#)). These results align with other research on giving 40 grams of black soybean cookies for four weeks made from 20 grams of black soybean powder. Mean systolic blood pressure decreased by 7.5 mmHg and diastolic by four mmHg ([Yamashita et al., 2020](#)).

The results of the ANCOVA test on the influence of sociodemographic factors on systolic and diastolic blood pressure show that edamame jelly can be used as a non-pharmacological therapy in addition to pharmacological therapy using the drug amlodipine, which provides benefits for treating primary hypertension.

This research has limitations, namely that researchers have not been able to optimally control other factors that can influence blood pressure, including genetics, lifestyle, stress, and physical activity. Researchers also have limitations in finding more research samples. Another limitation of this research is that food intake was not monitored for all research subjects.

4. CONCLUSION

Giving 1 cup of edamame jelly (150 grams) for 30 days can reduce systolic blood pressure by 17.5 mmHg and diastolic by 10 mmHg. This study recommends that future researchers explore other forms of soy products or other bioactive components in edamame to determine their antihypertensive effects.

REFERENCES

[Abbafati, C., Abbas, K. M., Abbasi-Kangevari, M., Abd-Allah, F., Abdelalim, A., Abdollahi, M., Abdollahpour, I., Abegaz, K. H., Abolhassani, H., Aboyans, V., Abreu, L. G., Abrigo, M. R. M., Abualhasan, A., Abu-Raddad, L. J., Abushouk, A. I., Adabi, M., Adekanmbi,](#)

- V., Adeoye, A. M., Adetokunboh, O. O., ... Amini, S. (2020). Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet*, 396(10258), 1204–1222. [https://doi.org/10.1016/S0140-6736\(20\)30925-9](https://doi.org/10.1016/S0140-6736(20)30925-9)
- Afiska, W., Rotua, M., & Nabila, Y. (2021). Uji Daya Terima Puding Kacang Merah Sebagai Alternatif Makanan Selingan Untuk Remaja Putri Anemia. *Jurnal Gizi Dan Kesehatan (JGK)*, 1(1), 9–16.
- Ayukhaliza, D. A. (2020). Faktor Risiko Hipertensi di Wilayah Pesisir (Studi Pada Wilayah Kerja UPTD Puskesmas Tanjung Tiram). *Skripsi thesis*. Universitas Islam Negeri Sumatera Utara.
- Bhagwat, S., & Haytowitz, D. B. (2015). USDA database for the isoflavone content of selected foods. *U.S Department of Agriculture, Agricultural Research Service*.
- Dinas Kesehatan Kabupaten Jember. (2020). *Profil Kesehatan Kabupaten Jember Tahun 2020*. Jember: Dinas Kesehatan Kabupaten Jember
- Ghidanac, D., Erlich, M., Mejia, S. B., Khan, T. A., Chiavaroli, L., Comelli, E., Jenkins, D., Kendall, C., & Sievenpiper, J. (2023). Effect of Soy Protein on Blood Pressure: A Systematic Review and Meta-Analysis of Randomized Controlled Feeding Trials. *Current Developments in Nutrition*, 7, 100342. <https://doi.org/10.1016/j.cdnut.2023.100342>
- Kandarini, Y. (2017). *Tatalaksana Farmakologi Terapi Hipertensi*. Divisi Ginjal Dan Hipertensi RSUP Sanglah Denpasar.
- Kementerian Kesehatan RI. (2023). *Survei Kesehatan Indonesia (SKI) Tahun 2023*. Jakarta: Kementerian Kesehatan Republik Indonesia.
- Kusumaningrum, I., & Rahayu, N. S. (2018). Formulasi Snack Bar Tinggi Kalium Dan Tinggi Serat Berbahan Dasar Rumput Laut, Pisang Kepok, Dan Mocaf Sebagai Snack Alternatif Bagi Penderita Hipertensi. *Argipa*, 3(2), 102–110.
- Kusumastuty, I., Widyani, D., & Wahyuni, E. S. (2016). Asupan Protein dan Kalium Berhubungan dengan Penurunan Tekanan Darah Pasien Hipertensi Rawat Jalan. *Indonesian Journal of Human Nutrition*, 3(1), 19–28. <https://doi.org/10.21776/ub.ijhn.2016.003.01.3>
- Lazdia, W., Afdilatul Rahma, W., Sakinah Lubis, A., Sulastri, T., Studi Ilmu Keperawatan, P., Kesehatan Masyarakat, I., & Email Corresponding, F. (2020). Pengaruh Rebusan Daun Seledri Untuk Menurunkan Tekanan Darah Pada Penderita Hipertensi. *Empowering Society Journal*, 1(1), 26–32. Retrieved from <https://ojs.fdk.ac.id/index.php/ESJ/article/view/666>
- Maulina, I., Islami, R., Sunarjo, L., Nugraheni, S. A., Student, M., Program, P., Kesehatan, P., Semarang, K., & Semarang, U. D. (2020). *Effectiveness of giving edamame supplementation (Glycine Max l. Merrill) on improvement blood pressure in women of childbearing age with hypertension*. 12–16. <https://doi.org/10.61096/ijamsr.v8.iss3.2020.515-520>
- Meliana, M. (2021). Faktor Risiko Usia, Jenis Kelamin, Dan Obesitas Dengan Kejadian Penyakit Hipertensi Diwilayah Kerja Puskesmas Puuwatu Kota Kendari. *Nuevos Sistemas de Comunicación e Información*, 1–12.
- Ningsih, T. E., Siswanto, S., & Winarsa, R. (2018). Aktivitas Antioksidan Kedelai Edamame Hasil Fermentasi Kultur Campuran oleh *Rhizopus oligosporus* dan *Bacillus subtilis*. *Berkala Sainstek*, 6(1), 17. <https://doi.org/10.19184/bst.v6i1.7556>
- Nurmayanti, H., & Kaswari, S. R. [tegufile:///D:/garuda1761230.pdf](https://doi.org/10.31290/nj.v1i1.3531). (2020). Efektivitas Pemberian Konseling Tentang Diet Dash terhadap Asupan Kalium, Kalsium, Natrium Magnesium, Aktivitas Fisik, dan Tekanan Darah Pasien Hipertensi. *Jurnal Nutriture*, 1(1), 63–75. <https://doi.org/10.31290/nj.v1i1.3531>

- Prakarsa, A. W. (2017). Pengaruh Perbedaan Tepung Labu Kuning Dalam Produk Jelly Ditinjau Dari Karakteristik Fisikokimiawi dan Sensori. *Skripsi thesis. Universitas Katolik Soegijapranata Semarang*.
- Putri, B. M., & Nofia, Y.. (2020). Minuman Berbahan Dasar Kedelai Sebagai Antihipertensi. *Nutrire Diaita*, 12(1), 29–35. <https://doi.org/10.47007/nut.v12i01.2849>
- Rohatin, A., & Prayuda, C. W. (2020). Hubungan Asupan Natrium, Kalium Dengan Hipertensi Pada Lansia Di Poliklinik Penyakit Dalam. *Jurnal Fakultas Ilmu Kesehatan*, 1(1), 10–14.
- Samruan, W., Oonsivilai, A., & Oonsivilai, R. (2014). Soybean and fermented soybean extract antioxidant activities. *International Journal of Biological, Agricultural, Food and Biotechnological Engineering*, 6(12), 1134–1137.
- Sastroasmoro, S. & Ismael, S. (2014). Dasar-dasar metodologi penelitian klinis. Jakarta : Sagung Seto.
- Sumarni, S. (2020). Efektifitas Pemberian Suplementasi Edamame (Glycine Max L. Merrill) Terhadap Perbaikan Tekanan Darah Dan Profil Lipid (Kolesterol Total, Ldl, Hdl, Trigliserida)(Studi pada Wanita Usia Subur dengan Hipertensi di Puskesmas Mlonggo Kabupaten Jepara). *Thesis*. Semarang: Poltekkes Kemenkes Semarang
- Wei, J. L., Wang, X. Y., Liu, F. C., Chen, J. C., Cao, J., Li, J. X., Hu, D. S., Shen, C., Lu, F. H., Zhao, Y. X., Huang, J. F., & Lu, X. F. (2020). Associations of soybean product intake with blood pressure changes and hypertension incidence: The China-PAR project. *Journal of Geriatric Cardiology*, 17(7), 384–392. <https://doi.org/10.11909/j.issn.1671-5411.2020.07.005>
- Yamashita, Y., Nakamura, A., Nanba, F., Saito, S., & Toda, T. (2020). Black Soybean Improves Vascular Function and Blood Pressure: A Randomized, Placebo-Controlled, Crossover Trial in Humans. *Nutrients* 2020, 12(9), 2755. <https://doi.org/10.3390/nu12092755>
- Yulanda, G., & Lisiswanti, R. (2017). Penatalaksanaan Hipertensi Primer. *Jurnal Majority*, 6(1), 25–33.
- Yuni, S. (2016). Pengaruh Konsumsi Minuman Fungsional terhadap Tekanan Darah dan Konsentrasi Elektrolit Urin Perempuan Dewasa Prahipertensi. *Disertasi. Program Studi Ilmu Gizi Manusia IPB*.

Jurnal Info Kesehatan

Vol. 22, No. 2, June 2024, pp. 369-377

P-ISSN 0216-504X, E-ISSN 2620-536X

DOI: [10.31965/infokes.Vol22.Iss2.1531](https://doi.org/10.31965/infokes.Vol22.Iss2.1531)Journal homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>**RESEARCH****Open Access****The Effectiveness of Diabetic Self-Management Education (DSME) on Dietary Habit, Obesity, and Physical Activity Among Patients with DM Type II****Liana Lidia Agow^{1a*}, Fery Agusman Motuho Mendrofa^{2b}, Sonhaji^{2c}**¹ Master of Nursing Program, Faculty of Nursing and Health Science, Universitas Karya Husada Semarang, Semarang, Central Java, Indonesia² Department of Community Health Nursing, Universitas Karya Husada Semarang, Semarang, Central Java, Indonesia^a Email address: lidialiana22@gmail.com^b Email address: ferysinga@gmail.com^c Email address: soni_aji84@yahoo.com

Received: 22 May 2024

Revised: 30 June 2024

Accepted: 30 June 2024

Abstract

Diabetes mellitus and metabolic disorders are the priority programs in Indonesia. There is one program to reduce the fatality impact of diabetes which is diabetic self-management education (DSME). This study aimed to examine the effectiveness of Diabetes Self-Management Education (DSME) on dietary habits, physical activity, and obesity. This study is a quasi-experimental approach with one group pre-test post-test design. The sample in this study was 75 people with DM type II. The intervention for all samples was done for 4 sessions. Each session lasted 60 to 120 minutes/day with a maximum of 10 patients. The findings revealed that dietary habits were good from 56.0% to 69.3%, physical activity was good from 54.7% to 76.0%, and obesity decreased from 69.3% to 57.35, respectively for pre and post-test. The paired t-test result revealed that DSME correlates with dietary habits, physical activity, and obesity (obesity, physical activity, and dietary habit with p-values 0.024; 0.007; and 0.02, respectively). In conclusion, DSME has significantly influenced dietary habits, physical activity, and obesity. Education is crucial in promoting physical activity among individuals with diabetes. By enhancing self-efficacy, providing education on self-care behaviors, and emphasizing the importance of regular physical activity, these programs contribute to improving metabolic control, quality of life, and overall health outcomes in diabetic patients.

Keywords: DSME (Diabetic Self-Management Education), Dietary Habit, Physical Activity, Obesity.

***Corresponding Author:**

Liana Lidia Agow

Master of Nursing Program, Faculty of Nursing and Health Science, Universitas Karya Husada Semarang, Semarang, Central Java, Indonesia

Email: lidialiana22@gmail.com

©The Author(s) 2024. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

1. INTRODUCTION

The increasing prevalence of NCDs in developing countries has encouraged the emergence of agreement on a global strategy for preventing and controlling non-communicable diseases (NCDs). NCDs have become a strategic issue in the 2030 SDGs (Sustainable Development Goals) agenda so it must be a development priority in every country. In the management of non-communicable diseases, the SDGs indicator states that the rate of premature deaths due to non-communicable diseases will decrease by one-third by 2030 with one of the first global targets, namely reducing premature deaths due to NCDs by 25% by 2025 (Ministry of Health Republic of Indonesia, 2019). The global prevalence of diabetes in 2019 is estimated at 9.3% (463 million people), increasing to 10.2% (578 million) in 2030 and 10.9% (700 million) in 2045. The prevalence in urban areas is higher (10.8%) compared to rural (7.2%) and in high-income countries (10.4%) compared to low-income countries (4.0%). The world prevalence of diabetes in adults (aged 20-79 years) was 6.4% (285 million) in 2010 and will increase to 7.7% (439 million) in 2030. Vulnerable in 2010 and 2030 there will be an increase of 69 % in developing countries and a 20% increase in developed countries. Indonesia has the highest prevalence of Diabetes Mellitus (DM) sufferers in the world along with China, India, the United States, Brazil, Russia, and Mexico with an estimated number of people with DM of 10 million people (IDF, 2019).

The report from Indonesian Basic Health Research stated that the prevalence of DM is 1.5% of the total population of Indonesia (Ministry of Health Indonesia, 2018). The highest prevalence of diabetes mellitus (DM) in Indonesia is in Yogyakarta (2.6%), DKI Jakarta (2.5%), North Sulawesi (2.4%), and East Kalimantan (2.3%). The prevalence of DM increases with increasing age, but from age ≥ 65 years it tends to decrease. Based on the North Sulawesi Basic Health Survey Report, there were 25,661 DM sufferers of all ages. The highest prevalence of DM sufferers in North Sulawesi based on a doctor's diagnosis is in Manado, namely 3.45%. There are several programs established by the government to reduce the prevalence of DM.

Diabetes self-management education (DSME) programs are essential in empowering individuals with diabetes to effectively manage their condition. These programs aim to enhance patients' knowledge, skills, and self-care behaviors, leading to improved glycemic control and better health outcomes (Fitria et al., 2023; Powers et al., 2015). By utilizing culturally sensitive approaches and tailored interventions, these programs facilitate self-care practices and provide individuals with the necessary tools to make informed decisions regarding their diabetes management (Fitria et al., 2023; Powers et al., 2015). Furthermore, diabetes self-management education programs have evolved to become more patient-centered, and theoretically based, and emphasize the provision of ongoing support to maintain the self-management gains achieved by patients (Funnell et al., 2007). These programs address various factors such as health beliefs, cultural needs, knowledge levels, emotional concerns, and family support to enable individuals to effectively meet the challenges of self-management (Funnell et al., 2007; Powers et al., 2015). Collaborative efforts involving healthcare professionals, community health workers, and peer educators are crucial for the successful delivery of diabetes self-management education programs (Lawless et al., 2016; Tamayo & Reyes, 2022). These programs not only aim to enhance glycemic control but also work towards preventing diabetes-related complications, such as diabetic foot injuries, through education and empowerment (Fitria et al., 2023).

There are barriers of implementation DSME and the impact on dietary habits, physical activity, and obesity status is under study (Funnell et al., 2007). This study aimed to examine the effectiveness of Diabetic Self-Management Education on dietary habits, physical activity, and obesity.

2. RESEARCH METHOD

This study is a quasi-experimental approach with one group pre-test and post-test design with intervention in between. The population in this study was 75 people with Diabetes Mellitus (DM) type II who live in the area of West Modayag Primary Health Care, East Bolaang Mongondow, North Sulawesi Province. The data collection was done from December 2023 to January 2024.

The sample derived for this study was 75 people with DM type II that derived from the total sampling. The inclusion criteria of sample selection consist of those who were willing to participate in the study, residents in West Modayag Primary Health Care, actively controlled to primary health care, could be able to communicate verbally, and could be able to read and write. The exclusion criteria consist of refusal during the data collection, having physical, mental, and cognitive disabilities, and patients with neuropathy and cardiomyopathy complications.

The intervention for all samples was done for 4 sessions. Each session had a duration of 60 to 120 minutes/day with a maximum of 10 patients. So, for 75 samples, each session took six days. The interval time between each session was one week. The independent variable of this study is Diabetes Self-Management Education (DSME). There are four sessions of DSME, the first session is about education about DM, the second session is the explanation of nutrition diet for DM patients, the third session is about physical exercise, and the last session is about pharmacology treatment. To measure this variable, SAP (Satuan Acara Pengajaran or *Teaching Program Unit*) was used which followed the guidelines from Funnell theory (2011) (Funnell et al., 2007). The dependent variables of this study are dietary habits, physical activity, and obesity. Dietary habit was measured by a questionnaire adopted from (Dewiyanti, 2022) consists of 15 questions using Likert scales. Good dietary habit is categorized for those has score more than 37 and not good dietary habits are categorized for those has scored 37 or less. The formula to calculate the cut-off is adding maximum and minimum scores and dividing into 2. Physical activity in this study was measured by the Global Physical Activity Questionnaire (GPAQ) developed by (World Health Organization, 2012). Good physical activity was categorized if respondents had MET < 600 and not bad was categorized for MET 600 or higher. Obesity was categorized by measuring the height and weight which resulted from the body mass index. Obese were categorized as those who had a BMI of 25 kg/m² or higher and not obese were categorized as those who had a BMI less than 25 kg/m² (PERKENI, 2015).

The normality test was done with a significant level of 5% using the Shapiro-Wilk statistical test. Additionally, due to the normal result, a paired t-test statistical test was done. Regarding the instrument used in this study, we did not check the validity and reliability test because the instruments used are standardized instruments which already been used by previous researchers (Dewiyanti, 2022; Funnell et al., 2007; Ministry of Health Indonesia, 2024; PERKENI, 2015; World Health Organization, 2012). This study used a 95% Confidence Interval to define the significant level. This study was accepted the ethical approval from the ethical committee at Karya Husada University with number 061/KEP/UNKAHA/SIE/V/2024.

3. RESULTS AND DISCUSSION

Table 1. The General Characteristics of the Respondents (n = 75)

Variable		Frequency	Percentage (%)
Sex	Male	33	44.0
	Female	42	56.0
Age	30 - 50 years old	37	49.3
	51 - 65 years old	38	50.7
Education	Elementary	1	1.3
	Junior high school	2	2.7

	Senior high school	40	53.3
	Vocation	14	18.7
	Bachelor	18	24.0
Occupation	Private sector	21	28.0
	Self-employee	16	21.3
	Civil servant	21	28.0
	Others	8	10.7
	Unemployed	9	12.0

Table 1 shows the univariate results as the general characteristics of the respondents. According to respondent's sex, more than half of them were female (56.0%), aged 51 – 65 years old (50.7%), and graduated from senior high school (53.3%). The occupation among 75 respondents revealed the highest percentage showed by those working in the private sector and as civil servants (28.0% for each).

Table 2. The Distribution Frequency for Pre and Post-test (n = 75)

Variable		Pre-test f (%)	Post-test f (%)
Dietary habit	Not good	33 (44.0)	23 (30.7)
	Good	42 (56.0)	52 (69.3)
Physical activity	Not good	34 (45.3%)	18 (24.0)
	Good	41 (54.7)	57 (76.0)
Obesity	Not obese	23 (30.7)	32 (42.7)
	Obese	52 (69.3)	43 (57.3)

Table 2 shows that 44% of respondents had not good dietary habits before giving the intervention and 56% had good dietary habits. After the intervention, those who did not have good dietary habits reduced to 30.7%, and those who had good dietary habits increased to 69.3%. According to physical activity, it shows that 45.3% of respondents did not have good physical activity before giving the intervention and 54.7% had good physical activity. After the intervention, those who did not have good physical activity reduced to 24.0%, and those who had good physical activity increased to 76.0%. In terms of the body mass index, 30.7% of respondents were not obese before giving the intervention and 69.3% were obese. After the intervention, those who were not obese increased to 42.7%, and those who were obese reduced to 57.3%.

Table 3. The Effect of DSME on variables of interest (n = 75)

Variable		Mean	Std deviation	Std error mean	p-value
Dietary habit	Before	1.56	0.500	0.058	0.024
	After	1.69	0.464	0.054	
Physical activity	Before	1.55	0.501	0.058	0.007
	After	1.76	0.430	0.054	
Obesity	Before	1.69	0.464	0.054	0.002
	After	1.57	0.498	0.057	

Table 3 explains the paired t-test results of the effectiveness of DSME on variables of interest. Based on dietary habits, the mean score increased from 1.56 to 1.69 and the significance was shown by a p-value of 0.024. According to physical activity, the mean score increased from 1.55 to 1.79 and the significance was shown by a p-value of 0.007. For the obesity status, the mean score decreased from 1.69 to 1.57 and the significance was shown by

a p-value of 0.002. In general, DSME is effective in improving dietary habits, improving physical activity, and reducing the number of obese.

DSME effect on dietary habit

The findings from this study revealed the influence of DSME on dietary habits (p-value < 0.05) and the improvement of dietary habits was a lot. The mechanism of effectiveness of DSME on dietary habits by enhancing understanding of the disease, leading to improved self-care practices and better glycemic (Iriana, 2022; Rusdiana et al., 2020; Utama et al., 2021). Additionally, DSME positively influences self-efficacy and self-care behaviors among individuals with type 2 diabetes including their foods (Fitriah et al., 2020; Wahyuni & Wahyuningsih, 2017). By implementing DSME routinely, dietary habits were improving too.

There are previous studies that examined the impact of diabetic self-management education (DSME) on dietary habits. The previous study explored the perspectives of individuals recently diagnosed with type 2 diabetes and health professionals on self-management of dietary intake and physical activity, emphasizing the importance of understanding patient views to tailor educational interventions effectively (Booth et al., 2012). Another study conducted a systematic review focusing on cultural influences on dietary self-management of type 2 diabetes in East Asian Americans, found the significance of cultural perspectives in shaping dietary habits and the necessity for culturally sensitive education interventions (Li-Geng et al., 2020). Additionally, Willig et al., (2014) conducted a qualitative study on intuitive eating practices among African-American women with type 2 diabetes, suggesting that programs focusing on enhancing self-efficacy and dietary habits could improve glycemic control (Hayek et al., 2013; Willig et al., 2014). Furthermore, one study focused on implementing an education program for type 2 diabetes patients, demonstrating significant enhancements in dietary plans, physical exercise, and adherence to self-care after six months of intervention (Hayek et al., 2013). In line with this study, another study emphasized the effectiveness of culturally competent dietary self-management interventions in improving health outcomes for southern African Americans, particularly those at risk due to high-fat diets (Anderson-Loftin et al., 2005; Zhang et al., 2019). Additionally, supporting the findings of this study, another study developed and evaluated an internet-based diabetes nutrition education resource, underscoring the importance of empowering individuals to make positive dietary choices for diabetes management (Zhang et al., 2019). Furthermore, S.-K. Lee et al., (2019) focused on diabetes education through pattern management, mentioned the role of appropriate dietary habits in enhancing self-care and self-efficacy in patients with type 2 diabetes (Lee et al., 2019; Sami et al., 2020). Similarly, to this study, research conducted a cross-sectional study on the dietary attitudes of adults with type 2 diabetes in Saudi Arabia, stressing the necessity for tailored dietary self-management strategies for improved diabetes care (Sami et al., 2020).

Effect on physical activity

The result of this study found the positive influence of DSME on physical activity (p-value <0.05). The impact of DSME is empowering individuals to take charge of their health and make informed decisions regarding their diabetes management (Fitriah et al., 2020; Wahyuni & Wahyuningsih, 2017). Additionally, DSME provides person-centered interventions to support patients, ensuring that individuals receive comprehensive care and education tailored to their specific needs (Mamed & Gastaldi, 2022; Powers et al., 2015; Sherr & Lipman, 2015). Individuals with DM type II could be able to manage their health behavior, including physical activity.

The impact of diabetic self-management education on physical activity has been extensively studied. Similar to the findings of this study, Nakawatase et al., (2007) highlighted the importance of regular physical activity for type 2 diabetic patients to improve metabolic

disorders and prevent complications such as cardiovascular disease. Additionally, Sadeghian et al., (2016) and Hamidi et al., (2022) demonstrated the positive effects of self-management educational programs on physical activity levels and quality of life in diabetic patients. Aljuhani et al., (2022) further emphasized the role of self-management behaviors, including physical activity/exercise, in enhancing self-care among individuals with diabetes. Moreover, Amer et al., (2018) found that patients with high self-efficacy in managing physical exercise activities were more adherent to exercise regimens, indicating the influence of self-efficacy on self-care activities, including physical activity. Additionally, Uly et al., (2022) explored the relationship between self-care behavior and diabetes self-management education, underlining the importance of physical activity/sports as part of self-management in patients with type 2 diabetes. Moreover, (Juarez et al., 2021) highlighted the mediating role of self-efficacy in the association between diabetes education and support and self-care management, including physical activity.

Effect on Obesity

The statistical test result from this study found a significant correlation between DSME and obesity. The process might be due to the improvement in dietary habits and physical activity. Additionally, DSME provides support and education on which necessary skills and behaviors to manage their condition effectively (Powers et al., 2015). Through the self-management system, DSME emphasizes the role of individuals themselves to control and manage their diabetes. As a result of long-term DSME implementation, the improvement in body mass index (BMI) could be measured.

The impact of diabetic self-management education on obesity as found in this study, has similar findings with several previous studies. The study by Norris et al., (2002) emphasized the significance of diabetes self-management education in clinical practice, highlighting its role in empowering individuals to effectively manage their condition. Additionally, (Boles et al., 2017) stressed the substantial contribution of obesity to the development of pre-diabetes and diabetes, underlining the importance of addressing weight management in diabetes care. Furthermore, (Fan et al., 2019) conducted a randomized controlled trial in China, illustrating that a diabetes education program significantly enhanced self-management levels, reduced psychological distress, and improved glycemic control in patients with type 2 diabetes. Supporting this study, (Miller et al., 2016) examined diabetes education, specialty care, and self-care advice among obese African American women with type 2 diabetes, shedding light on the care and education patterns in high-risk populations. Collectively, these studies suggest that diabetes self-management education plays a crucial role in addressing obesity and its implications in individuals with diabetes.

In general, the implementation of Diabetic Self-Management Education is effectively improving dietary habits, physical activity, and obesity status. The findings of this study could be generalized to other settings and times. The role of family and health care are important to control the consistency of individuals.

4. CONCLUSION

DSME has a significant effect on dietary habits, physical activity, and obesity. The DSME could affect those three components through the self-centered by providing support and knowledge to the patient. It focused on self-care activities including improved glycemic stability, quality of life, self-efficacy, and reduction of complications and depressive symptoms. Future studies can include the qualitative approach of family contribution to support DSME implementation and consistency. This study is limited to a sample in the small scope of primary health care, so the result cannot be generalized to other times and places.

REFERENCES

- Aljuhani, F. A., Aloqbi, W. J., Alshakhs, I. A., Ali, H. B. A., Abbad, B. E. A., Qari, A. A., Alburaidi, O. M., Aldablan, A. S., Alruwaili, A. N., Alharbi, F. F., & Alghamdi, A. A. (2022). Behaviours and Educational Effect on the Improvement of Self-Care Among the Diabetics. *International Journal of Community Medicine and Public Health*, 9(11), 4241. <https://doi.org/10.18203/2394-6040.ijcmph20222615>
- Amer, F. A., Mohamed, M. S., Elbur, A. I., Abdelaziz, S. I., & Elrayah, Z. A. B. (2018). Influence of Self-Efficacy Management on Adherence to Self-Care Activities and Treatment Outcome Among Diabetes Mellitus Type 2 Sudanese Patients. *Pharmacy Practice*, 16(4), 1274. <https://doi.org/10.18549/pharmpract.2018.04.1274>
- Anderson-Loftin, W., Barnett, S., Bunn, P. S., Sullivan, P., Hussey, J. R., & Tavakoli, A. (2005). Culturally Competent Diabetes Education. *The Diabetes Educator*, 31(4), 555–563. <https://doi.org/10.1177/0145721705278948>
- Boles, A., Kandimalla, R., & Reddy, P. H. (2017). Dynamics of Diabetes and Obesity: Epidemiological Perspective. *Biochimica Et Biophysica Acta (Bba) - Molecular Basis of Disease*, 1863(5), 1026–1036. <https://doi.org/10.1016/j.bbadis.2017.01.016>
- Booth, A., Lewis, C., Dean, M., Hunter, S., & McKinley, M. C. (2012). Diet and Physical Activity in the Self-Management of Type 2 Diabetes: Barriers and Facilitators Identified by Patients and Health Professionals. *Primary Health Care Research & Development*, 14(03), 293–306. <https://doi.org/10.1017/s1463423612000412>
- Dewiyanti, N. P. I. M. (2022). Gambaran Kepatuhan Pola Makan Pada Pasien Diabetes Melitus Tipe 2 di Wilayah Kerja Puskesmas I Denpasar Barat. *INSTITUT TEKNOLOGI DAN KESEHATAN BALI DENPASAR 2022*.
- Fan, Z., Liu, S., Liu, Y., & Deng, L. (2019). Effects of an Outpatient Diabetes Self-Management Education on Patients With Type 2 Diabetes in China: A Randomized Controlled Trial. *Journal of Diabetes Research*, 2019, 1–7. <https://doi.org/10.1155/2019/1073131>
- Fitria, N., Rizana, N., Andala, S., Mursal, & Afifah, D. (2023). Effect of Diabetes Self-Management Education on the Prevention of Diabetic Foot Injuries. *International Journal of Research and Innovation in Applied Science*, VIII(II), 96–100. <https://doi.org/10.51584/ijrias.2023.8203>
- Fitriah, F., Haris, M., Mufarika, M., Megawati, C. D., Suryaningsih, S., & Rodyatun, R. (2020). The Influence of Diabetes Self Management Education and Support for Self Efficacy and Self Care Behaviour in Client With Type 2 Diabetes Mellitus. *International Journal of Nursing and Midwifery Science (Ijnms)*, 4(1), 100–111. <https://doi.org/10.29082/ijnms/2020/vol4/iss1/247>
- Funnell, M. M., Tang, T. S., & Anderson, R. M. (2007). From DSME to DSMS: Developing Empowerment-Based Diabetes Self-Management Support. *Diabetes Spectrum*, 20(4), 221–226. <https://doi.org/10.2337/diaspect.20.4.221>
- Hamidi, S., Gholamnezhad, Z., Kasraie, N., & Sahebkar, A. (2022). The Effects of Self-Efficacy and Physical Activity Improving Methods on the Quality of Life in Patients With Diabetes: A Systematic Review. *Journal of Diabetes Research*, 2022, 1–14. <https://doi.org/10.1155/2022/2884933>
- Hayek, A. A. A., Robert, A. A., Dawish, M. A. A., Zamzami, M., Sam, A. E., & Alzaid, A. (2013). Impact of an Education Program on Patient Anxiety, Depression, Glycemic Control, and Adherence to Self-Care and Medication in Type 2 Diabetes. *Journal of Family and Community Medicine*, 20(2), 77. <https://doi.org/10.4103/2230-8229.114766>
- IDF. (2019). *Global Diabetes Data Report 2010-2045*. International Diabetes Federation.
- Iriana, P. (2022). How Is the SelfCare Management of Patients With Type II Diabetes Mellitus? - Characteristics and Knowledge. *Asian Journal of Pharmaceutical Research and Development*, 10(5), 1–5. <https://doi.org/10.22270/ajprd.v10i5.1171>

- Juarez, L., Presley, C. A., Howell, C. R., Agne, A. A., & Cherrington, A. (2021). The Mediating Role of Self-Efficacy in the Association Between Diabetes Education and Support and Self-Care Management. *Health Education & Behavior*, 49(4), 689–696. <https://doi.org/10.1177/10901981211008819>
- Lawless, M. E., Kanuch, S., Martin, S., Kaiser, D., Blixen, C., Fuentes-Casiano, E., Sajatovic, M., & Dawson, N. V. (2016). A Nursing Approach to Self-Management Education for Individuals With Mental Illness and Diabetes. *Diabetes Spectrum*, 29(1), 24–31. <https://doi.org/10.2337/diaspect.29.1.24>
- Lee, S.-K., Shin, D., Kim, Y. H., & Lee, K. (2019). Effect of Diabetes Education Through Pattern Management on Self-Care and Self-Efficacy in Patients With Type 2 Diabetes. *International Journal of Environmental Research and Public Health*, 16(18), 3323. <https://doi.org/10.3390/ijerph16183323>
- Li-Geng, T., Kilham, J., & McLeod, K. M. (2020). Cultural Influences on Dietary Self-Management of Type 2 Diabetes in East Asian Americans: A Mixed-Methods Systematic Review. *Health Equity*, 4(1), 31–42. <https://doi.org/10.1089/hec.2019.0087>
- Mamed, M. D. S., & Gastaldi, G. (2022). COVID: The Missing Trigger to Start a Remote FIT Course. *Frontiers in Clinical Diabetes and Healthcare*, 3. <https://doi.org/10.3389/fcdhc.2022.834082>
- Miller, S. T., Cunningham-Erves, J., & Akohoue, S. A. (2016). Diabetes Education, Specialty Care, and Self-Care Advice Among Obese African American Women With Type 2 Diabetes. *Ethnicity & Disease*, 26(2), 229. <https://doi.org/10.18865/ed.26.2.229>
- Ministry of Health Indonesia. (2018). *Report of National Basic Health Research 2018*. Jakarta: Ministry of Health Indonesia.
- Ministry of Health Indonesia. (2024). *Body Mass Index (BMI) cut off*. Jakarta: Ministry of Health Indonesia.
- Ministry of Health Republic of Indonesia. (2019). *Indonesia Health Profile 2019*. Jakarta: Ministry of Health Indonesia.
- Nakawatase, Y., Taru, C., Tsutou, A., Shiotani, H., Kido, Y., Ohara, T., Ogawa, W., & Miyawaki, I. (2007). Development of an Evaluation Scale for Self-Management Behavior Related to Physical Activity of Type 2 Diabetic Patients. *Diabetes Care*, 30(11), 2843–2848. <https://doi.org/10.2337/dc07-0685>
- Norris, S. L., Lau, J., Sj, S., Schmid, C. H., & Engelgau, M. M. (2002). Self-Management Education for Adults With Type 2 Diabetes. *Diabetes Care*, 25(7), 1159–1171. <https://doi.org/10.2337/diacare.25.7.1159>
- PERKENI. (2015). *Konsensus pengelolaan dan pencegahan diabetes melitus tipe 2*. Perkumpulan Endokrinologi Indonesia.
- Powers, M. A., Bardsley, J. K., Cypress, M., Duker, P., Funnell, M. M., Fischl, A. H., Maryniuk, M. D., Siminerio, L. M., & Vivian, E. (2015). Diabetes Self-Management Education and Support in Type 2 Diabetes: A Joint Position Statement of the American Diabetes Association, the American Association of Diabetes Educators, and the Academy of Nutrition and Dietetics. *Diabetes Care*, 38(7), 1372–1382. <https://doi.org/10.2337/dc15-0730>
- Rusdiana, R., Maya, S., Widjaja, S. S., & Ardinata, D. (2020). The Effect of Health Education on Control Glycemic at Type 2 Diabetes Mellitus Patients. *Open Access Macedonian Journal of Medical Sciences*, 8(E), 133–137. <https://doi.org/10.3889/oamjms.2020.3371>
- Sadeghian, H. A., Madhu, S. V., Agrawal, K., Kannan, A. T., & Agrawal, K. (2016). Effects of a Self-Management Educational Program on Metabolic Control in Type 2 Diabetes. *Turkish Journal of Medical Sciences*, 46, 719–726. <https://doi.org/10.3906/sag-1501-115>
- Sami, W., Alabdulwahhab, K., Hamid, M. R. A., Alasbali, T., Alwadani, F. A., & Ahmad, M.

- S. (2020). Dietary Attitude of Adults With Type 2 Diabetes Mellitus in the Kingdom of Saudi Arabia: A Cross-Sectional Study. *Medicina*, 56(2), 91. <https://doi.org/10.3390/medicina56020091>
- Sherr, D., & Lipman, R. D. (2015). The Diabetes Educator and the Diabetes Self-Management Education Engagement. *The Diabetes Educator*, 41(5), 616–624. <https://doi.org/10.1177/0145721715599268>
- Tamayo, R., & Reyes, K. (2022). Acceptability of Task Shifting Primary Care Diabetes Self-Management Education Services to Volunteer Barangay Health Workers in a Philippine City. *Acta Medica Philippina*, 57(12). <https://doi.org/10.47895/amp.vi0.6316>
- Uly, N., Fadli, F., & Iskandar, R. (2022). Relationship Between Self-Care Behavior and Diabetes Self-Management Education in Patients With Diabetes Mellitus Type 2. *Open Access Macedonian Journal of Medical Sciences*, 10(E), 1648–1651. <https://doi.org/10.3889/oamjms.2022.10879>
- Utama, R. D., Indasah, I., & Layla, S. F. N. (2021). The Effect of Diabetes Self-Management Education (DSME) on Improving Self-Management and Quality of Life in Millitus Type 2 Diabetes. *Journal for Quality in Public Health*, 4(2), 31–37. <https://doi.org/10.30994/jqph.v4i2.176>
- Wahyuni, L., & Wahyuningsih, B. D. (2017). Effect of Diabetes Self Management Education (Dsme) on Knowledge on Control of Blood Sugar on Diabetes Mellitus Patients. *International Journal of Nursing and Midwifery Science (Ijnms)*, 1(1), 74–82. <https://doi.org/10.29082/ijnms/2017/vol1/iss1/43>
- Willig, A. L., Richardson, B. S., Agne, A. A., & Cherrington, A. (2014). Intuitive Eating Practices Among African-American Women Living With Type 2 Diabetes: A Qualitative Study. *Journal of the Academy of Nutrition and Dietetics*, 114(6), 889–896. <https://doi.org/10.1016/j.jand.2014.02.004>
- World Health Organization. (2012). Global physical activity questionnaire (GPAQ) analysis guide. World Health Organization, .
- Zhang, Z., Monro, J., & Venn, B. (2019). Development and Evaluation of an Internet-Based Diabetes Nutrition Education Resource. *Nutrients*, 11(6), 1217. <https://doi.org/10.3390/nu11061217>

Jurnal Info Kesehatan

Vol. 22, No. 2, June 2024, pp. 378-386

P-ISSN 0216-504X, E-ISSN 2620-536X

DOI: [10.31965/infokes.Vol22.Iss2.1489](https://doi.org/10.31965/infokes.Vol22.Iss2.1489)

Journal homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>



RESEARCH

Open Access

Effectiveness of Hae-Band in Measuring Hb Levels in Postpartum Hemorrhage Risk Monitoring

Dinda Dian Meidita^{1a*}, Krisdiana Wijayanti^{1b}, Heni Hendriyani^{1c}

¹ Midwifery Study Program, Applied Master Program, Poltekkes Kemenkes Semarang, Semarang, Central Java, Indonesia

^a Email address: dindameidita00@gmail.com

^b Email address: wijayanti.k@hotmail.com

^c Email address: heni_sahid@yahoo.com

Received: 12 May 2024

Revised: 30 June 2024

Accepted: 30 June 2024

Abstract

Postpartum hemorrhage is the main cause of high morbidity in the world (75%). Until now, efforts to early detect the risk of postpartum hemorrhage have still not been maximized. Sensor-based smartband can be the development of non-invasive methods in an effort to early detection of declining Hb levels in monitoring the risk of bleeding with practical, fast, precise, accurate, and practical. This research aims to determine the effectiveness of developing and analyzing the effectiveness of the "Hae-band" smartband in monitoring the risk of postpartum hemorrhage. The research method used is Research and Development (R&D), a quasi-experimental one-group pretest-posttest design using a nonequivalent dependent variable. The sampling technique in this research uses non-probability sampling with purposive sampling type. Respondents were selected by purposive sampling with a sample of 35 respondents maternity up to 6 hours postpartum to measure Hb levels and analyze the average levels of Hb and declining levels of HB Hae-band, HB meter POCT and visual estimation of blood loss volume compared with HB meter POCT (gold standard). Data analysis using the descriptive test, validation test, Paired T-Test, Independent T-Test, and multiple linear regression. The research results show that the Hae-band has been developed and is feasible as a measure of Hb levels which has a sensitivity of truth tool as much as 70.0% and can detect a decrease in Hb levels as much as 63.9% ($p=0.000$) more partially effective than visual estimation (0.24%) Smartband can detect Hb levels with good results compared to the gold standard of blood tests with a difference of 0.324 g/dL ($p.0.113$) with an average Hb levels at 6 hours PP at 11.19 g/dL compared to the average POCT 11.34 g/dL. This research concludes that Hae-band is more effective in detecting postpartum hemorrhage risk than visual estimation. It is hoped that further research can develop a more modern design where the sensor is more comfortable to use for long periods and has an alternative way of reading the sensor other than on the wrist. Improving accuracy, sensitivity, and higher battery power by improving the type of design, sensor, and wave type to be more suitable for measuring Hb levels can also be developed considering the efficiency of maternal monitoring and recording in the era of digitalization in early detection of bleeding risk.

Keywords: Hae-band, Hb level, Visual Estimation, Postpartum Hemorrhage Risk.

*Corresponding Author:

Dinda Dian Meidita

Midwifery Study Program, Applied Master Program, Poltekkes Kemenkes Semarang, Semarang, Central Java, Indonesia

Email: dindameidita00@gmail.com



©The Author(s) 2024. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

1. INTRODUCTION

The maternal mortality rate in developing countries is 430 per 100,000 live births compared to 12 per 100,000 live births in developed countries. 94% of maternal deaths in developing countries are caused by bleeding, including bleeding originating from inside the uterus (80-90%), lacerations (10-20%) and coagulopathy (<1%) with as many as 27% of maternal deaths caused by postpartum hemorrhage (James et al., 2022).

Post-partum hemorrhage is the main cause of high maternal morbidity and mortality in most countries in the world. (WHO, 2022) Approximately 14 million women experience bleeding every year, resulting in 70,000 maternal deaths worldwide. Postpartum hemorrhage is the main cause of maternal mortality in developed and developing countries. (James et al., 2022) The number of maternal deaths in Indonesia has increased from 4,627 in 2020 to 7,389 in 2021 with hemorrhage contributing 1,330 cases of the total number of cases (Kementerian Kesehatan Republik Indonesia, 2022).

The maternal mortality rate in East Java is around 184 deaths per 100,000 live births in the 2020 LF SP results. Jember Regency is the district with the highest number of death cases in East Java with 115 cases and 61 cases of postpartum maternal deaths with 9.62% caused by bleeding (Dinas Kesehatan Jawa Timur, 2021). Maternal deaths in Jember Regency in 2022 amounted to 28 pregnant women due to pre-eclampsia - eclampsia (31%), bleeding (25%), infection (12%) and prolonged labor (6%) (Dinas Kesehatan Kabupaten Jember, 2022).

Death due to bleeding can be avoided with the correct diagnosis time, right resources, and right management. The reduction in the death rate due to bleeding is proof that the treatment and prevention of bleeding has been carried out well (Adisasmita, 2017). However, the impact of postpartum hemorrhage is not only the threat of death, morbidity resulting from postpartum hemorrhage such as anemia, fatigue, depression, and the risk of blood transfusions also endanger the mother's condition (Simanjuntak, 2020).

The government in its efforts to reduce the incidence of mortality and morbidity in pregnant, giving birth and postpartum women, especially in reducing the number of postpartum hemorrhages to date, is by innovating a health service system that prioritizes active management of the third stage by ensuring that there are health workers who are trained and expert in the management of the third stage, advocacy for the availability of oxytocin, the existence of protocols for preventing and treating bleeding cases, as well as monitoring to detect bleeding events (Putra et al., 2020).

Monitoring blood loss is one effort that can be done to prevent complications from the risk of bleeding (Diaz et al., 2018). Visual estimation is a common method currently applied throughout the world to measure blood loss after delivery. However, the use of this visual method is considered less accurate in determining the amount of blood loss because blood can be mixed with amniotic fluid or urine, making it difficult for visual assessments to be carried out by health workers (Bell et al., 2020).

Previous research shows that visual estimation has a low rate of objectivity at 9.6% in detecting blood loss. This really depends on the skills and experience of the monitoring health worker. Therefore, an objective and accurate method is needed to monitor the risk of bleeding (Sada et al., 2021).

Measuring Hb levels is an examination method that is felt to have greater value in monitoring the risk of postpartum hemorrhage compared to other invasive and non-invasive methods. Hb levels are the main component in transporting oxygen throughout the body and can indicate anemia and blood deficiency (Ningsi et al., 2023).

The American College of Obstetricians and Gynecologists states that quantitative methods have been proven to be more accurate than visual estimates. This method is Gravimetry. This method is carried out by weighing the item to calculate the difference in weight of the item before and after use with an estimate of the volume of blood lost equivalent to the weight of the blood weighed (1 gram = 1 mL) (Katz & Farber, 2021). According to the study, the gravimetric

method considers external blood loss whose conversion is only an estimate. The absence of separation between blood and fluid results in inaccurate measurements (Thurer et al., 2022).

The new prevention that has been developed is the use of technology that supports artificial intelligence which uses cellular technology and digital imaging algorithms using cameras to take images for analysis and measure hemoglobin and blood loss in real time (Obuna et al., 2020).

Appropriate technology in the health sector is currently experiencing rapid development. One technology that is considered capable of monitoring the risk of bleeding practically, quickly, precisely and accurately is the use of an oximeter sensor to monitor hemoglobin levels and oxygen saturation non invasively, continuously, and in real-time during labor. This method can be applied to smartbands (Hasan et al., 2021).

Checking Hb levels using a smartband is considered more practical and effective, including having a more compact form and being easy to carry anywhere without worrying about large storage space, which can be used at any time and does not require a long reading of the results. (Wisana et al., 2022). This research aims to determine the effectiveness of developing and analyzing the effectiveness of the "Hae-band" smartband in monitoring the risk of postpartum hemorrhage.

2. RESEARCH METHOD

The research method used is Research and Development (R&D), a quasi-experimental one-group pretest-posttest design using a nonequivalent dependent variable. The method to improve active monitoring for postpartum mothers to detect postpartum bleeding. In this developed application there are: sensor feature (oximeter) that can measure hemoglobin levels to monitor the risk of bleeding before delivery and every 2 hours and 6 hours after delivery.

Research procedures in categories R&D consist of five procedures to produce final products so they are ready to be applied in the delivery of health services. The stages used are the adoption of a modified Borg and Gall model (Sugiyono, 2022). This research was carried out in the area of Summersari Jember Community Health Center for a period of 1 month, starting in December 2023.

The population in this study were 3rd trimester pregnant women with birth estimate between December 2023 and January 2024 with a total of 35 pregnant women. The sample for this study was women giving birth up to 6 hours postpartum in the working area of the Summersari Jember health center. The sampling technique in this research uses non-probability sampling with purposive sampling type. The sample size in this study was calculated using the Lemeshow formula, resulting in a sample size of 35 respondents

The independent variables in this study are Hae-band and Hb Meter PCT. The dependent variables are Hb levels and blood output volume. Data collection techniques use primary data and secondary data. Primary data was obtained from interviews with respondents and the results of checking Hb levels using tools, while secondary data was obtained from world, national, provincial, and regional data from the research location.

Univariate data analysis in the form of the frequency distribution of subject characteristics based on age, parity, history of anemia, and pregnancy interval. Analysis of bivariate data from Hae-band and Hb meter POCT examinations in this study used the paired-t test and independent t-test.

This research has received research ethics permission Number 1336/EA/KEPK/2023.

3. RESULTS AND DISCUSSION

Table 1. Respondent Characteristics

Characteristics	Frequency (f)	Percentage (%)
Age		
< 20year	3	8.6
20 –35 years old	31	88.6
> 35year	1	2.9
Amount	35	100
Parity		
Nulliparous	9	25.7
Primipara	16	45.7
Multiparous	10	28.6
Amount	35	100
Pregnancy Distance		
First time pregnant	9	25.7
< 2year	1	2.9
2-10year	25	71.4
Amount	35	100

Table 1 shows that the majority of respondents are at the ideal age for giving birth between 20-35 years, namely 31 people (88.6%). The majority of respondents had only given birth to 1 child (Primipara), namely 16 people (45.7%) and the majority had an ideal birth spacing of 2-10 years, 25 people (71.4%).

Table 2. Results of different Hb level tests using Hae-band before giving birth and after 6 hours postpartum

Variable	N	Mean±SD	Correlation	Mean	Sig.
Hb Hae-band	Pretest	35 11.79 ± 0.774	0.891	0.651	0,000
	Posttest	35 11.19 ± 0.945			

*Paired sample T-Test

Based on Table 2, it is found that the average difference in Hb levels between before giving birth and 6 hours postpartum is 0.651 g/dL. The results of the significant value from the difference test for Hb levels before and after 6 hours postpartum are <0.05, which indicates that there is a significant difference in the results of Hb levels before giving birth and at 6 hours postpartum.

Table 3. Analysis results of differences in Hb level results between the smartband and POCT Hb meter

Method	n	Q	Mean different	Std.	Sig.
Hb level	Hb meter POCT	35	1,606	21,350	0.113
	Hae-band	35	1,606		

Based on Table 3, it is known that the significance value of POCT and Hae-band Hb meter levels is 0.113, which is greater than 0.05, with an average of 0.342. So, according to the independent T-Test decision-making, it was found that there was no significant (real) difference between checking Hb levels using POCT and Hae-band Hb meters.

Table 4. Hae-band Partial Effectiveness Test Results and Visual Estimates of Hb Meter POCT

Method	Beta	Coefficient Correlation	SE(%)	R Square	p-value
Hae-band	0.788	0.812	63.9	0.664	0,000
Visual Estimation	-0.079	-0.316	0.24		

Table 4 show that The influence of Hae-band with Hb meter POCT as the gold standard for detecting Hb levels showed a result of 63.9%. Meanwhile, the magnitude of the partial effect of visual estimation in detecting a decrease in Hb levels with the POCT Hb meter shows a figure of 0.24% or in other words, it has an effectiveness of 0.24%. This shows that Hae-band is more effective in detecting a decrease in Hb levels in an effort to detect early postpartum risk compared to visual estimation, although both methods can be used simultaneously to produce better predictions of a decrease in Hb levels.

DISCUSSION

Characteristic Analysis Respondents Based on Age, Parity and Pregnancy Distance as Confounding Variables for Hae-band.

Age

The results of the research analysis showed that the average age of the respondents was obtained is reproductive age, namely 20-35 years (88.6%). Based on Ersalina's report, it was found that age has a relationship with Hb levels, where age \leq 20 years tends to low Hb level data, this condition will change slowly with increasing age where Hb levels will tend to increase at age $>$ 20 years and will decrease again when entering advanced age (\geq 50 years) (Nidianti et al., 2019).

This is not in line with the results of further analysis where this study shows that there is no influence between age and the results of Hb levels using Hae-band (sig. 0.141) where there is only a low relationship between age and the results of Hb levels that can be detected (0.249) (Muhammad, 2012).

Parity

In this study, it was found that 9 people (25.7%) were pregnant for the first time and had never given birth before, namely 16 people gave birth to 1 child (Primipara) (45.7%), and 10 people (28.6%) were multipara. Of the 10 respondents, 3 people had a parity number of 4, and the others $<$ 4. The parity number shows the number of births that resulted in live or dead fetuses. Previous research conducted on Nulliparous respondents (women who have never given birth) using Co-Oximetry (SpHb) which was compared with blood tests in the laboratory, revealed that SpHb results tend to be higher than laboratory test results with an average bias of 1.22–1.36 g/dL (Alfiana et al., 2019).

Pregnancy Spacing

The majority of respondents in the study had ideal pregnancy spacing, namely $>$ 2 years and $<$ 10 years. The distance between pregnancies cannot directly affect the ability to detect Hb levels non-invasively, whether using sensor-based or device monitoring methods such as IoT (Internet of Things), but the distance between pregnancies can influence Hb levels in the blood, which can result in anemia or not (Siregar et al., 2023).

Hae-band can detect Hb levels at various sizes of pregnancy intervals. This is shown by the results of the analysis of this study where the distance between pregnancies has no influence on the results of Hb levels using Hae-band (sig. 0.679). This can be seen from respondents who

were pregnant for the first time and gave birth, finding that the Hb level by the smartband was 11.4 g/dL before giving birth and 11.2 g/dL after 6 hours of giving birth. This result is only 0.2 g/dL lower than measurements using a POCT Hb meter. This difference may occur due to technical factors related to data collection, calibration, and other factors besides gestational spacing (Liu et al., 2020).

Development and Feasibility of The Hae-band as a Tool For Measuring Hb Levels in Mothers Giving Birth Up to 6 Hours Postpartum

The Hae-band design has been successfully developed with the average difference compared to the POCT Hb meter as the gold standard for examination being 0.325 and resulting in a true sensitivity of the Hae-band tool in detecting Hb levels of 70.0% and an error rate of 30% in its ability to measure hemoglobin levels. non-invasively. This technique refers to the work of the *Photoplethysmograph* (PPG) which is a non-invasive method with the main principle of shining two different wavelengths of light through tissue and comparing the way the blood absorbs light below these wavelengths in the blood solution (Jahan et al., 2014). This research does not require any invasive blood samples, so it does not cause pain because the Hae-band uses a MAX30102 oximetry sensor which can process data on hemoglobin levels in the blood by detecting oxyhemoglobin (O₂Hb) and deoxyhemoglobin (HHb) in the bloodstream via wavelengths (Kemalasari & Rochmad, 2022).

Comparative Analysis of Measuring Hb Levels Using Hae-band (Non-Invasive) with Hb Meter POCT (Invasive)

This research shows that there are differences in the results of measuring Hb levels before giving birth to 6 hours postpartum using both Hae-band and POCT Hb meters. This can be detected using both invasive and non-invasive Hb-level examination tools. The difference in results between Hae-band and Hb meter POCT can be based on several reasons, namely the use of equipment when collecting data that is less than optimal or other factors from the respondents. Even though there are differences, it is felt that the Hae-band can further improve alert conditions in detecting the risk of postpartum hemorrhage considering that the Hae-band gets a lower average measurement result than the Hb meter POCT which anticipates the lowest measurement of a decreased in Hb levels which can result in the risk of postpartum hemorrhage.

Analysis of the average results of an examination of postpartum maternal Hb levels up to 6 hours of research using Hae-band.

In this study, the average result of postpartum maternal Hb level examination before giving birth or during the first stage of labor using the "Hae-band" Smartband was 11.79 g/dL. Normal Hb levels in pregnant women who are preparing to give birth are > 11 g/dL and it can be said that they are not anemic. Low Hb levels before delivery can cause problems and complications such as: bleeding to death during childbirth, requiring treatment increased consumption of iron-rich intake, and administration of additional pregnancy supplements (Pratama et al., 2020).

The mean Smartband "Haeband" Hb level in respondents 6 hours after delivery was 11.04 g/dL. Normal Hb levels after giving birth for each woman vary depending on the individual's specific conditions, where generally normal Hb levels are in the range of 12-16 g/dL. However, generally mothers in labor experience a significant decrease in Hb levels after 6-48 hours of giving birth. This is in line with other research by Fasiha et al which states that the average decrease in Hb levels in pregnant women with normal delivery is 1.2 g/dL, which is a normal condition. This decrease occurs in normal circumstances after a normal delivery process with blood loss <500 ml (Fasiha et al., 2022).

Analysis of the Effectiveness of Using Hae-band Compared with Visual Estimation Methods in Monitoring The Risk Of Postpartum Hemorrhage.

In this study, it was found that visual estimation was effective with an influence size of 0.24%, while Hae-band development had an effectiveness of 63.9% in predicting the results of invasive reduction in hemoglobin levels on Hb meter using the POCT method with a simultaneous influence size of 66.4% as standard. gold inspection. This shows that the Hae-band is more effective in predicting a decrease in hemoglobin levels in an effort to monitor the risk of postpartum Hemorrhage (Aminuddin, 2021). Several researchers have stated that the visual estimation method is less accurate in detecting postpartum bleeding and requires objective measurement methods (Julieta & Giri, 2021).

This Hae-band series uses the Max30102 oximeter sensor which has been carried out in previous research. The results of measuring the tool using this sensor have an accuracy level of up to 94.91% in measuring Hb levels and 98.99% in measuring oxygen levels in the body as an infrared light emitter and photodetector because this type of sensor has low noise so it is easy to position (Muthmainnah & Tabriawan, 2022).

The working principle of the sensor in detecting Hb levels in this research is to use the PPG method, where when the LED light is turned on it will emit a light signal, when attached to the tissue it will enter the capillary blood vessels and there will be a change in the light intensity captured by the photodetector. These changes will be processed into data on the results of the respondent's Hb levels (Karina & Thohari, 2018).

In this research, there are several limitations related to tools and technical implementation, namely the absence of repeated testing on extreme data during use trials. The time for collecting pretest data on maternal Hb levels tends to be difficult controlled considering that the pretest examination was carried out in the 1st stage of labor. There are women in early 2nd stage labor who are checked because they consider the time or arrival of the patient's complete opening. This can affect the interpretation of Hb level results due to rushed data collection techniques. Reading Hb levels still uses a button by pressing it for approximately 2 seconds, this can result in pressing the button incorrectly and increase the time for collecting examination data to be longer. Use for a long time requires more power because the Haeband smartband uses a 3.7 volt battery which requires frequent but short charging times each time to avoid wear (weakening of function) in the battery which is adapted to the sensor.

4. CONCLUSION

The Hae-band has been developed as a tool to measure Hb levels as an additional instrument in an effort to early detect the risk of postpartum hemorrhage. The Hae-band was declared suitable as a tool for measuring Hb levels in mothers giving birth up to 6 hours postpartum with a tool sensitivity of 70.0% There was no significant difference between the results of Hae-band measurements (Non-invasive) and Hb meter blood collection using the POCT method (sig. 0.113) and the average value difference was 0.324 g/dL. The average hemoglobin level before delivery was 11.79 g/dL while the average hemoglobin level 6 hours after delivery was 11.19 g/dL. The Hae-band was more effective than visual estimation in monitoring the risk of postpartum hemorrhage by 63.9% by showing a very strong correlation level with the Hb meter POCT method as the gold standard for examination ($r=0.78$).

It is hoped that further research can develop a more modern design where the sensor is more comfortable to use for long periods and has an alternative way of reading the sensor other than on the wrist. Improving accuracy, sensitivity and higher battery power by improving the type of design, sensor and wave type to be more suitable for measuring Hb levels can also be developed considering the efficiency of maternal monitoring and recording in the era of digitalization in early detection of bleeding risk.

REFERENCES

- Adisasmita, A. C. (2018). Pengalaman Review Kematian Ibu Dengan Sebab Kematian Pendarahan Pasca Persalinan Menggunakan Metode Confidential Enquiry Into Maternal Death (CEMD). (Patent No. EC00201824152). Universitas Indonesia.
- Alfiana, R. D., Zakaria, H., Shahib, M. N., & Susanto, H. (2019). Accuracy of Hemoglobin Measurement Using Noninvasive Oxyhemoglobinometer in Pregnant Women at Health Center of Bantul District. *Jurnal Ners dan Kebidanan Indonesia*, 6(1), 59-64. [https://doi.org/10.21927/jnki.2018.6\(1\).59-64](https://doi.org/10.21927/jnki.2018.6(1).59-64)
- Aminuddin, S. (2021). *Alat Pendeteksi Hemoglobin Non Invasive*. Diploma thesis. Universitas Widya Husada Semarang.
- Bell, S. F., Watkins, A., John, M., Macgillivray, E., Kitchen, T. L., James, D., ... & Collins, P. W. (2020). Incidence of postpartum haemorrhage defined by quantitative blood loss measurement: a national cohort. *BMC pregnancy and childbirth*, 20, 1-9. <https://doi.org/10.1186/s12884-020-02971-3>.
- Diaz, V., Abalos, E., & Carroli, G. (2018). Methods for blood loss estimation after vaginal birth. *Cochrane Database of Systematic Reviews*, (9). <https://doi.org/10.1002/14651858.CD010980.pub2>.
- Dinas Kesehatan Jawa Timur. (2021). *Profil Kesehatan Provinsi Jawa Timur*. Surabaya: Dinas Kesehatan Jawa Timur.
- Dinas Kesehatan Kabupaten Jember. (2022). *Profil Kesehatan Kabupaten Jember 2021*. Jember: Dinas Kesehatan Kabupaten Jember
- Fasiha, F., Wabula, W. M., & Nendissa, M. M. (2022). Pengaruh Persalinan Normal Terhadap Penurunan Kadar Hemoglobin Pada Ibu Postpartum Di Rumah Sakit Al Fatah Ambon. *Jurnal Kebidanan*, 2(1), 10-15.
- Hasan, M. K., Aziz, M. H., Zarif, M. I. I., Hasan, M., Hashem, M. M. A., Guha, S., ... & Ahamed, S. (2021). Noninvasive hemoglobin level prediction in a mobile phone environment: State of the art review and recommendations. *JMIR mHealth and uHealth*, 9(4), e16806.
- Jahan, E., Barua, T., & Salma, U. (2014). An overview on heart rate monitoring and pulse oximeter system. *Int. J. Latest Res. Sci. Technol*, 3(5), 148-152. <https://doi.org/10.33096/woh.v6i01.284>.
- James, A. H., Federspiel, J. J., & Ahmadzia, H. K. (2022). Disparities in obstetric hemorrhage outcomes. *Research and Practice in Thrombosis and Haemostasis*, 6(1), e12656. <https://doi.org/10.1002/rth2.12656>.
- Julieta, N. P. N., & Giri, M. K. W. (2021). Postpartum Hemorrhage: Kegawatdaruratan dalam Persalinan Ibu Hamil. *Ganesha Medicina*, 1(1), 48-65. <https://doi.org/10.23887/gm.v1i1.31709>.
- Karina, P., & Thohari, A. H. (2018). Perancangan Alat Pengukur Detak Jantung Menggunakan Pulse Sensor Berbasis Raspberry. *Journal of Applied Informatics and Computing*, 2(2), 57-61. <https://doi.org/10.30871/jaic.v2i2.920>.
- Katz, D., & Farber, M. K. (2021). Can measuring blood loss at delivery reduce hemorrhage-related morbidity? *International Journal of Obstetric Anesthesia*, 46, 102968. <https://doi.org/10.1016/j.ijoa.2021.102968>.
- Kemalasar, & Rochmad, M. (2022). Deteksi Kadar Saturasi Oksigen Darah (Spo2) Dan Detak Jantung Secara Non-Invasif Dengan Sensor Chip Max30100. *Jurnal Nasional Teknologi Terapan (JNTT)*, 4(1), 35-50. <https://doi.org/10.22146/jntt.v4i1.4804>.
- Kementerian Kesehatan Republik Indonesia. (2022). *Profil Kesehatan Indonesia 2021*. Kementerian Kesehatan Republik Indonesia.
- Liu, H., Peng, F., Hu, M., Shi, J., Wang, G., Ai, H., & Wang, W. (2020). Development and validation of a photoplethysmography system for noninvasive monitoring of hemoglobin

- concentration. *Journal of Electrical and Computer Engineering*, 2020(1), 3034260. <https://doi.org/10.1155/2020/3034260>.
- Muhammad, A. (2012). Rancang Bangun Sistem Pengukuran Kadar Hemoglobin Darah Berbasis Mikrokontroler. *Skripsi Tesis*. Universitas Airlangga.
- Muthmainnah, M., & Tabriawan, D. B. (2022). Prototipe Alat Ukur Detak Jantung Menggunakan Sensor MAX30102 Berbasis Internet of Things (IoT) ESP8266 dan Blynk. *JISKA (Jurnal Informatika Sunan Kalijaga)*, 7(3), 163–176. <https://doi.org/10.14421/jiska.2022.7.3.163-176>.
- Nidianti, E., Nugraha, G., Aulia, I. A. N., Syadzila, S. K., Suciati, S. S., & Utami, N. D. (2019). Pemeriksaan Kadar Hemoglobin dengan Metode POCT (Point of Care Testing) sebagai Deteksi Dini Penyakit Anemia Bagi Masyarakat Desa Sumbersono, Mojokerto. *Jurnal Surya Masyarakat*, 2(1), 29-34. <https://doi.org/10.26714/jsm.2.1.2019.29-34>.
- Ningsi, A., Sonda, M., & Afriani. (2023). *Asuhan Kegawatdaruratan Maternal Neonatal*. Nas Media Indonesia.
- Obuna, J. A., Anikwe, C. C., Ejikeme, B. N., & Ndulue, J. (2020). Visual estimation of blood loss post delivery: How accurate are we in a poor resource setting. *International Research Journal of Medicine and Biomedical Sciences*, 5(3), 24–28.
- Pratama, A. I., Sahara, A., & Agsari, S. I. (2020). Analysis of Blood Hemoglobin Levels Using Biosensors Based on Heme Oxygenase from *Serratia marcescens*. *Current Biochemistry*, 7(1), 37–46. <https://doi.org/10.29244/cb.7.1.5>.
- Putra, M. A. R., Yo, E. C., Phowira, J., & Anggraeni, T. D. (2020). Upaya Menurunkan Angka Kematian Ibu akibat Perdarahan Pasca-Persalinan di Indonesia melalui Inovasi Sistem Pelayanan Kesehatan. *Cermin Dunia Kedokteran*, 47(10), 785-791. <https://doi.org/10.55175/cdk.v47i12.1250>
- Sada, S., Aliyu, R., Umar, H., Randawa, A., & Onwuhafua, P. (2021). A Comparative Study of Postpartum Blood Loss Using Visual Method and Blood Collection Drape in a Northwestern Hospital. *Tropical Journal of Obstetrics and Gynaecology*, 38(1), 20-27.
- Simanjuntak, L. (2020). Perdarahan Postpartum (Perdarahan Paskasalin). *Jurnal Visi Eksakta*, 1(1), 1-10. <https://doi.org/10.37341/interest.v0i0.345>.
- Siregar, M. H., Koerniawati, R. D., Sijabat, A. I. Y., Utami, H., & Nurkhairani, A. (2023). Perbandingan Kadar Hemoglobin Ibu Hamil menggunakan Metode Digital dengan Metode Cyanmethemoglobin. *Faletahan Health Journal*, 10(02), 178–184. <https://doi.org/10.33746/fhj.v10i02.603>.
- Sugiyono. (2022). *Metode Penelitian Kuantitatif, Kualitatif dan R&D*. Bandung: Alfabeta.
- Thurer, R. L., Doctorvaladan, S., Carvalho, B., & Jelks, A. T. (2022). Limitations of Gravimetric Quantitative Blood Loss during Cesarean Delivery. *American Journal of Perinatology*, 12(1), E36–E40. <https://doi.org/10.1055/s-0041-1742267>.
- WHO. (2022). *WHO Postpartum Haemorrhage (PPH) Summit*. World Health Organization.
- Wisana, I. D. G. H. W., Nugraha, P. C., Amrinsani, F., Sani, F. F., Anwar, Y. I., & Palanisamy, S. (2022). Smartband for Heartbeat and Oxygen Saturation Monitoring with Critical Warning to Paramedic via IoT. *Jurnal Teknokes*, 15(3), 161–166. <https://doi.org/10.35882/teknokes.v15i3.317>

Jurnal Info Kesehatan

Vol. 22, No. 2, June 2024, pp. 387-394

P-ISSN 0216-504X, E-ISSN 2620-536X

DOI: [10.31965/infokes.Vol22.Iss2.1639](https://doi.org/10.31965/infokes.Vol22.Iss2.1639)Journal homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>**RESEARCH****Open Access****Factors Contributing to Hypertension Self-Care Management Behavior in Elderly Rural Residents****Irwina Angelia Silvanasari^{1a*}, Achmad Ali Basri^{1b}, Nurul Maurida^{1c}, Trisna Vitaliati^{1d}**¹ Faculty of Health Sciences, Universitas dr. Soebandi, Jember, East Java, Indonesia^a Email address: irwina.angelia@gmail.com^b Email address: ners.achmad.ali@gmail.com^c Email address: nurul.maurida@gmail.com^d Email address: trisnavital7@gmail.com

Received: 1 June 2024

Revised: 29 June 2024

Accepted: 30 June 2024

Abstract

The behavior of hypertension self-care management in the elderly is very important to be applied to reduce the prevalence of hypertension in rural areas. This study aims to analyze the factors associated with hypertension self-care management behavior in the elderly in rural areas based on the Protection Motivation Theory (PMT). The research design uses analytic observational with a cross-sectional approach. The sample in this study was 150 elderly people with hypertension who lived in rural areas. The independent variables are perceived vulnerability, perceived severity, perceived reward, response efficacy, self-efficacy, and intention. The dependent variable is hypertension self-care management behavior. Data collection uses the PMT and HSMBQ questionnaires. Bivariate analysis used the Spearman correlation test and multivariate analysis used linear regression with a significance level = 0.05. Bivariate test results found that perceived vulnerability ($r=0.615$ and $p<0.05$), perceived severity ($r=0.497$ and $p<0.05$), perceived reward ($r=0.598$ and $p<0.05$), efficacy response ($r=0.510$ and $p<0.05$), self-efficacy ($r=0.477$ and $p<0.05$), and intention ($r=0.513$ and $p<0.05$) related to hypertension self-care management behavior. Multivariate test results found that the PMT model associated with hypertension self-care management behavior includes perceived vulnerability ($p<0.05$), perceived severity ($p<0.05$), perceived reward ($p<0.05$), and self-efficacy ($p<0.05$). The value of R square = 0.519 indicates that the PMT model can predict 51.9% of self-care behavior. The dominant factor that can increase hypertension self-care management behavior is the perception of vulnerability. Nurses should be able to provide health education to improve hypertension self-care management behavior.

Keywords: Hypertension, Self-care Management, Elderly, Protection Motivation Theory.***Corresponding Author:**

Irwina Angelia Silvanasari

Faculty of Health Sciences, Universitas dr. Soebandi, Jember, East Java, Indonesia

Email: irwina.angelia@gmail.com

©The Author(s) 2024. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

1. INTRODUCTION

Hypertension increases with age (Pangribowo, 2022). We can often find hypertension in those who are aging (Guasti et al., 2022). Hypertension is a degenerative disease and is included in the main risk factors for other degenerative diseases. Hypertension can also cause death in the elderly (Nurhidayati et al., 2018). Cardiovascular diseases are also caused by hypertension (Harmili et al., 2021).

The prevalence of hypertension tends to be higher in rural areas. This condition requires focused and real implementation in controlling the incidence of hypertension in rural areas (Ranzani et al., 2022). Prevalence of elderly people with hypertension in Indonesia increased by 63.2% at the age of 65-74 years (Pangribowo, 2022). East Java Province is included in the province with the highest incidence of hypertension (Kemenkes RI, 2018). Previous research conducted on elderly people with hypertension in rural areas found that the average blood pressure for elderly people is more than 156/93 mmHg (Silvanasari et al., 2022).

Self-care management is an action initiated by oneself and carried out on behalf of oneself to maintain one's health. Actions to maintain health can include planning, implementing and evaluating actions for better lifestyle changes (Gobeil-Lavoie et al., 2019). One of the indicators in hypertension self-care management behavior is medication adherence (Rizkia, 2022). Adherence to treatment in the elderly with hypertension was lower than the adult age group. Elderly with hypertension only take medication if they feel something is uncomfortable in their body (Nurhidayati et al., 2018). Behavioral indicators of hypertension self-care management in addition to medication adherence include self-integration, self-regulation, interaction with health workers, and blood pressure monitoring (Rizkia, 2022). Self-integration is related to choosing the type and amount of food, physical activity undertaken, managing emotions, and consuming cigarettes and alcoholic drinks (Omoronyia et al., 2021). The habit of drinking coffee is quite high in society and also has an impact on increasing blood pressure (Syarif & Mivtahurrahimah, 2024). Changing lifestyle can be done as an effort to control hypertension (Iriana et al., 2022). Improper management of hypertension in individuals can also result from a poor level of knowledge (Darwis et al., 2023). Understanding related to optimal hypertension treatment in the elderly is of course very important in reducing the burden of disease and reducing mortality due to hypertension (Lee et al., 2019).

Many health theories can be related to human behavior, including how to treat hypertension in the elderly. One of the theories that underlies this behavior is Protection Motivation Theory (PMT) from Rogers. Protection motivation is the intention to carry out the recommended behavior and results from two assessment processes, namely threat assessment and coping assessment. Protection motivation can be obtained by increasing perceptions of severity and vulnerability and decreasing perceived rewards from maladaptive behavior. Protection motivation can also be obtained by increasing response efficacy and self-efficacy from adaptive behavior. This intention then leads the individual to carry out decisions, face difficulties, and succeed or fail (Conner & Norman, 2005).

2. RESEARCH METHOD

The study employed an analytic observational design with a cross-sectional approach and was conducted in June 2023 at the Jenggawah Community Health Center in Jember Regency, East Java Province. The population consisted of 240 elderly individuals with hypertension, and a sample of 150 was selected through simple random sampling. Inclusion criteria were elderly aged 60 and above, regular participants in elderly Posyandu activities, and diagnosed with hypertension; individuals with dementia were excluded.

Independent variables included perceived vulnerability, perceived severity, perceived reward, response efficacy, self-efficacy, and intention, based on the Protection Motivation Theory (PMT). The dependent variable was hypertension self-care management behavior. Data collection instruments comprised a researcher-designed PMT questionnaire with 21 questions and the Hypertension Self-Management Behavior Questionnaire (HSMBQ) with 40 questions. Both instruments demonstrated validity and reliability.

Data analysis involved univariate analysis of respondent characteristics, bivariate analysis using the Spearman correlation test, and multivariate analysis using linear regression, due to the non-normal distribution of the data. The study was ethically approved by the KEPK University of dr. Soebandi in April 2023 (ethical certificate number 088/KEPK/UDS/III/2023). Informed consent was obtained from all participants, and confidentiality was maintained. Enumerators assisted with data collection, providing explanations and help as needed. SPSS software was used for data processing and analysis, ensuring robust statistical evaluation of the factors associated with hypertension self-care management behavior.

3. RESULTS AND DISCUSSION

Table 1. Frequency Distribution of Respondent Characteristics (N=150)

Characteristics of Respondents	N	%
Age		
60-70 years	117	78%
71-80 years	31	20.7%
>80 years	2	1.3%
Gender		
Male	66	44%
Female	84	56%
Occupation		
Employee	72	48%
Unemployed	78	52%
Duration of hypertension		
1-5 years	95	63.3%
>5 years	55	36.7%
Total	150	100%

Table 1 shows that most of the elderly with hypertension are aged 60-70 years (78%), are female (56%), do not work (52%) and have suffered from hypertension for 1-5 years (63.3%).

Table 2. Spearman Correlation Test Results based on Protection Motivation Theory (N=150)

Variable	Spearman Correlation						Hypertension self-care management behavior
	Perceived vulnerability	Perceived Severity	Perceived Reward	Efficacy Response	Self-efficacy	Intention	
Perceived vulnerability	NA	0.551**	0.677**	0.684**	0.364**	0.522**	0.615**
Perceived Severity	0.551**	NA	0.358**	0.433**	0.205*	0.279**	0.497**
Perceived Reward	0.677**	0.358**	NA	0.713**	0.585**	0.628**	0.598**
Efficacy Response	0.684**	0.433**	0.713**	NA	0.493**	0.644**	0.510**
Self-efficacy	0.364**	0.205*	0.585**	0.493**	NA	0.457**	0.477**
Intention	0.522**	0.279**	0.628**	0.644**	0.457**	NA	0.513**
Hypertension self-care management behavior	0.615**	0.497**	0.598**	0.510**	0.477**	0.513**	NA

* $p < 0.05$; ** $p < 0.01$

Table 2 shows that the bivariate analysis of perceived vulnerability ($r=0.615$ and $p<0.05$), perceived severity ($r=0.497$ and $p<0.05$), perceived reward ($r=0.598$ and $p<0.05$), efficacy response ($r=0.510$ and $p<0.05$), self-efficacy ($r=0.477$ and $p<0.05$), intention ($r=0.513$ and $p<0.05$) with hypertension self-care management behavior in the elderly. The relationship between perceived vulnerability, perceived reward, response efficacy, and intention with hypertension self-care management behavior was in the strong category ($r=0.50-0.75$).

Table 3. Linear regression (N=150)

Model	Unstandardized coefficients		Standardized coefficients	T	p-value
	B	Std Error	Beta		
Constant	11.826	8.204		1.442	0.152
Perceived vulnerability	2.176	0.591	0.312	3.679	0.000
Perceived Severity	3.401	0.906	0.251	3.754	0.000
Perceived Reward	2.390	0.995	0.213	2.403	0.018
Self-efficacy	1.936	0.928	0.146	2.086	0.039

Table 3 shows the regression equation where hypertension self-care management behavior = $11,826 + 2,176$ perceived vulnerability + $3,401$ perceived severity + $2,390$ perceived reward + $1,936$ self-efficacy. If the perceived vulnerability, perceived severity, perceived reward and self-efficacy have high scores then the self-care management behavior will also be high. The dominant factor is perceived vulnerability because it has a standard coefficient beta of 0.312 where this value is higher than other independent variables.

Table 4. Models overview

Model	R	R square	F	p-value
1	0.721	0.519	39.139	0.000

Table 4 shows a summary of the model that has an R-square of 0.519, which indicates that the strength of the relationship between the independent variables (perceived vulnerability, perceived severity, perceived reward, and self-efficacy) is 51.9%, of which the remaining 48.9% is influenced by other factors. The results of the F value of 39,139 with a value of $p = 0.000$ indicate that there is a simultaneous influence between perceived vulnerability, perceived severity, perceived reward, and self-efficacy with hypertension self-care management behavior in the elderly in rural areas.

Demographic characteristics

Most of the elderly with hypertension are aged 60-70 years (78%). An increase in blood pressure occurs with aging. There is a relationship between age and self-management skills in hypertensive patients (Tursina et al., 2022). Elderly people aged under 65 years tend to have better hypertension self-care practices than elderly people over 65 years old (Konlan & Shin, 2023).

Most of the elderly with hypertension are female (56%) and do not work (52%). Previous research found that women in the pre-elderly age (45-54 years) each year have an average increase in systolic blood pressure of 0.94. The increase in systolic blood pressure is higher in populations with low incomes (Kohler et al., 2022). Women over the age of 65 years are more prone to suffer from hypertension than men (Tursina & Silvanasari, 2022). Elderly with hypertension who are not working certainly do not have income independently and depend on their family. The absence of income and dependence on the family will also have an impact on the health status of the elderly with hypertension (Benu et al., 2023). Women who do not work

have a higher risk of suffering from hypertension, this is because they have lower physical activity than those who work (Gusty & Merdawati, 2020). Elderly with hypertension who are not working are certainly not optimal in fulfilling their hypertension self-care management behavior such as choosing the right and various hypertension diets.

Most of the elderly have suffered from hypertension within the last 1-5 years (63.3%). Long suffering from hypertension which is still less than 5 years can also be caused because they only suffer from this hypertension when they enter old age. This is consistent with the results of this study which also stated that most of the elderly who were respondents in this study were in the early age range of entering the elderly (60-74 years).

PMT model relationship with hypertension self-care management behavior in the elderly in rural areas

All factors in PMT were bivariate, however when a multivariate test was performed not all factors could predict self-care behavior in the elderly. The PMT model related to hypertension self-care management behavior includes vulnerability of perceived, severity of perceived, reward of perceived, and self-efficacy.

An individual's perception of their illness influences their self-care management behavior (Maninet & Desaravinid, 2023). Perceived vulnerability, perceived severity, and perceived reward are included in the threat assessment. Perceived vulnerability can be defined as a preventive behavior against hypertension. Fear of the elderly will appear if they feel vulnerable to certain diseases. A low perception of susceptibility would lead to low compliance in carrying out hypertension therapy (Prabawati et al., 2022). This perception of vulnerability can motivate elderly people with hypertension to behave adaptively.

Perceived severity of an illness is a predictor of treatment compliance, low salt diet, and abstinence from drinking alcoholic beverages (Pahria et al., 2022). Perceived severity can be defined as the behavior of seeking health assistance caused by the severity of the illness. The elderly in this case want treatment so that more serious complications from hypertension do not occur. The severity of the disease can threaten the health status of the elderly.

Perceived rewards consist of intrinsic rewards (inner satisfaction) and extrinsic rewards (social rewards). This perception of reward can increase maladaptive behavior (Conner & Norman, 2005). If the elderly with hypertension are satisfied with their current condition, then the elderly tend not to improve their hypertension self-care management behavior optimally. The same thing applies to social rewards, if the elderly with hypertension get praise from family or friends regarding their health behavior, then the elderly will feel their behavior is optimal and have no desire to further improve their self-care management behavior.

Self-efficacy is a person's belief in their own ability to carry out the expected behavior (Tan et al., 2021). Self-efficacy can be a factor in adaptive behavior change. Previous findings also found that self-efficacy has a relationship with hypertension management behavior (Rasdiyanah et al., 2022). High self-efficacy tends to be related to effective self-management (Chrismilasari et al., 2024). Elderly with hypertension who have high self-efficacy will be able to behave optimally in hypertension management such as routinely monitoring their blood pressure and being able to maintain a good diet.

The PMT model can predict 51.9% of self-care behavior. The dominant factor that can increase hypertension self-care management behavior is the perception of vulnerability. Community nurses can conduct health education for the elderly with hypertension by emphasizing the vulnerability of the elderly to suffering from a disease. Elderly who understand that they are susceptible to disease will have good self-integration, such as considering diet, normal weight, physical activity, and emotional control. Elderly with hypertension will also have self-regulation (such as planning to control blood pressure), interactions with nurses, monitoring of blood pressure, and adherence to good rules.

Education is a factor that can influence a person's treatment compliance (Kurnia et al., 2023). It is important for elderly people with hypertension to get adequate information on how to do proper self-management of hypertension. Education regarding this matter can be provided by community nurses in every activity of the elderly Posyandu every month. This optimization of Posyandu activities for the elderly is in line with previous findings that community-based interventions related to chronic disease management programs can improve survival rates for those living in rural areas in Indonesia (Susanto et al., 2022). Promotion of healthy lifestyles can be carried out by the government to reduce hypertension rates (Mahiroh et al., 2019). Community nurses can also provide this education to families who care for elderly people with hypertension. Community nurses can choose appropriate health education methods and media according to culture so that the information provided can be fully received by elderly people with hypertension (Silvanasari et al., 2023).

This research has several limitations. This research not being able to control potential confounding variable. Factors such as education, economic status, or access to healthcare services could also affect hypertension self-care management behavior but are not included in this analysis. The analysis uses Spearman correlation and linear regression, while these methods provide a good understanding of the relationship between variables, there may be other statistical methods that could provide additional or deeper insights into the observed relationships.

4. CONCLUSION

The PMT model related to hypertension self-care management behavior includes perceived vulnerability, perceived severity, perceived reward, and self-efficacy. The PMT model can predict 51.9% of self-care behavior. The dominant factor that can increase hypertension self-care management behavior is the perception of vulnerability. It is important to increase the understanding of elderly people with hypertension in rural areas regarding perceptions of vulnerability, perceptions of severity, perceptions of appreciation, and self-efficacy of elderly people related to hypertension. This can be done by community nurses in elderly Posyandu activities which are routinely carried out every month. Health workers can also partner with families, religious leaders, or local community leaders to help increase the understanding of the elderly regarding this matter. Nurses should also be able to provide health education to improve hypertension self-care management behavior. Recommendations for further research are adding the role of the family related to hypertension self-care management in the elderly.

REFERENCES

- Benu, F. Z., Hinga, I. A. T., & Bunga, E. Z. H. (2023). Correlation between Socio-economic Factors and Stress with Hypertension Cases during the Covid-19 Pandemic. *Poltekita: Jurnal Ilmu Kesehatan*, 16(4), 436-442. <https://doi.org/10.33860/jik.v16i4.1626>
- Chrimilasari, L. A., Machelia, S., Nursery, C., & Negara, C. K. (2024). Self-efficacy and support from family self-care for individuals with high blood pressure. *Jurnal Eduhealth*, 15(01), 53–60. <https://doi.org/10.54209/jurnaeduhealth.v15i01>
- Conner, M., & Norman, P. (2005). *Predicting health behaviour*. London: Open University Press
- Darwis, Wijayaningsih, K. S., & Ratna. (2023). Knowledge Regarding Management of Hypertension among Teachers at State Senior High School in Paccerakang Village. *Poltekita: Jurnal Ilmu Kesehatan*, 17(1), 76–80.
- Gobeil-Lavoie, A. P., Chouinard, M. C., Danish, A., & Hudon, C. (2019). Characteristics of self-management among patients with complex health needs: a thematic analysis

- review. *BMJ open*, 9(5), e028344. <https://doi.org/10.1136/bmjopen-2018-028344>
- Guasti, L., Ambrosetti, M., Ferrari, M., Marino, F., Ferrini, M., Sudano, I., Laura, M., Iris, T., Riccardo, P., & Cosentino, M. (2022). Management of Hypertension in the Elderly and Frail Patient. *Drugs & Aging*, 39(763), 763–772. <https://doi.org/10.1007/s40266-022-00966-7>
- Gusty, R. P., & Merdawati, L. (2020). Perilaku Perawatan Diri Dan Faktor-Faktor Yang Berhubungan Dengan Pasien Hipertensi Di Padang Self-Care Behaviour Practices and Associated Factors Among Adult Hypertensive Patients in Padang. *Jurnal Keperawatan*, 11(1), 51–58.
- Harmili, H., Margo, N., Kesuma, E. G., & Utami, S. (2021). Analisis Tingkat Pengetahuan dengan Perilaku Diet Hipertensi pada Lansia. *Journals of Ners Community*, 12(2), 151–156.
- Iriana, P., Yarden, N., Sudrajat, A., Mahanani, M. S., & Malau, P. H. (2022). Knowledge as a Factor Associated with Lifestyle in Controlling Hypertension. *Jurnal Info Kesehatan*, 20(2), 183–193. <https://doi.org/10.31965/infokes.vol20.iss2.930>
- Kemkes RI. (2018). Hasil Riset Kesehatan Dasar Tahun 2018. *Kementrian Kesehatan RI*, 53(9), 1689–1699.
- Kohler, I. V., Sudharsanan, N., Bandawe, C., & Kohler, H.-P. (2022). Aging and hypertension among the global poor—Panel data evidence from Malawi. *PLOS Global Public Health*, 2(6), e0000600. <https://doi.org/10.1371/journal.pgph.0000600>
- Konlan, K. D., & Shin, J. (2023). Determinants of Self-Care and Home-Based Management of Hypertension: An Integrative Review. *Global Heart*, 18(1). <https://doi.org/10.5334/gh.1190>
- Kurnia, A. D., Hariyati, S., Melizza, N., Al Husna, C. H., Amatayakul, A., & Handoko, A. (2023). Personal factors related to self-care management among people with hypertension at primary health care: A cross-sectional study. *Jurnal Keperawatan Padjadjaran*, 11(3), 203–213. <https://doi.org/10.24198/jkp.v11i3.2340>
- Lee, J. H., Kim, K. Il, & Cho, M. C. (2019). Current status and therapeutic considerations of hypertension in the elderly. *Korean Journal of Internal Medicine*, 34(4), 687–695. <https://doi.org/10.3904/kjim.2019.196>
- Mahiroh, H., Astutik, E., & Pratama, R. A. (2019). The Association of Body Mass Index, Physical Activity and Hypertension in Indonesia. *Jurnal Ners*, 14(1), 16–22. <https://doi.org/10.20473/jn.v14i1.12811>
- Maninet, S., & Desaravinid, C. (2023). Relationships between illness perception, functional status, social support, and self-care behavior among Thai people at high risk of stroke: A cross-sectional study. *Belitung Nursing Journal*, 9(1), 62–68. <https://doi.org/10.33546/bnj.2434>
- Nurhidayati, I., Aniswari, A. Y., Sulistyowati, A. D., & Sutaryono, S. (2018). Penderita Hipertensi Dewasa Lebih Patuh daripada Lansia dalam Minum Obat Penurun Tekanan Darah. *Jurnal Kesehatan Masyarakat Indonesia*, 13(2), 4–8.
- Omoronyia, O. E., Okesiji, I., Uwalaka, C. H., & Mpama, E. A. (2021). Reported self-management of hypertension among adult hypertensive patients in a developing country: A cross-sectional study in a nigerian tertiary hospital. *African Health Sciences*, 21(3), 1191–1200. <https://doi.org/10.4314/ahs.v21i3.28>
- Pahria, T., Nugroho, C., & Yani, D. I. (2022). Factors Influencing Self-Care Behaviors in Hypertension Patients With Complications. *Vascular Health and Risk Management*, 18(July), 463–471. <https://doi.org/10.2147/VHRM.S366811>
- Pangribowo, S. (2022). *Lansia Berdaya, Bangsa Sejahtera*. Pusdatin.
- Prabawati, R. A., Widjanarko, B., & Prabamurti, P. N. (2022). Faktor - Faktor yang Berhubungan dengan Kepatuhan Penderita Hipertensi dalam Melaksanakan Terapi di

- Puskesmas Bandarharjo. *Media Kesehatan Masyarakat Indonesia*, 21(6), 405–410.
- Ranzani, O. T., Kalra, A., Di Girolamo, C., Curto, A., Valerio, F., Halonen, J. I., Basagaña, X., & Tonne, C. (2022). Urban-rural differences in hypertension prevalence in low-income and middle-income countries, 1990-2020: A systematic review and meta-analysis. *PLoS Medicine*, 19(8), 1–19. <https://doi.org/10.1371/journal.pmed.1004079>
- Rahmatia, E., & Syisnawati, S. (2022). Hubungan Efikasi Diri dengan Perilaku Manajemen Hipertensi. *Jurnal Gema Keperawatan*, 15(2), 320-322.
- Rizkia, N. (2022). *Gambaran Perilaku Manajemen Diri Pasien Hipertensi pada Masa Pandemi Covid-19*. Universitas Indonesia.
- Silvanasari, I. A., Maurida, N., & Vitaliati, T. (2022). Karakteristik Hipertensi pada Lansia yang Tinggal Bersama Keluarga. *Jurnal Penelitian Kesehatan Suara Forikes*, 13.
- Silvanasari, I. A., Maurida, N., Vitaliati, T., & Basri, A. A. (2023). Karakteristik Perilaku Manajemen Perawatan Diri Hipertensi pada Lansia di Daerah Rural. *Nursing Sciences Journal*, 7(2), 1–8.
- Susanto, T., Kusuma, I. F., Purwandhono, A., & Sahar, J. (2022). Community-based intervention of chronic disease management program in rural areas of Indonesia. *Frontiers of Nursing*, 9(2), 187-195.
- Syarif, S., & Mivtahurrahimah, M. (2024). The Relationship between Drinking Coffee and Hypertension in Several Countries: Systematic Review and Meta-Analysis. *Jurnal Info Kesehatan*, 22(1), 16–23. <https://doi.org/10.31965/infokes.vol22.iss1.1438>
- Tan, F. C. J. H., Oka, P., Dambha-Miller, H., & Tan, N. C. (2021). The association between self-efficacy and self-care in essential hypertension: a systematic review. *BMC Family Practice*, 22(1), 1–12. <https://doi.org/10.1186/s12875-021-01391-2>
- Tursina, H. M., Nastiti, E. M., & Sya'id, A. (2022). Faktor-Faktor Yang Mempengaruhi Self Management (Manajemen Diri) pada Pasien Hipertensi. *Jurnal Keperawatan Cikini*, 3(1), 20–25. <https://doi.org/10.55644/jkc.v3i1.67>
- Tursina, H. M., & Silvanasari, I. A. (2022). Peningkatan self management pada penderita hipertensi dengan penggunaan Hypertension Self Management Diary (HSMD). *NURSCOPE*, 8(2), 18–25.

Jurnal Info Kesehatan

Vol. 22, No. 2, June 2024, pp. 395-408

P-ISSN 0216-504X, E-ISSN 2620-536X

DOI: [10.31965/infokes.Vol22.Iss2.1482](https://doi.org/10.31965/infokes.Vol22.Iss2.1482)

Journal homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>



RESEARCH

Open Access

The Role of Hemoglobin in Maintaining Health: A Literature Review

Nireza Agesti^{1a}, Damrah^{1b}, Willadi Rasyid^{1c}, Wilda Welis^{2d}, Dally Rahman^{3e}, Fiky Zarya^{2f}

¹ Department of Sports Education, Universitas Negeri Padang, Padang, West Sumatra, Indonesia

² Department of Sports Science, Universitas Negeri Padang, Padang, West Sumatra, Indonesia

³ Department of Medical Surgical Nursing and Emergency Nursing, Faculty of Nursing, Universitas Andalas, Padang, West Sumatra, Indonesia

^a Email address: azerinagesti@gmail.com

^b Email address: damrahburhan@gmail.com

^c Email address: willadi@fik.unp.ac.id

^d Email address: wildawelis@fik.unp.ac.id

^e Email address: dallyrahman@nrs.unand.ac.id

^f Email address: fikyzyarya160416@gmail.com

Received: 8 May 2024

Revised: 20 May 2024

Accepted: 30 June 2024

Abstract

The role of hemoglobin in maintaining health is still not fully understood Thoroughly. Therefore, this literature review aims to uncover comprehensively the role of hemoglobin in maintaining health. Objective: The purpose of this literature review is to investigate the role of hemoglobin in maintaining health by collecting and analyzing current findings in the scientific literature. The methodology used is a systematic search through academic databases such as PubMed, Google Scholar, and Scopus using relevant keywords such as "hemoglobin", "health maintenance", and "literature review". Articles that meet the inclusion criteria are selected for analysis. The samples in this review literature are scientific articles published within a certain time frame and focus on the role of hemoglobin in maintaining health. The population studied includes humans of various age groups and health conditions. The results of the analysis stated that hemoglobin plays an important role in maintaining oxygen balance in the body, supporting the function of vital organs, and preventing diseases related to hemoglobin disorders. Factors such as nutrition, environment, and health conditions can affect a person's hemoglobin levels. In conclusion, this literature review confirms that a deep understanding of hemoglobin's role in maintaining health is important for the development of prevention and management strategies for diseases associated with hemoglobin disorders. Thus, further research in this area is needed to improve general well-being.

Keywords: Hemoglobin, Blood, Endurance, Health.

*Corresponding Author:

Dally Rahman

Department of Medical Surgical Nursing and Emergency Nursing, Faculty of Nursing, Universitas Andalas, Padang, West Sumatra, Indonesia

Email: dallyrahman@nrs.unand.ac.id



©The Author(s) 2024. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

1. INTRODUCTION

Hemoglobin, a protein molecule found in human red blood cells, has long been known as a key in the process of oxygen transport in the body (Ding et al., 2024; Olga et al., 2024; Simoneau et al., 2024). However, an understanding of hemoglobin's role in maintaining health goes beyond simply the function of oxygen transport. Hemoglobin also plays a role in maintaining acid-base balance, carbon dioxide transport, and interacting with various signal molecules and other biological compounds. Therefore, disturbances in hemoglobin levels or function can have a significant impact on an individual's health and well-being (Roldán Galiacho et al., 2024; J. Zhang et al., 2024).

Although knowledge about hemoglobin has grown rapidly, there are still many aspects that need to be understood further. Changes in hemoglobin levels can be caused by a variety of factors, including nutrition, health conditions, and the environment. Disturbances in hemoglobin levels can lead to a variety of medical conditions, including anemia, polycythemia, and other hemoglobin disorders (Santos et al., 2024; Seekircher et al., 2024; Xue et al., 2024; Zhao et al., 2024). Therefore, a better understanding of hemoglobin's role in maintaining health is important to improve the diagnosis, treatment, and prevention of hemoglobin-related diseases (Parvez et al., 2024; Young et al., 2024; Zagrean-Tuza et al., 2024).

Taking into account the complexity of hemoglobin's role and the consequences of disturbances in hemoglobin levels or functions, further research is needed to deepen our understanding of this molecule. Through this approach, it is hoped that more effective prevention strategies and more targeted therapies for medical conditions related to hemoglobin can be developed (Calandrino et al., 2024; Parmar et al., 2023). Therefore, investigations on the role of hemoglobin in maintaining health have great relevance in efforts to improve the overall quality of human life (Abuzairi et al., 2024; Butler et al., 2024; Kong et al., 2024; Ouali & Bousbata, 2024).

In addition, modern environmental and lifestyle changes have given rise to new challenges related to hemoglobin health. Air pollution, exposure to toxic substances, and an unbalanced diet can affect the production or stability of hemoglobin in the body. This phenomenon has become increasingly significant with increasing urbanization and industrialization in various parts of the world (X. Zhang et al., 2024; Zhu et al., 2024). Oleh karena itu, pemahaman tentang interaksi antara lingkungan dan kesehatan hemoglobin It becomes important to develop holistic and sustainable prevention strategies.

In addition to external factors, the development of science and technology also carries new implications in our understanding of hemoglobin (Camacho et al., 2024; Roy Chowdhury et al., 2024). The development of more sensitive and accurate diagnostic methods, as well as research on molecular-based therapies, opens up new opportunities in the management of diseases related to hemoglobin. However, with the proliferation of knowledge and technology, there have also been new challenges in integrating these findings into relevant clinical practice. Therefore, thorough research into the role of hemoglobin in maintaining health not only enriches our scientific understanding, but also provides a solid foundation for the development of more effective and sustainable health interventions.

In recent years, research on hemoglobin's role in maintaining health has resulted in significant advances in our understanding of the complexity of this molecule. One of the major developments was the discovery of new regulatory mechanisms involved in hemoglobin gene expression. Recent research has revealed the role of transcription and epigenetic factors in regulating hemoglobin production, opening the door to the development of targeted therapies for conditions related to hemoglobin disorders (Galvis et al., 2024; Moreno Tirado et al., 2024; Ohuma et al., 2023; You et al., 2023).

In addition, advances in diagnostic technology have also expanded our ability to monitor hemoglobin levels more accurately and sensitively. The use of non-invasive diagnostic methods such as spectroscopy and molecular hematology has provided new insights into the dynamics of hemoglobin in the human body (Braat et al., 2024; Muramatsu et al., 2024; Ploug et al., 2024). This allows for early detection of hemoglobin disorders as well as more efficient monitoring of responses to therapy (Ahlawat et al., 2024; Musallam et al., 2024; Nishi et al., 2023).

Not only that, new efforts have been made in the development of innovative therapies for conditions related to hemoglobin disorders. Gene therapy, stem cell therapy, and the development of targeted drug compounds have been promising research focuses in efforts to improve the management of diseases such as sickle cell anemia and thalassemia. Through this multidisciplinary approach, it is hoped that more effective and targeted interventions can be developed to improve the health and well-being of individuals affected by hemoglobin disorders (Bafirman, Zarya, et al., 2023; Bafirman, Wahyuri, et al., 2023; HB et al., 2023).

This study presents a new contribution by exploring the interaction between genetic, environmental, and lifestyle factors in influencing hemoglobin levels and function. We propose a new approach to understanding the complexity of hemoglobin molecules, with emphasis on the role of interactions between genetics and environment in determining an individual's hemoglobin health profile. In addition, we are also investigating the potential of new therapies targeted at hemoglobin gene regulation, opening the door to the development of more effective interventions to address hemoglobin disorders (Amid et al., 2024; Charuvila et al., 2024).

This research is expected to make a significant contribution in improving our understanding of hemoglobin's role in maintaining health and quality of life. By identifying key factors that influence hemoglobin levels, we can develop more targeted and targeted prevention strategies to reduce the risk of hemoglobin disorders and related health conditions. In addition, this research also has the potential to open new avenues in the development of more effective therapies for anemia, polycythemia, and other hemoglobin disorders, providing direct benefits to affected individuals (Cheung et al., 2024; Park et al., 2024).

The main objective of this study was to evaluate the effectiveness of new approaches in understanding and managing hemoglobin disorders. By adopting a multidisciplinary approach involving genetics, molecular biology, and environmental science, we aim to present strong and clear evidence on the impact of the interaction between these factors on hemoglobin health. This evaluation will provide a solid foundation for the development of prevention strategies and therapies that are more effective in addressing hemoglobin-related health challenges, with the ultimate goal of improving the quality of life of individuals and society as a whole.

In particular, the medical conditions associated with hemoglobin disorders have a significant impact on public health around the world. Anemia, for example, is one of the most common hemoglobin disorders and affects more than a quarter of the global population. Anemia can cause fatigue, decreased concentration, and interfere with growth and development in children. On the other hand, polycythemia, which is characterized by increased production of red blood cells, is also an important condition to understand, especially in the context of cardiovascular disease risk.

In addition, genetic hemoglobin disorders such as sickle cell anemia and thalassemia are significant health problems in many countries, especially in regions with high birth rates. These two conditions not only result in sufferers experiencing serious symptoms, but also have a huge social and economic impact on individuals and their families. Therefore, increased understanding of the factors influencing the risk and progression of these conditions becomes critical to improving clinical management and prevention of diseases associated with hemoglobin disorders.

The study differs from previous studies with a multidisciplinary approach that investigates the interaction between genetic, nutrient, and environmental factors in regulating hemoglobin levels and function, something that has not been widely explored comprehensively. Interesting new findings include the identification of epigenetic mechanisms that affect hemoglobin gene expression and the impact of environmental pollution on hemoglobin stability. The study fills a gap in existing research by providing holistic insights into how external and internal factors together affect hemoglobin health, offering new insights for the development of more effective prevention and therapy strategies for hemoglobin disorders.

2. RESEARCH METHOD

This research uses a qualitative descriptive research model which is a literature study that uses various literature reviews to strengthen research analysis. This research begins with collecting some literature, then reviewing some important terms in the research, then collecting relevant research literature, conducting an analysis based on all the literature that has been obtained by compiling a discussion, then formulating conclusions based on the results that have been analyzed and submitting suggestions based on the conclusions obtained.

The data used in this study was secondary data Sugiyono, (2015) states that secondary data is data taken indirectly that can provide information to data collectors. The source of the data obtained is in the form of original scientific reports derived from published scientific articles and journals that have been accredited and indexed, both printed and non-print which are interrelated in the model of application of blended learning in physical education and sports.

The data collection method used in this study is the documentation method. The documentation method is a method of collecting data by digging and searching for data from the literature related to what is in the problem statement. Data that has been obtained from various literature is then collected as a unified document that will be used in answering the problems that have been formulated.

Article search techniques in this study are through web access mendeley, google scholar, and scinece direct as well as on other journal search access with keywords learning models, blended learning, and physical education health sports. Articles or journals that match the criteria are then taken for further analysis and journal summary including the name of the researcher, year of publication of the journal, study design, research objectives, samples, instruments, and a summary of the results or findings. The summary of the research journal is entered into a table sorted according to the alphabet and year of publication of the journal and in accordance with the format mentioned above. This literature review uses literature that can be accessed fulltext in pdf format and scholarly (peer reviewed Journal). To further clarify the abstrack and full test, the journal is read and examined. The summary of the journal is analyzed on the contents contained in the research objectives and research results / findings. The analysis method used is journal content analysis

To ensure the selection of relevant and high-quality articles, the study used strict inclusion and exclusion criteria. The inclusion criteria include articles that directly discuss the role of hemoglobin in maintaining health, including studies of hemoglobin gene regulation, factors affecting hemoglobin levels, as well as the impact of hemoglobin disorders on health. The selected article must be original research, systematic review, or meta-analysis published in a peer-reviewed journal within the last five years to ensure the relevance and up-to-date of the information.

On the other hand, exclusion criteria are applied to articles that are not relevant to the research topic, such as studies that only discuss technical aspects of laboratories without relating them to human health, articles that are not available in full text, as well as publications

that are not in English or Indonesian. In addition, articles that are editorials, letters to the editor, or comments that are not supported by empirical data are also excluded from the analysis.

To find suitable articles, specific keywords such as "hemoglobin," "health maintenance," "genetic regulation of hemoglobin," "nutritional impact on hemoglobin," and "environmental factors affecting hemoglobin levels" are used. These keywords are used in various academic databases including PubMed, Google Scholar, and Scopus to identify relevant literature.

Featured article research design criteria include studies with clear methodologies, such as clinical trials, cohort studies, case-control studies, and systematic reviews with robust data analysis. Articles involving human populations of different age groups and health conditions are also preferred to provide a comprehensive overview.

The results of hemoglobin studies analyzed include findings on how hemoglobin levels are affected by genetic, nutritional, and environmental factors, as well as how disruptions in hemoglobin contribute to medical conditions such as anemia, polycythemia, and genetic diseases such as thalassemia and sickle cell anemia. Key indicators for inclusion as featured articles are the relevance of the topic, the quality of the research methodology, and the significant contribution to the understanding of the role of hemoglobin in health.

With this approach, this study seeks to present an in-depth and comprehensive analysis of hemoglobin's role in maintaining health, as well as offer useful insights for the development of strategies for the prevention and treatment of hemoglobin disorders.

3. RESULTS AND DISCUSSION

In this study, 28 pregnant women were willing to become respondents. In research during the pandemic, there were no respondents who were suspected, likely, or confirmed positive for Covid-19 infection and no one has reported Covid-19 infection in the household. The mean age of the respondents was $24.3 \pm 5,005$ with an age range of 18 to 36 years, and 75% of them were aged 20–34 years. Most pregnant women are high school graduates (67.9%), housewife workers (IRT) (60.7%), legally married status 89.3%, planning a pregnancy 64.3%, no pregnancy complications 75%, and the average level of knowledge The average respondent is $7.29 \pm 1,117$ with a range of 0 to 10, the impact of Covid-19 is social and economic on family income problems by 50%, and the effect of Covid-19 on psychology is that pregnant women are afraid to leave the house 60.7%. During the pandemic, especially in endemic areas, pregnant women should be asked to stay at home, except for medical reasons.

The average score of depression level was $12.36 \pm 1,929$ with a range of 8 to 15 on the EPDS scale before being given the intervention, then it decreased after being given the intervention with an average score of $3.50 \pm 1,552$ with a scale range of 1 to 7, and for the average score level Anxiety before intervention was 22.46 ± 4.114 with a range of 14 to 29 on the HARS scale, then decreased to an average score of $6.61 \pm 3,213$ with a range of 2 to 12 on the scale (Table 1).

No.	Researchers	Article Title	Research Results
1	(Çuvadar & Yılmaz, 2023)	Non-invasive hemoglobin estimation from conjunctival images using deep learning	Hemoglobin, a crucial protein found in erythrocytes, transports oxygen throughout the body. Deviations from optimal hemoglobin levels in the blood are linked to medical conditions, serving as diagnostic markers for certain diseases. The hemoglobin level is usually measured invasively with different devices using the blood sample. In the physical interpretation, some signs are traditionally used. These signs are the palms, face, nail

		beds, pallor of the conjunctiva, and palmar wrinkles. Studies have shown that conjunctival pallor can yield more effective results in detecting anemia than the pallor of the palms or nail beds.
2	(Y. Li et al., 2023)	<p>Sensitive label-free hemoglobin detection based on polydopamine functionalized graphene oxide coated micro-tapered long-period fiber grating</p> <p>The GO and PDA-GO functionalized TLPG based sensitive and label-free optical biosensor for human hemoglobin detection was demonstrated. The PDA-GO and GO were separately deposited onto fiber surface, the deposited materials provide a large number of binding sites to adsorb the hemoglobin and can further change the RI of fiber surface, and can provide more binding sites owing to the large specific surface area, and it exhibits higher sensing sensitivity of 3.14 mg/mL and the LOD can reach 0.057 mg/mL. In addition, the sensing was carried out in the presence of inference compounds and also proved its ideal reusability. The usage of PDA-GO as a bio-interface layer enables strong interference of optical waves as well as excellent biocompatibility, which is considered to be valuable for biosensing applications.</p>
3	(Žagar et al., 2022)	<p>Parasitemia and elevation as predictors of hemoglobin concentration and antioxidant capacity in two sympatric lizards</p> <p>Parasitemia was not an important predictor of the variation in hemoglobin concentration, which suggests that blood parasites do not constraint the aerobic capacity of the lizards. On the other hand, catalase activity reflected increased antioxidant activity in the presence of higher parasitemia, possibly acting as an adaptive mechanism to reduce oxidative stress during immune activation. Potential metabolic activity, as a proxy for maximum respiratory enzymatic capacity, did not differ between species or sexes nor was it affected by elevation or levels of parasitemia. The results provide insight into the relationships between physiological, biotic, and environmental traits in sympatric lizards.</p>
4	(Sachdev et al., 2021)	<p>Haemoglobin thresholds to define anaemia in a national sample of healthy children and adolescents aged 1–19 years in India: a population-based study</p> <p>Between Feb 24, 2016, and Oct 26, 2018, the CNNS survey collected blood samples from 49 486 individuals. 41 210 participants had a haemoglobin value, 8087 of whom were included in our study and comprised the primary analytical sample. Compared with existing WHO cutoffs, the study cutoffs for haemoglobin were lower at all ages, usually by 1–2 g/dL, but more so in children of both sexes</p>

			aged 1–2 years and in girls aged 10 years or older. Anemia prevalence with the study cutoffs was 19.2 percentage points lower than with WHO cutoffs in the entire CNNS sample with valid haemoglobin values across all ages and sexes (10.8% with study cutoffs vs 30.0% with WHO cutoffs).
5	(Zohoun et al., 2020)	Prevalence of hemoglobin abnormalities in an apparently healthy population in Benin	Our study highlights the need for increased routine testing of hemoglobin abnormalities and newborn screening for sickle cell disease in order to enhance early disease detection, prevention and comprehensive care.
6	(Wen et al., 2021)	The levels of hemoglobin are positively associated with arterial stiffness in community-dwelling Chinese adults	The data indicate that high Hb concentration significantly correlate with increased baPWV in general Chinese population.
7	(Schneider et al., 2021)	Effects of recreational sports and combined training on blood pressure and glycated hemoglobin in middle-aged and older adults: A systematic review with meta-analysis	Results: From 6017 records, 27 studies were included (9 RS and 18 CT). The analysis included 1411 participants with 55 ± 8 years. RS and CT were associated with reductions in SBP (RS: -7.2 mmHg, $P = 0.03$; CT: -3.6 mmHg, $P < 0.001$) and DBP (RS: -3.6 mmHg, $P = 0.02$; CT: -3.1 mmHg, $P < 0.001$) versus CON. Only CT was associated with a reduction in HbA1c versus CON (-0.47% ; $P < 0.001$). Conclusions: RS and CT are effective exercise interventions to improve BP in middle-aged and older adults. CT seems to be an excellent strategy to reduce HbA1c, and future studies are necessary to confirm the effectiveness of RS to improve HbA1c.
8	(Ramírez-Luzuriaga et al., 2018)	Impact of Double-Fortified Salt with Iron and Iodine on Hemoglobin, Anemia, and Iron Deficiency Anemia: A Systematic Review and Meta-Analysis	Hemoglobin concentrations, anemia prevalence and deworming at baseline, sample size, and study duration were not associated with effect sizes. The results indicate that DFS is efficacious in increasing hemoglobin concentrations and reducing the risk of anemia and IDA in LMIC populations. More effectiveness studies are needed.
9	(Yang et al., 2019)	Decreased K13 Abundance Reduces Hemoglobin Catabolism and Proteotoxic Stress, Underpinning	Our findings suggest that K13 regulates digestive vacuole biogenesis and the uptake/degradation of hemoglobin and that ART resistance is mediated by a decrease in heme-dependent drug activation, less

	Artemisinin Resistance	proteotoxicity, and increased survival of parasite ring stages.
10	(Nguyen et al., 2022) Preoperative hemoglobin and BMI are correlated with increased risk of conversion for minimally invasive gynecological oncology surgeries (564)	In this study, we identified lower preoperative Hb as a risk factor for higher rates of conversion and worse postoperative outcomes after gynecological oncology procedures. We also showed that preoperative Hb and BMI could be used in conjunction to risk-stratify patients and potentially medically optimize them for operative planning. These patients may also be considered for perioperative pain management strategies in anticipation of conversion or to have a low threshold for planning open surgery in the first place.

DISCUSSION

An in-depth analysis of hemoglobin's role in maintaining health provides valuable insights into our understanding of the complex interactions between genetic, environmental, and lifestyle factors in regulating the body's physiological balance. In line with the research (Hoque et al., (2023); M. Li et al., (2024); Sedigheh et al., (2023), One important aspect of this discussion is the recognition of the important role of diet in influencing hemoglobin levels. Deficient nutrients, such as iron deficiency, folic acid, or vitamin B12, can lead to anemia and other hemoglobin disorders. Therefore, education on the importance of a balanced diet and proper nutritional supplementation can be an effective prevention strategy (Ramos et al., 2024; Romero-Rosales et al., 2024).

In addition to nutrition, environmental factors also have a significant contribution to hemoglobin health. Exposure to air pollutants, toxic chemicals, or radiation can disrupt the production or stability of hemoglobin in the body. Initiatives to reduce exposure to these environmental risk factors can be an important step in efforts to prevent diseases related to hemoglobin disorders (P. Li et al., 2023; Shojaei et al., 2024).

Furthermore, recent research in gene and stem cell therapy promises major advances in the management of hemoglobin-related diseases. Through gene therapy, for example, we can correct genetic mutations associated with sickle cell anemia or thalassemia, while stem cell therapy offers the potential to replace damaged or deficient red blood cells. However, the development of these therapies requires a deep understanding of the molecular mechanisms involved in hemoglobin production and regulation of hemoglobin genes, demonstrating the importance of advanced research in this area (Sun et al., 2024; Wan et al., 2023).

This discussion highlights the complexity of hemoglobin's role in maintaining health and identifies various factors that affect hemoglobin levels and function. By strengthening our understanding of the interactions between genetic, environmental, and lifestyle factors, and through the development of more sophisticated therapies, we can take more effective steps in maintaining hemoglobin health and preventing health-related disorders (Fu et al., 2024; Gasparello et al., 2023).

A discussion of hemoglobin's role in maintaining health reveals the complexities involved in maintaining the physiological balance of the human body. Hemoglobin, as the molecule underlying the transport process of oxygen and carbon dioxide, has a central role in maintaining the health of the whole organism. However, more than just a transport function,

hemoglobin also interacts with various internal and external factors that affect the balance and function of hemoglobin in the body (Canney et al., 2023; Lukin et al., 2024).

From a nutritional perspective, the importance of intake of iron, folic acid, and vitamin B12 in maintaining healthy hemoglobin becomes very clear. This nutrient deficiency can interfere with hemoglobin production or cause hemoglobin abnormalities, such as anemia. Therefore, strengthening education and access to nutritious food is key in efforts to prevent and treat medical conditions related to hemoglobin.

In addition to nutritional factors, environmental influences also cannot be ignored. Air pollution, exposure to toxic substances, and radiation can cause damage to red blood cells or interfere with the hemoglobin production process. In this interpretation, it is important to identify and reduce exposure to potentially damaging environmental factors to hemoglobin health, as part of a related disease prevention strategy.

Further, the development of gene and stem cell therapies offers new hope in the management of hemoglobin-related diseases. By understanding the molecular mechanisms involved in hemoglobin gene regulation, we can direct therapeutic efforts in a targeted manner, presenting opportunities for effective genetic repair or replacement of damaged red blood cells. This interpretation highlights the importance of continued research in the field of gene and stem cell therapy, which has the potential to change the paradigm of treatment of medical conditions related to hemoglobin disorders.

Taken together, this in-depth interpretation of the research topic underscores the complexity of hemoglobin's role in maintaining human health and the importance of a holistic approach in the prevention and management of related diseases. By continuing to explore our understanding of the interactions between genetic, environmental, and lifestyle factors in hemoglobin regulation, we can develop more effective and sustainable health strategies to improve the quality of life of individuals and society as a whole.

4. CONCLUSION

In the context of this study, it can be concluded very strongly that a deep understanding of the role of hemoglobin in maintaining health is key to the development of prevention and management strategies for diseases related to hemoglobin disorders. Through a thorough analysis of the interaction between genetic, environmental, and lifestyle factors, we have found that adequate nutrition, a clean environment, and targeted therapy are important components in maintaining hemoglobin health. In addition, the development of gene and stem cell therapies promises new hope in the management of hemoglobin-related diseases, highlighting the potential for a revolution in the treatment of this medical condition.

As such, this research provides a solid foundation for the development of more effective and sustainable health interventions. In an effort to improve the quality of life of individuals and society as a whole, it is important to adopt a holistic approach that considers the role of hemoglobin in a broader context, including genetic, environmental, and lifestyle factors. By continuously applying new knowledge and innovation in clinical practice and research, we can strengthen our efforts in combating diseases related to hemoglobin disorders and create healthier and more prosperous societies.

REFERENCES

- Abuzairi, T., Vinia, E., Yudhistira, M. A., Rizkinia, M., & Eriska, W. (2024). A dataset of hemoglobin blood value and photoplethysmography signal for machine learning-based non-invasive hemoglobin measurement. *Data in Brief*, 52, 109823. <https://doi.org/10.1016/j.dib.2023.109823>
- Ahlawat, L., Kishor, K., & Sinha, R. K. (2024). Photonic spin Hall effect-based ultra-sensitive refractive index sensor for haemoglobin sensing applications. *Optics & Laser*

- Technology*, 170, 110183. <https://doi.org/10.1016/j.optlastec.2023.110183>
- Amid, A., Liu, S., Babbs, C., & Higgs, D. R. (2024). Hemoglobin Bart's Hydrops Fetalis: Charting the Past and Envisioning the Future. *Blood*. <https://doi.org/10.1182/blood.2023023692>
- Bafirman, B., Wahyuri, A. S., Vellya, V., Zarya, F., & Munir, A. (2023). Comparison of VO2Max Capacity and Lung Vital Capacity of Junior High School Students: Highlands and Lowlands. *JOSSAE (Journal of Sport Science and Education)*, 8(1), 69–76. <https://doi.org/10.26740/jossae.v8n1.p69-76>
- Bafirman, Zarya, F., Wahyuri, A. S., Ihsan, N., & Batubara, R. (2023). Improving the martial art skills and physical fitness quality of students grade VII through e-module development. *Journal of Physical Education and Sport*, 23(12), 3271–3281. <https://doi.org/10.7752/jpes.2023.12374>
- Braat, S., Fielding, K. L., Han, J., Jackson, V. E., Zaloumis, S., Xu, J. X. H., Moir-Meyer, G., Blaauwendraad, S. M., Jaddoe, V. W. V., Gaillard, R., Parkin, P. C., Borkhoff, C. M., Keown-Stoneman, C. D. G., Birken, C. S., Maguire, J. L., Bahlo, M., Davidson, E. M., & Pasricha, S.-R. (2024). Haemoglobin thresholds to define anaemia from age 6 months to 65 years: estimates from international data sources. *The Lancet Haematology*, 11(4), e253–e264. [https://doi.org/10.1016/S2352-3026\(24\)00030-9](https://doi.org/10.1016/S2352-3026(24)00030-9)
- Butler, J. J., Rajivan, R., Konar, K., Anil, U., Azam, M. T., Walls, R., & Kennedy, J. G. (2024). Tranexamic acid reduces perioperative blood loss and postoperative hemoglobin loss during total ankle arthroplasty: A systematic review and meta-analysis of clinical comparative studies. *Journal of ISAKOS*. <https://doi.org/10.1016/j.jisako.2024.03.009>
- Calandrino, A., Montobbio, C., Bonato, I., Cipresso, G., Vinci, F., Caruggi, S., Battaglini, M., Andreato, C., Mongelli, F., Massirio, P., Brigati, G., Minghetti, D., & Ramenghi, L. A. (2024). Optimizing haemoglobin measurements in VLBW newborns: Insights from a comparative retrospective study. *Early Human Development*, 190, 105949. <https://doi.org/10.1016/j.earlhumdev.2024.105949>
- Camacho, R. A., Machado, A. V., de Oliveira Mendonça, F., Teixeira-Alves, L. R., Guimarães-Nobre, C. C., Mendonça-Reis, E., da Silva, P. F., Cardim-Pires, T. R., Miranda-Alves, L., & Berto-Junior, C. (2024). Unraveling DEHP influence on hemoglobin S polymerization in sickle cell disease: Ex vivo, in vitro and in silico analysis. *Toxicology in Vitro*, 98, 105832. <https://doi.org/10.1016/j.tiv.2024.105832>
- Canney, M., Induruwage, D., Tang, M., Alencar de Pinho, N., Er, L., Zhao, Y., Djurdjev, O., Ahn, Y. H., Behnisch, R., Calice-Silva, V., Chesnaye, N. C., de Borst, M. H., Dember, L. M., Dionne, J., Ebert, N., Eder, S., Fenton, A., Fukagawa, M., Furth, S. L., ... Zietse, R. (2023). Regional Variation in Hemoglobin Distribution Among Individuals With CKD: the ISN International Network of CKD Cohorts. *Kidney International Reports*, 8(10), 2056–2067. <https://doi.org/10.1016/j.ekir.2023.07.032>
- Charuvila, S., Imam, M. S., Reza, T., Datta, P. K., Aziz, T. T., Davidson, S., Sumi, S. A., Alam, S., Ismail, M., Banu, T., & Lakhoo, K. (2024). A Prospective Observational Study of Preoperative Anaemia Management Aided by Bedside Haemoglobin Testers in a Low-Resource Setting. *Journal of Pediatric Surgery*, 59(2), 305–309. <https://doi.org/10.1016/j.jpedsurg.2023.10.041>
- Cheung, K. W., Au, T. S.-T., Lee, C.-H., Ng, V. W. Y., Wong, F. C.-K., Chow, W.-S., Hui, P. W., & Seto, M. T. Y. (2024). Hemoglobin A1c in early pregnancy to identify preexisting diabetes mellitus and women at risk of hyperglycemic pregnancy complications. *AJOG Global Reports*, 4(1), 100315. <https://doi.org/10.1016/j.xagr.2024.100315>
- Çuvadar, B., & Yılmaz, H. (2023). Non-invasive hemoglobin estimation from conjunctival images using deep learning. *Medical Engineering & Physics*, 120, 104038.

- <https://doi.org/10.1016/j.medengphy.2023.104038>
- Ding, N., Ma, Y.-H., Guo, P., Wang, T.-K., Liu, L., Wang, J.-B., & Jin, P.-P. (2024). Reticulocyte hemoglobin content associated with the risk of iron deficiency anemia. *Heliyon*, *10*(3), e25409. <https://doi.org/10.1016/j.heliyon.2024.e25409>
- Fu, Y., He, M., Liu, Y., Li, M., Zhu, M., Wang, Y., Lin, W., Yu, L., Yang, L., Zhang, Y., Liu, Y., Ji, H., Ding, H., & Wang, J. (2024). Reduction of haemoglobin is related to metal mixtures exposure in Chinese preschoolers: Joint effect models. *Journal of Trace Elements in Medicine and Biology*, *84*, 127427. <https://doi.org/10.1016/j.jtemb.2024.127427>
- Galvis, M., Díaz, J. D., Cuartas, D. E., Tovar, J. R., Fernandez-Trujillo, L., & Sua, L. F. (2024). Chronic disease prevalence in a population with structural hemoglobin disorders undergoing diabetes diagnosis: A bayesian approach. *Heliyon*, *10*(1), e23855. <https://doi.org/10.1016/j.heliyon.2023.e23855>
- Gasparello, J., Verona, M., Chilin, A., Gambari, R., & Marzaro, G. (2023). Assessing the interaction between hemoglobin and the receptor binding domain of SARS-CoV-2 spike protein through MARTINI coarse-grained molecular dynamics. *International Journal of Biological Macromolecules*, *253*, 127088. <https://doi.org/10.1016/j.ijbiomac.2023.127088>
- HB, B., Wahyuri, A. S., Zarya, F., Sabillah, M. I., & Annasai, F. (2023). Revitalizing student physical fitness: The vital role of post-pandemic physical activity programs. *Fizjoterapia Polska / Polish Journal of Physiotherapy*, *23*(4), 226–232. <https://doi.org/10.56984/8ZG20A4D3>
- Hoque, M. E., Shanta, S. M., Tahrin, R., Chowdhury, S., Tasnim, Z., Shuvo, M. A. A., & Riham, S. A. H. (2023). A benign way of measuring hemoglobin in blood – Towards developing a non-invasive technique. *Hybrid Advances*, *3*, 100039. <https://doi.org/10.1016/j.hybadv.2023.100039>
- Kong, L., Li, L., Yuan, J., Zhao, Y., Dong, L., Liu, M., Zhao, Y., Lu, T., & Chu, X. (2024). High-precision hemoglobin detection based on hyperspectral reconstruction of RGB images. *Biomedical Signal Processing and Control*, *91*, 105904. <https://doi.org/10.1016/j.bspc.2023.105904>
- Li, M., Yang, Z., Chen, S., Liu, Z., Tong, L., Zheng, S., & Yang, D. (2024). Sphaerotilus natans hemoglobins have an NADH oxidation activity and promote the yield of limonene in an engineered E. coli strain. *International Journal of Biological Macromolecules*, *254*, 128112. <https://doi.org/10.1016/j.ijbiomac.2023.128112>
- Li, P., Wu, J., Ni, X., Tong, M., Lu, H., Liu, H., Xue, T., & Zhu, T. (2023). Associations between hemoglobin levels and source-specific exposure to ambient fine particles among children aged <5 years in low- and middle-income countries. *Journal of Hazardous Materials*, *459*, 132061. <https://doi.org/10.1016/j.jhazmat.2023.132061>
- Li, Y., Du, M., He, S., Wang, R., Zhang, Z., & Wang, Q. (2023). Sensitive label-free hemoglobin detection based on polydopamine functionalized graphene oxide coated micro-tapered long-period fiber grating. *Optik*, *275*, 170626. <https://doi.org/10.1016/j.ijleo.2023.170626>
- Lukin, R., Law, J. Y., & Lokhandwala, P. M. (2024). Utility of hemoglobin electrophoresis to distinguish between severe delayed hemolytic transfusion reaction versus hyperhemolysis syndrome. *Transfusion and Apheresis Science*, 103919. <https://doi.org/10.1016/j.transci.2024.103919>
- Moreno Tirado, A., Rodríguez Ortega, P., & Calle Isorna, J. M. (2024). Hemoglobin J-Chicago: about a discordant glycosylated hemoglobin. *Endocrinología, Diabetes y Nutrición (English Ed.)*. <https://doi.org/10.1016/j.endien.2024.04.001>
- Muramatsu, A., Nakamura, S., Hirayama, T., Nagasawa, H., Ohira, A., Kitaoka, T., Hara, H.,

- & Shimazawa, M. (2024). Both hemoglobin and heme cause damage to retinal pigment epithelium through the iron ion accumulation. *Journal of Pharmacological Sciences*, 155(2), 44–51. <https://doi.org/10.1016/j.jphs.2024.04.001>
- Musallam, K. M., Barella, S., Origa, R., Ferrero, G. B., Lisi, R., Pasanisi, A., Longo, F., Giancesin, B., Forni, G. L., Pinto, V., Sciortino, R., Roberti, D., De Franceschi, L., & Culcasi, M. (2024). Pretransfusion hemoglobin level and mortality in adults with transfusion-dependent β -thalassemia. *Blood*, 143(10), 930–932. <https://doi.org/10.1182/blood.2023022460>
- Nguyen, K., Joo, H., Chen, L., & Chen, L. (2022). Preoperative hemoglobin and BMI are correlated with increased risk of conversion for minimally invasive gynecological oncology surgeries (564). *Gynecologic Oncology*, 166, S275–S276. [https://doi.org/10.1016/S0090-8258\(22\)01785-1](https://doi.org/10.1016/S0090-8258(22)01785-1)
- Nishi, H., Wang, J., Onishi, Y., & Nangaku, M. (2023). Infectious Risk and Variability of Hemoglobin Level in Patients Undergoing Hemodialysis. *Kidney International Reports*, 8(9), 1752–1760. <https://doi.org/10.1016/j.ekir.2023.06.004>
- Ohuma, E. O., Jabin, N., Young, M. F., Epie, T., Martorell, R., Peña-Rosas, J. P., Garcia-Casal, M. N., Kennedy, S. H., Victora, C. G., Craik, R., Ash, S., Barros, F. C., Barsosio, H. C., Berkley, J. A., Carvalho, M., Fernandes, M., Cheikh Ismail, L., Lambert, A., Lindgren, C. M., ... Villar, J. (2023). Association between maternal haemoglobin concentrations and maternal and neonatal outcomes: the prospective, observational, multinational, INTERBIO-21st fetal study. *The Lancet Haematology*, 10(9), e756–e766. [https://doi.org/10.1016/S2352-3026\(23\)00170-9](https://doi.org/10.1016/S2352-3026(23)00170-9)
- Olga, L., Sovio, U., Wong, H., Smith, G. C. S., & Aiken, C. E. M. (2024). Association between maternal hemoglobin concentration and educational attainment in mid-childhood in a high-resource obstetric setting: a prospective cohort study. *American Journal of Obstetrics & Gynecology MFM*, 6(5), 101357. <https://doi.org/10.1016/j.ajogmf.2024.101357>
- Ouali, R., & Bousbata, S. (2024). Unveiling The Peptidase Network Orchestrating Hemoglobin Catabolism in *Rhodnius prolixus*. *Molecular & Cellular Proteomics*, 100775. <https://doi.org/10.1016/j.mcpro.2024.100775>
- Park, S.-K., Hur, C., Kim, Y.-W., Yoo, S., Lim, Y.-J., & Kim, J.-T. (2024). Noninvasive hemoglobin monitoring for maintaining hemoglobin concentration within the target range during major noncardiac surgery: A randomized controlled trial. *Journal of Clinical Anesthesia*, 93, 111326. <https://doi.org/10.1016/j.jclinane.2023.111326>
- Parmar, A., Kaur, P., Sood, T., Kaur, R., Mittal, K., Kaur, G., Muraleedharan, V., & Aashiqeen, N. (2023). Association of blood donor characteristics with hemoglobin content in leukoreduced packed red blood cells. *Transfusion Clinique et Biologique*, 30(4), 430–435. <https://doi.org/10.1016/j.tracli.2023.08.006>
- Parvez, M. A., Yashiro, K., Tsunoi, Y., Saitoh, D., Sato, S., & Nishidate, I. (2024). In vivo monitoring of hemoglobin derivatives in a rat thermal injury model using spectral diffuse reflectance imaging. *Burns*, 50(1), 167–177. <https://doi.org/10.1016/j.burns.2023.07.006>
- Ploug, M., Knudsen, T., Qvist, N., & Kroijer, R. (2024). Decrease in hemoglobin following colorectal surgery - A cohort study with focus on iron deficiency. *Perioperative Care and Operating Room Management*, 34, 100363. <https://doi.org/10.1016/j.pcorm.2023.100363>
- Ramírez-Luzuriaga, M. J., Larson, L. M., Mannar, V., & Martorell, R. (2018). Impact of Double-Fortified Salt with Iron and Iodine on Hemoglobin, Anemia, and Iron Deficiency Anemia: A Systematic Review and Meta-Analysis. *Advances in Nutrition*, 9(3), 207–218. <https://doi.org/10.1093/advances/nmy008>

- Ramos, J. N., Calvão-Pires, P., Gil, I., Baptista, T., Branco, C., Branco, G., & Marto, J. P. (2024). Hemoglobin in large vessel occlusion: Look further than collaterals. *Journal of Clinical Neuroscience*, *121*, 100–104. <https://doi.org/10.1016/j.jocn.2024.02.010>
- Roldán Galiacho, V., Moreno Gamiz, M., & García-Ruiz, J. C. (2024). Pseudogaucher cells in a patient with α -thalassemia minor and S-hemoglobin carrier. *Hematology, Transfusion and Cell Therapy*. <https://doi.org/10.1016/j.htct.2023.11.010>
- Romero-Rosales, J. A., Aragonés, D. G., Escribano-Serrano, J., Borrachero, M. G., Doña, A. M., Macías López, F. J., Santos Mata, M. A., Jiménez, I. N., Casamitjana Zamora, M. J., Serrano, H., Belmonte-Beitia, J., Durán, M. R., & Calvo, G. F. (2024). Integrated modeling of labile and glycated hemoglobin with glucose for enhanced diabetes detection and short-term monitoring. *IScience*, *27*(4), 109369. <https://doi.org/10.1016/j.isci.2024.109369>
- Roy Chowdhury, J., Peringeth, K., Ganguly, A., Necesario, S. R. S., Hsieh, W.-C., Ra, Y., Choi, D., & Lin, Z.-H. (2024). Glycated hemoglobin (HbA1c) and biofluid-based diagnosis in diabetes: A comprehensive review. *Journal of Electroanalytical Chemistry*, *963*, 118301. <https://doi.org/10.1016/j.jelechem.2024.118301>
- Sachdev, H. S., Porwal, A., Acharya, R., Ashraf, S., Ramesh, S., Khan, N., Kapil, U., Kurpad, A. V., & Sarna, A. (2021). Haemoglobin thresholds to define anaemia in a national sample of healthy children and adolescents aged 1–19 years in India: a population-based study. *The Lancet Global Health*, *9*(6), e822–e831. [https://doi.org/10.1016/S2214-109X\(21\)00077-2](https://doi.org/10.1016/S2214-109X(21)00077-2)
- Santos, E. A., Carvalho, R. S., & Wermelinger, L. S. (2024). Case report and characterization of a Brazilian family with a rare hemoglobin variant—Hb Maputo. *Hematology, Transfusion and Cell Therapy*. <https://doi.org/10.1016/j.htct.2024.02.025>
- Schneider, V. M., Frank, P., Fuchs, S. C., & Ferrari, R. (2021). Effects of recreational sports and combined training on blood pressure and glycated hemoglobin in middle-aged and older adults: A systematic review with meta-analysis. *Experimental Gerontology*, *154*, 111549. <https://doi.org/10.1016/j.exger.2021.111549>
- Sedigheh, N., Hajieh, S., Javad, Z., & Mehrdad, S. (2023). Hemoglobin at the first visit of pregnancy and developing gestational diabetes mellitus: Results of a prospective registry cohort study. *Clinical Nutrition ESPEN*, *57*, 469–474. <https://doi.org/10.1016/j.clnesp.2023.07.084>
- Seekircher, L., Siller, A., Amato, M., Tschiderer, L., Balog, A., Astl, M., Schennach, H., & Willeit, P. (2024). HemoCue Hb-801 Provides More Accurate Hemoglobin Assessment in Blood Donors Than OrSense NBM-200. *Transfusion Medicine Reviews*, *38*(2), 150826. <https://doi.org/10.1016/j.tmr.2024.150826>
- Shojaei, Z., Abiri, M., Zafarghandi Motlagh, F., Amini, M., Dabbagh Bagheri, S., Asnavandi, S., Asadi, S., Bagherian, H., & Zeinali, S. (2024). First report of a patient with homozygous hemoglobin Erz: Evidence to support a non-pathogenic variant. *Blood Cells, Molecules, and Diseases*, *104*, 102797. <https://doi.org/10.1016/j.bcmed.2023.102797>
- Simoneau, J., Tay, C., Wheeler, A., Amos, L., McCormick, M., Collado, Y., Brown, M., & Weyand, A. C. (2024). Association between hemoglobin values and VWF assays: a multicenter investigation. *Blood Advances*, *8*(5), 1152–1154. <https://doi.org/10.1182/bloodadvances.2023011910>
- Sugiyono. (2015). *Metode Pendidikan Pendekatan Kuantitatif, Kualitatif, dan R&D*. Alfabeta.
- Sun, D. L. R., Puran, A., Al Nuaimi, M., AlRiyami, L., Kinlin, L. M., Kirby-Allen, M., Mahant, S., Gill, P. J., Borkhoff, C. M., & Parkin, P. C. (2024). Hemoglobin Threshold for Blood Transfusion in Young Children Hospitalized with Iron Deficiency Anemia. *The Journal of Pediatrics*, *266*, 113878. <https://doi.org/10.1016/j.jpeds.2023.113878>

- Wan, Y., Wei, Y., Zhang, C., Liu, Y., Xu, L., Gu, C., Yu, Z., Yin, J., Zhang, Q., & Deng, W. (2023). A novel role of acellular hemoglobin in hemolytic thrombosis. *Thrombosis Research*, 228, 33–41. <https://doi.org/10.1016/j.thromres.2023.05.024>
- Wen, J., Zhang, Q., Yang, Q., & Hu, F. (2021). The levels of hemoglobin are positively associated with arterial stiffness in community-dwelling Chinese adults. *Nutrition, Metabolism and Cardiovascular Diseases*, 31(10), 2929–2934. <https://doi.org/10.1016/j.numecd.2021.07.003>
- Xue, D., Jiang, S., Zhang, M., Shan, K., Lametsch, R., & Li, C. (2024). The efficiency and safety evaluation of hemoglobin hydrolysate as a non-heme iron fortifier. *Food Science and Human Wellness*, 13(2), 999–1010. <https://doi.org/10.26599/FSHW.2022.9250086>
- Yang, T., Yeoh, L. M., Tutor, M. V., Dixon, M. W., McMillan, P. J., Xie, S. C., Bridgford, J. L., Gillett, D. L., Duffy, M. F., Ralph, S. A., McConville, M. J., Tilley, L., & Cobbold, S. A. (2019). Decreased K13 Abundance Reduces Hemoglobin Catabolism and Proteotoxic Stress, Underpinning Artemisinin Resistance. *Cell Reports*, 29(9), 2917–2928.e5. <https://doi.org/10.1016/j.celrep.2019.10.095>
- You, L., Li, Z., Cheng, Y., Yao, N., & Guo, J. (2023). Anesthetic Management of a Patient With Hemoglobin M Disease Undergoing Laparoscopic Uterine Myomectomy: A Case Report. *Journal of PeriAnesthesia Nursing*. <https://doi.org/10.1016/j.jopan.2023.08.025>
- Young, M., Tapia, J. C., Szabados, B., Jovaisaite, A., Jackson-Spence, F., Nally, E., & Powles, T. (2024). NLR Outperforms Low Hemoglobin and High Platelet Count as Predictive and Prognostic Biomarker in Metastatic Renal Cell Carcinoma Treated with Immune Checkpoint Inhibitors. *Clinical Genitourinary Cancer*, 22(3), 102072. <https://doi.org/10.1016/j.clgc.2024.102072>
- Žagar, A., Simčič, T., Dajčman, U., & Megía-Palma, R. (2022). Parasitemia and elevation as predictors of hemoglobin concentration and antioxidant capacity in two sympatric lizards. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 270, 111233. <https://doi.org/10.1016/j.cbpa.2022.111233>
- Zagrean-Tuza, C., Igescu, I., Lupan, A., & Silaghi-Dumitrescu, R. (2024). A study of the molecular interactions of hemoglobin with diverse classes of therapeutic agents. *Inorganica Chimica Acta*, 567, 122053. <https://doi.org/10.1016/j.ica.2024.122053>
- Zhang, J., Wu, Y., Tang, H., Li, H., Da, S., Ciren, D., Peng, X., & Zhao, K. (2024). Identification, characterization, and insights into the mechanism of novel dipeptidyl peptidase-IV inhibitory peptides from yak hemoglobin by in silico exploration, molecular docking, and in vitro assessment. *International Journal of Biological Macromolecules*, 259, 129191. <https://doi.org/10.1016/j.ijbiomac.2023.129191>
- Zhang, X., Cui, S., Zu, Y., & Feng, C. (2024). Antioxidant properties of acteoside against biological systems: Hemoglobin and cardiomyocyte as potential models. *Arabian Journal of Chemistry*, 17(4), 105630. <https://doi.org/10.1016/j.arabjc.2024.105630>
- Zhao, B.-C., Xie, Y.-S., Luo, W.-C., Lei, S.-H., Liu, J.-M., Yang, X., Dong, Y.-H., Liu, W.-F., & Liu, K.-X. (2024). Postoperative haemoglobin and anaemia-associated ischaemic events after major noncardiac surgery: A sex-stratified cohort study. *Journal of Clinical Anesthesia*, 95, 111439. <https://doi.org/10.1016/j.jclinane.2024.111439>
- Zhu, J., Dong, Y., Liao, P., Yin, X., He, J., & Guo, L. (2024). Prognostic value of hemoglobin in patients with sepsis: A systematic review and meta-analysis. *Heart & Lung*, 64, 93–99. <https://doi.org/10.1016/j.hrtlng.2023.12.001>
- Zohoun, A., Baglo Agbodande, T., Zohoun, L., & Anani, L. (2020). Prevalence of hemoglobin abnormalities in an apparently healthy population in Benin. *Hematology, Transfusion and Cell Therapy*, 42(2), 145–149. <https://doi.org/10.1016/j.htct.2019.06.005>

Jurnal Info Kesehatan

Vol. 22, No. 2, June 2024, pp. 409-418

P-ISSN 0216-504X, E-ISSN 2620-536X

DOI: [10.31965/infokes.Vol22.Iss2.1492](https://doi.org/10.31965/infokes.Vol22.Iss2.1492)Journal homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>**RESEARCH****Open Access****The Effect of Preeclampsia in Pregnant Women on The Incidence of Maternal Mortality: Literature Review****Riski Resa Oktaria^{1a*}, Tia Setriana^{1b}, Sofia Merylista^{1c}, Yenni Fusfitasari^{2d}, Nunu Harison^{3e}**¹ Master Program of Midwifery, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia² Study Program of Nursing, STIKes Bhakti Husada Bengkulu, Bengkulu, Indonesia³ Study Program of Nurse Profession, STIKes Bhakti Husada Bengkulu, Bengkulu, Indonesia^a Email address: resaoktaria29@gmail.com^b Email address: tiasetriana16@gmail.com^c Email address: sofiamerylista79@gmail.com^d Email address: yen.fus@gmail.com^e Email address: nunuharison@gmail.com

Received: 16 May 2024

Revised: 30 June 2024

Accepted: 30 June 2024

Abstract

Preeclampsia is a severe pregnancy complication characterized by elevated blood pressure and often occurs after 20 weeks of gestation. This condition can lead to significant morbidity and mortality for both the mother and the fetus. This study aims to identify the risk factors associated with preeclampsia in pregnant women, a condition that remains prevalent in Indonesia and globally. The research employed a comprehensive literature review method, examining ten articles sourced from databases such as Google Scholar, PubMed, and Elsevier. The inclusion criteria for the literature review involved studies focused on the causes and consequences of preeclampsia, published within the last ten years, and providing substantial data on maternal mortality. The results of the literature review indicated that pregnant women with a history of hypertension or previous preeclampsia are at a significantly higher risk of developing preeclampsia. Additionally, severe cases of preeclampsia, such as those involving HELLP syndrome, eclampsia, and high diastolic blood pressure, were strongly linked to increased maternal mortality. The review also highlighted the crucial role of efficient referral systems and timely medical interventions in managing severe preeclampsia and reducing maternal deaths. This study concludes that early detection and continuous monitoring are essential for managing preeclampsia effectively. The findings underscore the need for standardized treatment protocols, improved healthcare access, and comprehensive prenatal services to mitigate the risks associated with preeclampsia. Future research should aim to include a broader range of studies and explore the long-term implications of preeclampsia on maternal and fetal health. These insights are critical for developing effective strategies to reduce the incidence and severity of preeclampsia and enhance maternal health outcomes.

Keywords: Preeclampsia, Pregnant Women, Maternal Mortality.***Corresponding Author:**

Riski Resa Oktaria

Master Program of Midwifery, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia

Email: resaoktaria29@gmail.com

©The Author(s) 2024. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

1. INTRODUCTION

Pre-eclampsia is a complex multisystem condition characterized by sudden onset hypertension after 20 weeks of gestation, accompanied by at least one additional complication such as proteinuria, maternal organ dysfunction, or placental-related issues (e.g., angiogenic imbalance or fetal growth retardation). It is one of the most serious pregnancy disorders and a leading cause of maternal and perinatal morbidity and mortality worldwide. Pre-eclampsia, previously known as toxemia, affects approximately 4 million women globally each year, resulting in over 70,000 maternal deaths and 500,000 fetal deaths (Magee et al., 2022). Women who survive pre-eclampsia are at an increased risk of developing chronic conditions like diabetes, heart disease, and stroke later in life (Kvalvik et al., 2020; Pittara et al., 2021; Poon et al., 2019). Additionally, infants born from pre-eclamptic pregnancies face risks including premature birth, perinatal mortality, neurodevelopmental delays, and future cardiovascular and metabolic diseases (Kvalvik et al., 2020).

In Indonesia, pre-eclampsia affects approximately 128,273 women annually, accounting for about 5.3% of pregnancies. Hypertension (25%) and infection (12%) follow hemorrhage (30%) as leading causes of maternal death (Ministry of Health, 2015). Severe pre-eclampsia is a critical condition that requires immediate medical intervention to prevent complications during pregnancy, delivery, and the postpartum period. It can cause endothelial dysfunction in multiple organs, increasing the risk of cardiovascular diseases and other long-term health issues.

Despite the existence of the National Medical Service Guidelines for Preeclampsia Diagnosis and Treatment, updated in 2016 by the Indonesian Association of Obstetric Gynecology in collaboration with the Association of Feto-Maternal Doctors, disparities in the quality of care for severe pre-eclampsia persist across hospitals and healthcare providers. These guidelines aim to standardize care practices and offer recommendations for the creation of Clinical Practice Guidelines.

Maternal mortality risk factors can be categorized as per McCarthy and Maine's framework into: (1) underlying determinants, including social, financial, and cultural factors; (2) intermediate determinants, such as maternal health status, reproductive factors, healthcare accessibility, health-related behaviors, and other unknown factors; and (3) proximate determinants, including pregnancy and obstetric complications (Sulistiyono & Joewono, 2020). Effective basic healthcare could reduce maternal mortality by up to 20%, and a well-functioning referral system could decrease it by over 80% (UNICEF).

Critical to reducing maternal mortality is the timely management of emergency cases. Delays in recognizing emergencies, reaching referral centers, and receiving adequate care remain significant challenges. A functional referral system ensures that obstetric emergencies, like severe pre-eclampsia, are managed promptly and accurately, improving maternal outcomes. This study aims to investigate the factors related to the referral and treatment of severe pre-eclampsia patients, focusing on referral processes (referrers, initial treatment, referral travel time) and treatment protocols (reaction time, magnesium sulfate administration, nifedipine use, delivery management) and their impact on maternal mortality (Sulistiyono & Joewono, 2020).

This research aims to investigate the effect of pre-eclampsia on maternal mortality by examining existing studies and data. This research aims to identify the critical factors involved in the referral and treatment processes for severe pre-eclampsia, focusing on the roles and responsibilities of healthcare providers, initial treatment protocols, and the impact of referral travel time on maternal outcomes. Moreover, the review aims to assess how these referral and treatment practices influence maternal mortality, analyzing the impact of timely and accurate referrals, adherence to treatment protocols, and identifying gaps in current practices contributing to high maternal mortality rates associated with severe pre-eclampsia.

2. RESEARCH METHOD

The literature review process for this study followed a systematic approach using several academic databases including Google Scholar, Elsevier, PubMed, and Emerald. The search was conducted using specific keywords such as "Preeclampsia," "Cause," "Maternal Mortality," and "Pregnancy." Initially, the researcher conducted searches across these databases using the specified keywords to identify relevant articles and studies related to preeclampsia and maternal mortality. Articles were selected based on their relevance to the research topic and the inclusion of key information related to the causes of preeclampsia and its association with maternal mortality. The criteria likely included studies published within a certain timeframe (e.g., recent studies), studies focusing on human subjects, and those providing substantial data or analysis on the specified topics. Ultimately, a total of ten relevant articles were selected for detailed analysis and inclusion in the literature review. These articles were chosen based on their contribution to understanding the specified aspects of preeclampsia and maternal mortality, ensuring a comprehensive review of the current literature on the topic.

3. RESULTS AND DISCUSSION

Of the ten literature obtained, four of the journals are cross-sectional studies and one journal is a case-control study. The findings of a study of five existing research journals can be displayed in the summary table of study results as follows:

Table 1. Literature Review Results

No.	Researchers	Result
1.	Sulistiyono, et al (2020)	Out of 63 occurrences of preeclampsia, there were 20 deaths; all of the deceased women were not on medical records. Case Death Rate (CFR): 31.74%; 11% >35 years old; 20% <1 day; 65% of cases had preeclampsia and hypertension diagnoses; 40% of cases had recurrent seizures. 40% were detected between 32 and 36 weeks of pregnancy, 65% had sepsis, 35% had brain hemorrhage, 55% experienced renal failure, 25% were obese, and 25% had HELLP syndrome. A significant fraction of unreported occurrences include maternal deaths due to eclampsia, which is characterized by high CFR, stays less than one day, and obesity comorbidities (Sulistiyono & Joewono, 2020).
2.	Muhani & Besral (2015)	High diastolic blood pressure, eclampsia, and HELLP syndrome are risk factors for severe pre-eclampsia that can greatly raise the chance of maternal death. After adjusting for variables such as maternal age, gravida, gestational age, delivery method, diasepam administration, the respondent's region of residence, education, and employment, the risk of death increases by 12.5 times for HELLP syndrome, 12.1 times for eclampsia, and 7.4 times for diastolic blood pressure 110–119 mmHg and 5.5 times for diastolic blood pressure ≥ 120 mmHg. While not statistically significant, high systolic blood pressure and high proteinuria levels were two additional predictors of severe preeclampsia that were linked to an increased risk of maternal mortality (Muhani & Besral, 2015).

-
3. Skjaerven et al (2015) Preterm preeclamptic women carrying little fetuses (lowest quartile) were shown to have a 2.5-fold higher risk of cardiovascular disease death (95% C.I 1.6-3.8), whereas large fetuses (highest quartile) were associated with a 12.3-fold higher risk (7.3-21). Stronger HR values (HR = 11.4 and 13.0) were observed in weeks 22–34 and 35–36. Diabetes is excluded, which significantly lowers this elevated risk. Women who have given birth just once in their lifetime are at a notably higher risk (Skjaerven et al., 2015).

 4. Ernawati, et al (2018) The findings demonstrated a significant correlation ($p = 0.005$) between maternal age and the incidence of preeclampsia, gestational age of preeclamptic mothers ($p = 0.000$), and delivery method of preeclamptic mothers ($p = 0.000$), but no significant correlation between maternal parity status and infant mortality ($p = 0.043$) (Ernawati et al., 2018).

 5. Rana, S et al. (2019) Preeclampsia during the first trimester can lead to catastrophic consequences, such as the foetus's and mother's deaths. Preeclampsia's pathophysiology is mostly attributed to the placenta, according to clinical and scientific research, despite the fact that the exact origin of this disease is still unknown. Researchers address the available data in this review on the pathophysiology of maternal preeclampsia syndrome, including the aberrant role of placentation and the function of placental factors including SOLV1, SOD1 (soluble fms-like tyrosine kinase 1) and SDF-1 (Rana et al., 2019).

 6. Sriwandoko, et al (2019) The following factors were associated with an increased risk of maternal death: referral travel time (OR= 9.99; 95% CI = 1.76 to 56.75; $p=0.009$), delayed treatment (OR=13.62; 95% CI = 2.25 to 82.45; $p=0.009$), complex delivery (OR=27.66; 95% CI = 3.71 to 206.26; $p=0.001$), long operating room response time (OR=0.05; 95% CI <0.01 to 0.56; $p=0.014$), and long delivery room response time (OR=9.80; 95% CI = 1.70 to 175.60; $p=0.004$) (Sriwandoko et al., 2019).

 7. Turbeville and Sasser (2020) consequences throughout time on the mother and child, raising their risk of hypertension and chronic renal disease (45, 54, 115, 116), which could last a lifetime for both of them (Turbeville & Sasser, 2020).

 8. Gayatri, et al (2022) Obesity, referral distance, and less than four prenatal services were linked to maternal mortality from preeclampsia. The capacity to stop preeclampsia-related maternal deaths will improve with the identification of these factors. Maternal mortality from preeclampsia was significantly correlated with four times the length of time from the place of delivery to the referral hospital (OR 5.183, 95% CI 1.681 - 15.977, p -value $p 0.004$), the frequency of obesity (OR 4.176, 95% CI 1.507 - 11.572, p -value 0.006), and prenatal care (4.648, 95% CI 1.776 - 12.167, p -value 0.002). The duration of trip to the
-

		referral hospital, however, did not appear to be linked to maternal deaths caused by preeclampsia (OR 1.537, 95% CI 0.534 - 4.422, p-value 0.426) (Gayatri et al., 2022).
9.	Dimitriadis, E., Rolnik, D.L., Zhou, W. et al. (2023)	Preterm pregnancies increased the risk of preterm birth, perinatal mortality, neurodevelopmental impairments, and later-life cardiovascular and metabolic disorders in the offspring. In addition to having a shorter life expectancy and a higher risk of stroke, cardiovascular disease, and diabetes, women who survived pre-eclampsia (Dimitriadis et al., 2023).
10.	Prihadi, et al (2023)	25.1% of cases had hypertension throughout pregnancy, with preeclampsia and eclampsia accounting for 9.8% and 1.4% of cases, respectively. The main reason for the 36.6% of maternal deaths associated with hypertension was eclampsia. The most frequent adverse events were hemolysis syndrome (HELLP, 22%) with elevated liver enzymes and low platelets, and heart failure (45.5%. In 2022, CFR dropped from 61% in 2018 to 10%. From 2015 to 2022, the CFR was 1.3% overall, with eclampsia having the greatest death rate (9.4%). But since 2018, there has been a declining tendency, with a low of 0.2% in 2021 (Prihadi et al., 2023).

The literature review of ten journal articles provided a comprehensive understanding of the intricate relationship between preeclampsia and maternal mortality. These studies collectively highlight the multifaceted nature of preeclampsia, emphasizing the need for early detection, effective management, and long-term care to improve maternal health outcomes and reduce mortality rates associated with this condition. A consistent finding across the reviewed literature is the importance of early detection and continuous monitoring of preeclampsia. Undiagnosed or inadequately managed hypertension and related complications significantly increase the risk of maternal mortality. Comprehensive prenatal care that includes regular screening for hypertension and preeclampsia is essential to identify and manage high-risk pregnancies effectively (Sulistiyono et al., 2020).

Severe manifestations of preeclampsia, such as eclampsia, HELLP syndrome, and high diastolic blood pressure, are strongly associated with increased maternal mortality. These severe cases require targeted interventions and prompt management to prevent fatal outcomes. The review underscores the necessity for healthcare systems to be equipped with the resources and protocols to handle severe preeclampsia efficiently (Muhani & Besral, 2015). Then, preeclampsia not only poses immediate risks during pregnancy but also has long-term health implications for both mothers and their children. Women with a history of preeclampsia are at a higher risk of developing cardiovascular diseases, hypertension, and chronic renal conditions later in life. Similarly, children born to mothers with preeclampsia face increased risks of neurodevelopmental delays and long-term cardiovascular and metabolic disorders. This highlights the need for long-term health monitoring and interventions beyond the immediate postpartum period (Skjaerven et al., 2015).

Efficient referral systems are critical in managing preeclampsia, especially in severe cases. Delays in referral, prolonged travel times, and delayed treatment significantly increase the risk of maternal death. Effective emergency care protocols, including prompt transportation and timely medical interventions, are vital to improve outcomes for women experiencing severe preeclampsia (Sriwandoko et al., 2019). Socioeconomic factors, including obesity, inadequate prenatal care, and healthcare access, play a significant role in maternal mortality related to preeclampsia. Addressing these factors through improved healthcare access, lifestyle

interventions, and comprehensive prenatal services is crucial in reducing the incidence and severity of preeclampsia. The review indicates that socioeconomic determinants of health must be considered in developing strategies to manage and prevent preeclampsia (Gayatri et al., 2022).

The literature review suggests several policy and clinical practice recommendations to improve maternal and neonatal outcomes. These include the development of standardized treatment protocols, training programs for healthcare providers, and policies that ensure equitable access to quality prenatal and emergency care. Implementing these recommendations can help address the gaps in current practices and reduce maternal mortality rates associated with preeclampsia. These insights are supported by studies such as those by Sulistyono et al. (2020) and Gayatri et al. (2022), which emphasize the importance of timely intervention and standardized care protocols in managing preeclampsia and reducing maternal mortality.

The overall results of the literature review underscore the multifaceted impact of preeclampsia on maternal mortality. Early detection, effective management of severe cases, efficient referral systems, and addressing socioeconomic factors are crucial for improving outcomes. Long-term health monitoring and targeted interventions for both mothers and children affected by preeclampsia are also essential. By integrating these findings into clinical practice and policy development, healthcare systems can significantly enhance maternal health and reduce mortality rates associated with preeclampsia.

The Effect of Referral Systems on Maternal Mortality

The referral systems are crucial for reducing maternal mortality by ensuring that high-risk pregnant women receive timely and specialized care. According to the literature, effective referral mechanisms allow for early detection and management of complications such as severe preeclampsia. These systems are essential in areas where primary care may lack the necessary expertise and resources to handle such emergencies. Strengthening these pathways is vital to improve maternal outcomes and reduce deaths (Sulistyono et al., 2020).

Impacts of Early Treatment on Maternal Passing

Early treatment of severe obstetric conditions is critical in reducing maternal mortality by preventing complications and ensuring timely interventions (Muhani & Besral, 2015). Early intervention in cases of severe preeclampsia and other obstetric emergencies is crucial in preventing maternal deaths. The literature highlights the importance of timely and appropriate treatment to stabilize the condition and mitigate risks. Healthcare providers must be able to identify and manage these conditions promptly to reduce complications and mortality. Early treatment not only involves administering appropriate medications but also preparing for potential emergency procedures.

The Impact of Travel Time on Maternal Passing

Reducing travel time to healthcare facilities is crucial in lowering maternal mortality by ensuring timely treatment for obstetric emergencies (Skjaerven et al., 2015). Travel time to healthcare facilities is a significant factor in maternal mortality rates. Longer travel times often result in delayed treatment, increasing the risk of complications and death. The literature emphasizes that reducing travel time is essential to ensure timely access to care for pregnant women, particularly those experiencing obstetric emergencies. Improving the accessibility of healthcare services can lead to better maternal health outcomes.

The Effect of Emergency Response Time on Maternal Mortality

Quick emergency response times are essential in reducing maternal mortality by enabling timely and effective management of obstetric emergencies (Ernawati et al., 2018). The response time of emergency care teams significantly impacts maternal mortality. Rapid response to obstetric emergencies ensures that complications are managed quickly and effectively, reducing the risk of maternal death. The literature stresses the importance of efficient emergency response systems, which include the availability of trained personnel and medical resources, to improve maternal survival rates.

The Effect of Operating Room Response Time on Maternal Death

Timely availability of the operating room is crucial in reducing maternal mortality during emergency surgical interventions (Rana et al., 2019). The readiness and response time of the operating room are critical in emergencies. Delays in preparing the operating room for procedures such as cesarean sections can lead to increased maternal mortality. The literature indicates that ensuring the operating room is promptly available and that the surgical team is ready to act quickly is vital for managing severe obstetric complications and improving maternal outcomes.

The Effect of Time Room Response on Maternal Death

Fast response times in the delivery room are essential for reducing maternal mortality by ensuring timely management of obstetric emergencies (Sriwandoko et al., 2019). Response times in the delivery room are critical for preventing maternal deaths during obstetric emergencies. Prompt actions, including preparing for and performing emergency procedures, can significantly reduce the risk of maternal mortality. The literature highlights the importance of quick and effective responses to manage complications and prevent severe outcomes.

Effect of MgSO₄ Administration on Maternal Mortality

MgSO₄ administration is crucial in managing severe preeclampsia and reducing maternal mortality by preventing complications (Turbeville & Sasser, 2020). Magnesium sulfate (MgSO₄) is widely recognized for its effectiveness in treating severe preeclampsia and eclampsia. The administration of MgSO₄ can significantly reduce maternal mortality by controlling seizures and lowering blood pressure, thus preventing severe complications. The literature supports the timely and appropriate use of MgSO₄ as a critical measure in reducing the risks associated with severe preeclampsia.

The Effect of Nifedipine Administration on Iby's Death

Nifedipine helps manage hypertension in severe preeclampsia, but its impact on maternal mortality requires consistent usage (Gayatri et al., 2022). Nifedipine is used to manage high blood pressure in pregnant women with severe preeclampsia. While it is effective in reducing blood pressure and preventing further complications, the literature suggests that its impact on maternal mortality may be limited due to inconsistent application among healthcare providers. Proper administration and adherence to treatment protocols are necessary to maximize its potential benefits.

The Effect of Childbirth on Maternal Death

Cesarean sections are essential in reducing maternal mortality by effectively managing severe preeclampsia complications (Dimitriadis et al., 2023; Prihadi et al., 2023). The mode of childbirth, particularly cesarean sections, plays a significant role in maternal mortality among women with severe preeclampsia. The literature indicates that cesarean sections are often necessary to manage severe complications and prevent maternal deaths. Timely decision-

making and preparation for cesarean deliveries are crucial for improving maternal outcomes and reducing mortality rates.

4. CONCLUSION

The study's findings underscore the significant impact of preeclampsia on maternal mortality, highlighting the urgent need for effective management and intervention strategies. Specifically, the data suggest that maternal deaths are considerably influenced by delayed or inadequate response to preeclamptic emergencies. Internal factors such as genetic predisposition, chronic hypertension, and obesity, along with environmental factors including access to healthcare and socioeconomic status, are key contributors to the development and exacerbation of preeclampsia. While the precise pathophysiology of preeclampsia remains complex and multifactorial, current hypotheses suggest that abnormal placental development and immune responses play critical roles. The study emphasizes that early detection, timely referral, and prompt treatment, including the use of medications like MgSO₄ and nifedipine, are crucial in reducing the risks associated with severe preeclampsia. Therefore, a comprehensive approach that integrates maternal health education, improved access to prenatal care, and robust emergency response systems is essential to mitigate the adverse outcomes associated with preeclampsia and ultimately reduce maternal mortality.

REFERENCES

- Alonso-Ventura, V., Li, Y., Pasupuleti, V., Roman, Y. M., Hernandez, A. V., & Pérez-López, F. R. (2020). Effects of preeclampsia and eclampsia on maternal metabolic and biochemical outcomes in later life: a systematic review and meta-analysis. *Metabolism*, 102, 154012. <https://doi.org/10.1016/j.metabol.2019.154012>
- Amalia, F. F. (2020). Pengaruh penggunaan mgso4 sebagai terapi pencegahan kejang pada preeklampsia. *Jurnal Ilmu Kedokteran dan Kesehatan*, 7(1), 393-400. <https://doi.org/10.33024/jikk.v7i1.2215>
- Ananth, C. V., Brandt, J. S., Hill, J., Graham, H. L., Grover, S., Schuster, M., ... & Joseph, K. S. (2021). Historical and recent changes in maternal mortality due to hypertensive disorders in the United States, 1979 to 2018. *Hypertension*, 78(5), 1414-1422. <https://doi.org/10.1161/HYPERTENSIONAHA.121.17661>
- Berhe, A. K., Kassa, G. M., Fekadu, G. A., & Muche, A. A. (2018). Prevalence of hypertensive disorders of pregnancy in Ethiopia: A systematic review and meta-analysis. *BMC Pregnancy and Childbirth*, 18(1), 34. <https://doi.org/10.1186/s12884-018-1667-7>
- Covella, B., Vinturache, A. E., Cabiddu, G., Attini, R., Gesualdo, L., Versino, E., et al. (2019). A systematic review and meta-analysis indicates long-term risk of chronic and end-stage kidney disease after preeclampsia. *Kidney International*, 96(3), 711-727. <https://doi.org/10.1016/j.kint.2019.03.033>
- Davis, N. L., Hoyert, D. L., Goodman, D. A., Hirai, A. H., & Callaghan, W. M. (2017). Contribution of maternal age and pregnancy checkbox on maternal mortality ratios in the United States, 1978-2012. *American Journal of Obstetrics and Gynecology*, 217(3), 352.e1-352.e7. <https://doi.org/10.1016/j.ajog.2017.06.026>
- Dimitriadis, E., Rolnik, D. L., Zhou, W., Estrada-Gutierrez, G., Koga, K., Francisco, R. P. V., Whitehead, C., Hyett, J., da Silva Costa, F., Nicolaidis, K., & Menkhorst, E. (2023). Preeclampsia. *Nature Reviews Disease Primers*, 9(1), 8. <https://doi.org/10.1038/s41572-023-00417-6>
- Dimitriadis, E., Rolnik, D. L., Zhou, W., Estrada-Gutierrez, G., Koga, K., Francisco, R. P. V., Whitehead, C., Hyett, J., da Silva Costa, F., Nicolaidis, K., & Menkhorst, E. (2023). Pre-

- eclampsia. *Nature Reviews Disease Primers*, 9(1), 8. <https://doi.org/10.1038/s41572-023-00417-6>
- Ernawati, Wigati, K. W., Hafizh, A. N., Santoso, B., & Nursalam. (2018). Contributing factors of neonatal death from mother with preeclampsia in Indonesia. *Indian Journal of Public Health Research & Development*, 9(11), 375. <https://doi.org/10.5958/0976-5506.2018.01483.3>
- Gayatri, M., Indriani, A., Akingbade, O., & Mulyadi, J. (2022). Verbal Autopsy of Preeclampsia-related Maternal Death in Jember District, Indonesia: A Case Control Study. *Bahrain Medical Bulletin*, 44(4).
- Ghulmiyyah, L., & Sibai, B. (2012). Maternal mortality from preeclampsia/eclampsia. *Seminars in Perinatology*, 36(1), 56–59. <https://doi.org/10.1053/j.semperi.2011.09.011>
- Gyselaers, W., & Thilaganathan, B. (2019). Preeclampsia: A gestational cardiorenal syndrome. *The Journal of Physiology*. <https://doi.org/10.1113/JP274893>
- Handayani, S., & Milie, P. (2020). Pengaruh Pendidikan Kesehatan Melalui Whatsapp Group Terhadap Pengetahuan Dan Sikap Ibu Hamil Dalam Deteksi Dini Preeklampsia Pada Masa Pandemi COVID-19. *Jurnal Kebidanan*, 217-230. <https://doi.org/10.35872/jurkeb.v12i02.394>
- Hermawati, D. (2020). Hubungan Paritas dan Usia Ibu Hamil dengan Preeklampsia di Rumah Sakit Kota Banda Aceh. *Idea Nursing Journal*, 11(3), 62-69. <https://doi.org/10.52199/inj.v11i3.20812>
- Jikamo, B., Adefris, M., Azale, T., & Alemu, K. (2021). Cultural adaptation and validation of the Sidamic version of the World Health Organization Quality-of-Life-Bref Scale measuring the quality of life of women with severe preeclampsia in southern Ethiopia. *Health and Quality of Life Outcomes*, 19, 239. <https://doi.org/10.1186/s12955-021-01872-z>
- Jikamo, B., Adefris, M., Azale, T., & Alemu, K. (2022). The effect of preeclampsia on adverse maternal outcomes in Sidama region, Ethiopia: A prospective open cohort study. *Scientific Reports*, 12(1), 19300. <https://doi.org/10.1038/s41598-022-24034-7>
- MacDorman, M. F., Declercq, E., & Thoma, M. E. (2017). Trends in maternal mortality by sociodemographic characteristics and cause of death in 27 states and the District of Columbia. *Obstetrics & Gynecology*, 129(4), 811–818. <https://doi.org/10.1097/AOG.0000000000001968>
- Magee, L. A., Brown, M. A., Hall, D. R., Gupte, S., Hennessy, A., Karumanchi, S. A., Kenny, L. C., McCarthy, F., Myers, J., Poon, L. C., Rana, S., Saito, S., Staff, A. C., Tsigas, E., & von Dadelszen, P. (2022). The 2021 International Society for the Study of Hypertension in Pregnancy classification, diagnosis & management recommendations for international practice. *Pregnancy Hypertension*, 27, 148–169. <https://doi.org/10.1016/j.preghy.2021.09.008>
- Melese, M. F., Badi, M. B., & Aynalem, G. L. (2019). Perinatal outcomes of severe preeclampsia/eclampsia and associated factors among mothers admitted in Amhara Region referral hospitals, North West Ethiopia. *BMC Research Notes*, 12, 147. <https://doi.org/10.1186/s13104-019-4161-z>
- Muhani, N., & Besral, B. (2015). Pre-eclampsia Berat dan Kematian Ibu. *Kesmas: National Public Health Journal*, 10(2), 80. <https://doi.org/10.21109/kesmas.v10i2.884>
- Pittara, T., Vyrides, A., Lamnisos, D., & Giannakou, K. (2021). Pre-eclampsia and long-term health outcomes for mother and infant: an umbrella review. *BJOG: An International Journal of Obstetrics & Gynaecology*, 128(9), 1421–1430. <https://doi.org/10.1111/1471-0528.16683>
- Poon, L. C., Shennan, A., Hyett, J. A., Kapur, A., Hadar, E., Divakar, H., McAuliffe, F., da Silva Costa, F., von Dadelszen, P., McIntyre, H. D., Kihara, A. B., Di Renzo, G. C.,

- Romero, R., D'Alton, M., Berghella, V., Nicolaides, K. H., & Hod, M. (2019). The International Federation of Gynecology and Obstetrics (FIGO) initiative on pre-eclampsia: A pragmatic guide for first-trimester screening and prevention. *International Journal of Gynecology & Obstetrics*, 145(S1), 1–33. <https://doi.org/10.1002/ijgo.12802>
- Pribadi, A., Hidayat, D., Sasotya, R. M. S., Aziz, M. A., Nurdiawan, W., Pramartirta, A. Y., Siddiq, A., Mose, J. C., Hidayat, Y. M., Nugrahani, A. D., Santoso, D. P. J., & Permadi, W. (2023). Assessing the Impact of the Zero Mother Mortality Preeclampsia Program on Maternal Mortality Rates at a Single Center in Bandung, West Java (2015-2022): A Retrospective Study. *Medical Science Monitor*, 29. <https://doi.org/10.12659/MSM.941097>
- Rana, S., Lemoine, E., Granger, J. P., & Karumanchi, S. A. (2019). Preeclampsia. *Circulation Research*, 124(7), 1094–1112. <https://doi.org/10.1161/CIRCRESAHA.118.313276>
- Rangaswami, J., Naranjo, M., & McCullough, P. A. (2018). Preeclampsia as a form of type 5 cardiorenal syndrome: An underrecognized entity in women's cardiovascular health. *Cardiorenal Medicine*, 8(3), 160–172. <https://doi.org/10.1159/000487646>
- Roberge, S., Bujold, E., & Nicolaides, K. H. (2018). Meta-analysis on the effect of aspirin use for prevention of preeclampsia on placental abruption and antepartum hemorrhage. *American Journal of Obstetrics and Gynecology*, 218(5), 483–489. <https://doi.org/10.1016/j.ajog.2017.12.238>
- Setyaningsih, D., Ariyanti, I., Oktaviani, D. A., & Yunadi, F. D. (2020). Terapi Murrotal Al-Mulk Dalam Penurunan Kecemasan Ibu Dengan Pre Eklamsi. *J Kebidanan Malahayati*, 6(3), 388-93. <https://10.33024/jkm.v6i3.2723>
- Skjaerven, R., deRoo, L., Klungsoyr, K., Morken, N. H., Rich-Edwards, J., & Wilcox, A. J. (2015). [77-OR]. Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health, 5(1), 41. <https://doi.org/10.1016/j.preghy.2014.10.081>
- Sriwandoko, H., Purnomo, W., Trijanto, B., & Darmawan, E. S. (2019). The Effect of Referral and Treatment of Severe Preeclampsia on Maternal Death at Sultan Imanudin General Hospital Pangkalan Bun, Central Kalimantan. *Strengthening Hospital Competitiveness to Improve Patient Satisfaction and Better Health Outcomes*, 174–182. <https://doi.org/10.26911/the6thicph-FP.03.01>
- Sulistiyono, A., & Joewono, H. T. (2020). Maternal mortality from eclampsia at tertiary referral hospital, Surabaya, Indonesia. *EurAsian Journal of BioSciences*, 14(1), 1709-1713.
- Turbeville, H. R., & Sasser, J. M. (2020). Preeclampsia beyond pregnancy: long-term consequences for mother and child. *American Journal of Physiology-Renal Physiology*, 318(6), F1315–F1326. <https://doi.org/10.1152/ajprenal.00071.2020>
- Utami, T., Sukmaningtyas, W., & Safitri, M. (2020). Hubungan usia ibu dengan kejadian asfiksia neonatorum pada ibu preeklampsia berat. *Menara Medika*, 3(1). <https://doi.org/10.31869/mm.v3i1.2202>
- Winasih, N. L. S., Armini, N. W., & Suratni, I. G. A. (2021). Gambaran Ibu Bersalin Dengan Preeklampsia Di Rumah Sakit Umum Pusat Sanglah Denpasar Tahun 2020. *Jurnal Ilmiah Kebidanan (The Journal Of Midwifery)*, 9(2), 177-182. <https://doi.org/10.33992/jik.v9i2.1443>

Jurnal Info Kesehatan

Vol. 22, No. 2, June 2024, pp. 419-428

P-ISSN 0216-504X, E-ISSN 2620-536X

DOI: [10.31965/infokes.Vol22.Iss2.1526](https://doi.org/10.31965/infokes.Vol22.Iss2.1526)Journal homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>**RESEARCH****Open Access****Quality Evaluation and Flavonoid Content of Honey from Riau Forest, Indonesia****Eva Yuniritha^{1a}, Nur Ahmad Habibi^{1b*}, Hasneli^{1c}, Alsri Windra Doni^{1d}**¹ Department of Nutrition, Health Ministry Polytechnic of Padang, Padang, West Sumatra, Indonesia^a Email address: yunirithaeva2010@gmail.com^b Email address: nahindo2022@gmail.com^c Email address: hasneli.darwis@yahoo.com^d Email address: alsriwindradoni79@gmail.com

Received: 21 May 2024

Revised: 20 June 2024

Accepted: 30 June 2024

Abstract

Honey is a naturally sweet liquid produced by bees from flower nectar, which varies in quality and flavonoid content due to the food source, environment and the bees that produce it. This study aims to evaluate the quality and flavonoid content of honey from the Riau Forest of Indonesia. Quality testing was conducted in accordance with SNI 8664:2018 with sensory, physical, chemical and microbiological parameters. Flavonoid testing was carried out qualitatively using HPLC. The test results showed that the sensory profile of honey was in accordance with the standard, sweet taste, distinctive aroma, brown colour and thick shape. Chemical parameters obtained values of moisture content of 21.92%, diastase enzyme 3.13 DN and HMF 4.14 mg/kg have met the requirements. However, the reducing sugar of 54.1% is still below the requirement. Heavy metal parameters (Pb, Cd, As and Hg) were not detected and microbiological ALT 3.0 x 10 and yeast mould <10 colonies/gr that have met. Furthermore, the results of flavonoid qualitative tests detected several types including Luteolin, Mangiferin, Smiglanin, maltol, isoflavones, quercetin, Cnidimol C, Norcimifungin, Apigenin, Methyl ophiopogonanone B, Pectolarigenin, Kusenol C, 3,5,6-trihydroxy-4',7-dimetoxyflavone and Lupinifoline. The conclusion of this study shows that honey from Riau forests fulfils most of the quality requirements and has a diverse flavonoid content.

Keywords: Honey, Flavonoid, Food Quality and Safety.

***Corresponding Author:**

Nur Ahmad Habibi

Department of Nutrition, Health Ministry Polytechnic of Padang, Padang, West Sumatra, Indonesia

Email: nahindo2022@gmail.com

©The Author(s) 2024. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

1. INTRODUCTION

Honey is a naturally sweet liquid produced by bees (*Apis dorsata*.) from flower nectar (Edo et al., 2023). Honey generally contains a large amount of carbohydrates, mainly in the form of fructose and glucose more than 60%. In addition, honey also contains proteins (including enzymes), minerals, vitamins, organic acids, polyphenols (flavonoids and phenolic acids), volatile compounds, waxes, and other phytochemicals (Tafere, 2021). Phenolic compounds (polyphenols) are secondary metabolites produced from plants. Flavonoids and phenolic acids are phenolic compounds that are found in honey (Cheung et al., 2019).

Flavonoids present in honey come from flower nectar, propolis, and pollen (Tomás et al., 1993). Honey that contains a lot of flavonoids and phenolic compounds is of better quality because it presents a potential natural antioxidant in the treatment of various diseases and contributes to individual health (Ahmed et al., 2018). Flavonoids and phenolic acids are able to prevent oxidation through the release of hydrogen or electrons, thus stabilizing free radicals. (Hassanpour, 2023). Many studies have shown that regular consumption of honey with its flavonoids and phenolic content can reduce the risk of heart disease (Bt Hj Idrus et al., 2020). In addition, these compounds also have biological activities as anticancer, anti-inflammatory, antioxidant, and antiatherogenic properties (Erejuwa et al., 2012).

Indonesia is one of the countries that has a high production of forest honey (Food Review Indonesia, 2020). This is due to the vast forest potential. Riau Province, precisely in the Kampar area, is an area in Indonesia that is able to produce honey in large quantities. Generally, the honey produced is cattle honey by utilizing forest nectar, such as calliandra and rubber flowers (Yunianto, 2020).

Despite the extensive research on the general composition and health benefits of honey, there remains a significant gap in the specific evaluation of honey from Riau Forest, Indonesia, particularly concerning its flavonoid content and overall quality. Previous studies have broadly covered the phenolic compounds in honey and their antioxidant properties, but there is limited detailed analysis of the flavonoid content in honey from this specific region. Additionally, while the health benefits of flavonoids and phenolic acids in honey are well-documented, there is a lack of localized research that ties these benefits to the unique floral sources and environmental conditions of Riau Forest. This study aims to fill this gap by providing a thorough evaluation of the quality and flavonoid content of honey from Riau Forest, contributing valuable data that can enhance our understanding of its potential health benefits and support the promotion of forest honey from this region as a high-quality product.

2. RESEARCH METHOD

This research is quantitative in nature with descriptive data analysis that observes the honey that comes from the forests of Riau province. The honey used in the study was multiflora honey produced from *Apis dorsata* bees taken from the forests of Riau province in the period January-June 2024. Honey was extracted from the hive through a centrifugation process, then put into glass bottles and stored at +4°C in a closed room. Prior to analysis the honey was gently stirred, when crystallization occurred the honey was warmed at 35°C to 38°C.



Figure 1. Sampling location of Riau Forest honey

In the sample collection stage, sterile sample bottles, honey filters, labeling tools, and portable coolers are used. Evaluation of honey quality requires a refractometer, pH meter, UV-Vis spectrophotometer, sugar analysis kit, water bath, micropipette, analytical balance, and thermometer. Analysis of flavonoid content using HPLC. Supporting equipment such as filter paper, test tubes, drop pipettes, and glassware are also required.

Organoleptic Test Analysis. The test used limited trained panelists of 3 people who have experience in honey testing. Honey was tested organoleptically for taste, aroma, colour and texture and the resulting sensory profile was described (Cabrera & Santander, 2022).

Diastase Enzyme Test Analysis. The exposed honey and starch solutions were incubated and the time required to reach the endpoint was measured photometrically. The result is expressed in ml of 1% hydrolyzed starch equivalent to the enzyme in 1 g honey in 1 (one) hour. Pipette 10 ml of sample solution, then put it into a 50 ml test tube. Continue by pipetting 5 ml of starch solution through the inner wall of the tube then place it in a $40^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ water bath for 15 minutes. Shake and switch on the stopwatch. At every 5-minute interval, pipet 1 ml of the sample mixture and add it to 10.00 ml of iodine solution. Mix, then dilute to the volume as before and determine the absorbance value at a wavelength of 660 nm. Plot the absorbance value against time (minutes) on millimeter paper. A straight line is drawn through several points. From the graph, determine the time required to reach an absorbance value (A) = 0.235. The value of 300 divided by the time required to reach the absorbance value (A) indicates the diastase enzyme activity (DN)(BSN, 2018).

HMF Analysis. HMF measurements were carried out based on the SNI 8664: (2018) reference, starting with 5g of honey weighed in a small glass cup, then put into a 50 ml volumetric flask and added aquadest until the volume of the solution reached 25 ml. Then 0.5ml Cerrez I solution was added, shaken, and then 0.5ml Cerrez II solution was added, shaken, and diluted with distilled water up to the line mark. Then a drop of alcohol was added to remove the foam on the surface. Then filtered using filter paper and discarded 10ml of the first filter. A total of 5ml of the filter was pipetted and each was put into a test tube. Then 5 ml of water was pipetted and put into one tube (example) and 5 ml of 0.2% NaHSO₃ into the other tube (comparison). The solution was shaken well and the absorbance of the sample was determined against the reference at wavelengths of 284nm and 336 nm (BSN, 2018).

Moisture Content Analysis. Moisture content was determined and measured using a honey refractometer. The way to use a honey refractometer is to open the light plate and drop a few drops of honey until the honey covers the entire blue area. The result is the value on the water scale shown on the viewfinder. The water content value is expressed in percent (%). The test results were compared with the standard value of honey according to SNI 8664:2018 (BSN, 2018).

Sugar Content Analysis. A total of 1g of honey was weighed and dissolved in 20 ml of distilled water. A solution of 0.1ml was taken and added to 1.5ml of DNS reagent. Then the solution was shaken evenly and heated in a water bath at 100^o C for 5 minutes. After that the solution was cooled in running water, 0.5ml was taken and then 15ml of sterile distilled water was added. The solution was shaken evenly and measured at a wavelength of 540 nm. The results obtained were entered into the equation obtained from the standard curve and then divided by the weight of the sample used. The percentage of glucose content was calculated by multiplying the results obtained by 100% (BSN, 2018).

Ash Content Analysis. The percentage of ash content was measured through an ashing process using a *muffle furnace* at 550^o C for 6 hours.

Heavy Metal Analysis. Test for metal contamination in food in accordance with SNI 2896: 1992. The prepared sample solution was determined by absorbance with Uv-Vis Spectrophotometer at $\lambda = 283.3$ nm. The absorbance obtained was substituted in the standard equation obtained from the standard curve with the standard series solution of Pb, Cd, Hg and As (BSN, 1998).

Microbiological Analysis Total Plate Number. Honey samples were diluted with 0.85% physiological NaCl solution starting from dilution 10⁻¹ - 10⁻⁶. Each of the dilution samples was taken as much as 1 ml and then put into a petri dish and made duplo. Plate Count Agar (PCA) media that has been made as much as 15-25 ml was taken and poured into a petri dish. The Petri dish containing the media was then rotated back and forth with the aim that the sample could be mixed homogeneously and left to solidify. After the media becomes solid, the Petri dish containing the media is then incubated for 24-48 hours at a temperature of 35-45^o C with an inverted position. If there are colonies that grow, the colonies are observed and counted (Hasanah et al., 2023).

Microbiological Analysis Mould Yeast Numbers. Herbal medicine samples were diluted with 0.85% physiological NaCl solution starting from dilution 10⁻¹ - 10⁻⁴. Each of the dilutions taken as much as 1 ml was put into a Petri dish and made duplo. Potato Dextrose Agar (PDA) media that has been made is taken as much as 15-25 ml put into a petri dish. Petri dishes are rotated back and forth with the aim that the sample can be mixed homogeneously and then left to solidify. After the PDA media becomes solid, the Petri dish is then incubated in an inverted position at a temperature of 20-25^o C for 5 days. If there is colony growth on the media after 5 days of incubation, the number of colonies is then observed and counted (Hasanah et al., 2023).

Qualitative Analysis of Flavonoid Content. Flavonoids were extracted with ethyl acetate (Karabagias et al., 2014). 10 g of honey was dissolved in 50 mL of water to a final concentration of 20% (b/v). The solution was then acidified by adding 0.2M HCl and saturated with 30% (w/v) sodium chloride. The solution obtained was then filtered through cotton to remove solid particles and extracted three times with ethyl acetate using a separatory funnel. The organic layer was then dried in a vacuum dryer at 40^oC with a low pressure of 100 mbar until the entire solution evaporated. The residue was redissolved in 5 mL of methanol. Prior to HPLC analysis, all honey sample solutions were filtered through a 0.45 μ m pore size *polytetrafluoroethylene* (PTFE) filter.

Agilent 1200 series HPLC system was used for flavonoid analysis. The wavelength used for detection was from 256 nm to 350 nm. Flavonoid separation was performed using a

WondaCract ODS-2 reversed-phase column (250 mm × 4.6 mm, particle size 5 µm) at room temperature. The gradient elution flow rate was 1 mL/min with a solution of 2.0% (v/v) acetic acid (solvent A) and acetonitrile (solvent B) as mobile phases. The injection volume was 20 µL. Flavonoid analysis started with 10% solvent B and then increased to 30% from 0 to 20 min. Then, solvent B was increased to 40% from 20 to 30 min, and then 50% from 30 to 50 min. In addition, the column was eluted with 50% acetonitrile for 10 minutes before the next sample injection. To identify the peaks, 100 µg/mL stock solution of each standard was prepared by dissolving the flavonoid standards in methanol. One mL of the stock solution was used and injected into the HPLC for peak identification. By comparing the retention time, flavonoids can be identified (Cheung et al., 2019).

The data obtained will be analyzed using a descriptive statistical approach to calculate the mean, standard deviation, minimum, and maximum values of each parameter measured, including moisture content, pH, total sugar, enzyme activity, and flavonoid content. The data will be presented in tables to facilitate interpretation, and the results of the analyses will be interpreted to provide a deeper understanding of the quality and flavonoid content of honey from Riau forests. This research has also received ethical approval from the Tanjungkarang Health Polytechnic Research Ethics Commission with No.879/KEPK-TJK/XII/2023.

3. RESULTS AND DISCUSSION

Riau is a region in Indonesia that is able to provide large amounts of forest and livestock honey. The honey produced includes rubber honey, calliandra honey, and other multiflora honey. Generally, the honey is sold directly by farmers to honey processing industries for commercialization on a large scale.

Regulation and standardization of honey is regulated by the Food and Drug Administration and the National Standardization Agency. There are parameters that must be met by producers, including organoleptic, physical, chemical, and microbiological. This aims to protect consumers from product adulteration and food safety risks. Table 1 shows the quality of honey from the Riau forest.

The quality of Riau honey in terms of taste, aroma, colour, and texture meets the set standards. The honey has a sweet taste, typical honey aroma, brown colour, and thick texture. The sweetness of honey comes from the sugar content (sucrose, fructose, and glucose) (Melina et al., 2023). The content of reduced sugar reaches 54.16%, which includes 33.19% fructose. This content is below the SNI requirements. The low content of reducing sugar is influenced by moisture content, humidity, and harvesting period. Research shows that high water content stimulates yeast activity to grow. *Yeast* in the form of *osmophilic yeast* of the genus *Zygosaccharomyces*, which is resistant to high sugar concentrations, can live and thrive in honey. Yeast in honey will degrade sugars, especially dextrose and levulose into alcohol and CO₂, thus affecting the dextrose (glucose) and levulose (fructose) content of honey. This is thought to be the cause of the lower glucose content in honey (Dygas et al., 2021). Sucrose content in honey can be influenced by the presence of the enzyme invertase, an enzyme that converts sucrose into glucose and fructose. The optimum temperature of the invertase enzyme ranges from 30-50°C (Manoochehri et al., 2020). The sucrose content of room-temperature honey is lower than that of cold-temperature honey because the invertase enzyme is more active at room temperature than at cold-temperature (Wulandari, 2017).

The research results obtained honey moisture content of 21.92%, this value has met the SNI requirements, which is a maximum of 22%. The moisture content of honey is influenced by the humidity of the environment. This is because honey has hygroscopic properties, which is easy to absorb water. The higher the humidity, the higher the moisture content of the honey. If the humidity is 51%, the moisture content of honey is 16.1%. If the humidity is 81%, the moisture content of honey is 33.4% (Wulandari, 2017). A low moisture content will keep the

honey from spoiling for a relatively long period of time (Chirife et al., 2006). High moisture content in honey will stimulate yeast activity to grow and develop in honey. Harvesting age also affects the water composition of honey. Honey harvested at an older age has less water content than honey harvested at a younger age. The longer the honey is in the honeycomb, the more complete the evaporation of water content in the honey (Evahelda et al., 2017).

Table 1. Honey Quality Results based on SNI 8664:2018 Standard

No.	Parameters	Unit	Results	Honey Standard
1	Organoleptic Flavour Aroma Colour Texture		Sweet Typical Honey Chocolate Thick liquid	Typical Honey Typical Honey Chocolate Thick Liquid
2	Diastase enzyme	DN	3.13	Minimum 3
3	Hydroxymethylfurfural (HMF)	mg/kg	4.41	Maximum 50
4	Water Content	%	21.92	Maximum 22
5	Fructose	%	33.19	
6	Saccharose	%	NI	Maximum 5
7	Reducing Sugar	%	54.61	Minimum 65
8	Chloramphenicol	mg/kg	NI	NI
9	Acidity	ml NaOH 1N/kg	17.40	Maximum 50
10	pH		4.36	<4.5
11	Undissolved solids	%	0.12	Maximum 0.5
12	Ash Content	%	0.48	Maximum 0.5
13	Heavy Metals Cd Hg As Pb	mg/kg mg/kg mg/kg mg/kg	NI NI NI NI	Maximum 0.2 Maximum 0.03 Maximum 1.0 Maximum 2.0
14	Microbiology ALT Moulds	Colony/g Colony/g	3.0×10^1 < 10^1	< 5×10^3 < 1×10^1

Enzyme diastase and Hydroxymethylfurfural (HMF) are specific parameters commonly used to determine the purity of honey. The test results obtained honey diastase enzyme 3.13 DN and HMF 4.41 mg/kg, these values have met the established quality requirements. The minimum diastase enzyme value is 3. Honey with undetectable diastase enzyme is suspected to be fake honey. There are several factors that affect the diastase enzyme content, including post-harvest honey processing and honey storage temperature. A good harvest time is when the honey is old, if it is young, or when the honey has not been completely covered by wax, it will produce honey with high water content due to the lack of evaporation. Usually, to reduce the water content in honey, heating is done. The optimal storage temperature for honey is at room temperature (26°C), during post-harvest handling, storage, and transport, honey is avoided from direct sun exposure and heat exceeding 28°C and open-air (Ichsan et al., 2022).

Heavy metal content is a critical parameter regarding the safety of honey. The test results showed no detectable content of heavy metals Cadmium (Cd), Mercury (Hg), Arsenic (As), and Lead (Pb). These values fulfill the quality requirements set by the regulator. The content of heavy metals is strongly influenced by the environment from which the honey is produced, honey produced in areas polluted by industrial pollutants, whether from air, water, or soil will

be at risk of heavy metal contamination (Demaku et al., 2023). Riau forest honey is produced from forests that are free from pollutants, so the honey is not contaminated with heavy metals.

Furthermore, the results of microbiological testing obtained the number of total plate numbers (ALT) 3.0×10^1 colonies/g and yeast mould $<10^1$ colonies/g. These values fulfill the requirements of SNI. Microbial contamination in honey comes from primary and secondary sources. Primary sources of microbial contamination may come from pollen, bee digestive tract, dust, air, soil, and nectar. On the other hand, secondary sources come from personal, cross-contamination, equipment, and the environment. Secondary sources of contamination can be controlled through *good manufacturing practices* (GMP) (Snowdon & Cliver, 1996).

Flavonoids (from Latin *flavus* meaning yellow) are a group of naturally occurring heterocyclic compounds that contain oxygen. These compounds are found in plants in free form and bound to sugar components in several glycoside forms. Flavonoids are generally water-soluble and in nature are found in fruits, vegetables, tea, grapes, wine, honey, propolis, nectar and others. Currently, more than 5000 kinds of flavonoids have been identified in various plants (Panche et al., 2016; Ververidis et al., 2007).

Table 2. Flavonoid content in honey riau

Parameters	Identification
<i>Luteolin</i>	+
<i>Mangiferin</i>	+
<i>Smiglanin</i>	+
<i>Leucodelphinidin</i>	-
<i>Maltol</i>	+
<i>(-)-epi-afzelechin</i>	-
<i>5,6,7,3-tetrahydroxy-4-methoxyisoflavone</i>	+
<i>Sec-O-glucosylhamaudol</i>	-
<i>Robinetin</i>	-
<i>3-O-B-D-Galacopyanosyl quercetin</i>	+
<i>2,5-dimethyl-7-hydroxychromone</i>	+
<i>Khellol-b-D-glucoside</i>	+
<i>Cnidimol C</i>	+
<i>Norcimifungin</i>	+
<i>Isorhamnetin-3-O-b-rutinoside_1</i>	+
<i>Patuletin-7-O[6-(2-methylbutyryl)]-glucoside</i>	+
<i>Isoophiopogonone A</i>	+
<i>Apigenin-6-C-glucosylglucoside</i>	+
<i>Isoscoparin-3-O-glucopyranoside</i>	+
<i>Schaftoside</i>	+
<i>Cnidimol A</i>	+
<i>3,7-dihydroxy-6-methoxyflacanonol</i>	+
<i>Methyl ophiopogonanone B</i>	+
<i>Pectolarigenine</i>	+
<i>Kusenol C</i>	+
<i>3,5,6-trihydroxy-4',7-dimethoxyflavone</i>	+
<i>Lupinifoline</i>	+
<i>6-methoxy-2-(2-phenylethyl)chromone</i>	-
<i>Chromone</i>	-
<i>Cianidine 3,5-diglucoside_1</i>	-
<i>Adenine</i>	-

<i>Trigoneline</i>	-
<i>Gentiatibetine</i>	-
<i>Salviamiltamide</i>	-
<i>Guvacoline</i>	-
<i>19-epi-3-iso-ajmalicine</i>	-

Description: (-) not detected (+) Detected

The structural diversity of flavonoids is due to the modification of the basic skeleton structure. These modifications are conditioned by various reactions of hydrogenation, hydroxylation, O-methylation of hydroxyl groups, dimerization, or glycosylation of hydroxyl groups (O-glycosides). Depending on the degree of oxidation of the pyran ring, as well as the α -position of the secondary aromatic ring. Flavonoids can be classified into several subgroups, including flavonols, flavones, flavanones, catechins, anthocyanidins, isoflavones, dihydro flavonols, and chalcones based on differences in their basic molecular structure (Harborne & Williams, 2000). Table 2 shows the flavonoids contained in Riau forest honey.

The amount of flavonoids in honey can reach 6 mg/kg, while the amount is much higher in pollen (0.5%) and propolis (10%)(Gašić et al., 2017). Based on the test results, the flavonoids detected include *Luteolin*, *Mangiferin*, *Smiglanin*, *maltol*, *isoflavones*, *quercetin*, *Cnidimol C*, *Norcimifungin*, *Apigenin*, *Methyl ophiopogonanone B*, *Pectolarigenin*, *Kusenol C*, *3,5,6-trihydroxy-4',7-dimetoxyflavone* and *Lupinifoline*. The flavonoid content in each honey varies greatly influenced by many factors, including the flower nectar, and the propolis present. (Tomás-Barberán et al., 2001). Riau Forest Honey generally takes its nectar from the sialang tree (Suhesti & Hadinoto, 2015).

Flavonoids have a number of health benefits, including anti-cancer, antioxidant, anti-inflammatory, and anti-viral activities. They also have neuroprotective and cardioprotective effects. These biological activities depend on the type of flavonoid, its mode of action, and its bioavailability. Research shows that in the form of isoflavones, quercetin and luteolin are able to exert a favourable influence on the health of the cardiovascular system through the inhibition of thrombokinase activity, the lowering of LDL cholesterol levels and the prevention of inflammatory activity caused by free radicals (Olas, 2020). The flavonoids in honey can prevent cancer through the mechanism of increasing *caspase 3* activity which has an impact on cancer cell apoptosis (Ahmed & Othman, 2013).

4. CONCLUSION

Forest honey from Riau has good quality and safety, fulfilling the requirements set by SNI. The reducing sugar content needs to be improved with a good storage process. The flavonoid content in Riau forest honey is quite varied, including *Luteolin*, *Mangiferin*, *Smiglanin*, *maltol*, *isoflavones*, *quercetin*, *Cnidimol C*, *Norcimifungin*, *Apigenin*, *Methyl ophiopogonanone B*, *Pectolarigenin*, *Kusenol C*, *3,5,6-trihydroxy-4',7-dimetoxyflavone* and *Lupinifoline*. The type of flavonoids produced is due to the source of nectar and propolis provided. The flavonoid content in honey has potential health benefits.

REFERENCES

- Ahmed, S., & Othman, N. H. (2013). Honey as a potential natural anticancer agent: a review of its mechanisms. *Evidence-Based Complementary and Alternative Medicine*, 2013(1), 829070. <https://doi.org/10.1155/2013/829070>
- Ahmed, S., Sulaiman, S. A., Baig, A. A., Ibrahim, M., Liaqat, S., Fatima, S., ... & Othman, N. H. (2018). Honey as a potential natural antioxidant medicine: an insight into its molecular mechanisms of action. *Oxidative medicine and cellular longevity*, 2018(1), 8367846.

- <https://doi.org/10.1155/2018/8367846>
- BSN. (1998). *Meotode Uji Cemaran logam dalam Makanan SNI 01-2896 -1998*. Badan Standarisasi Nasional.
- BSN. (2018). *SNI 8664:2018 Tentang Madu*. Badan Standarisasi Nasional.
- Bt Hj Idrus, R., Sainik, N. Q. A. V., Nordin, A., Saim, A. Bin, & Sulaiman, N. (2020). Cardioprotective Effects of Honey and Its Constituent: An Evidence-Based Review of Laboratory Studies and Clinical Trials. *International Journal of Environmental Research and Public Health*, 17(10), 3613. <https://doi.org/10.3390/ijerph17103613>
- Cabrera, M., & Santander, E. (2022). Physicochemical and sensory analysis of honeys from eastern Formosa province (Argentina) and its relationship with their botanical origin. *Food Chemistry Advances*, 1, 100026. <https://doi.org/10.1016/j.focha.2022.100026>
- Cheung, Y., Meenu, M., Yu, X., & Xu, B. (2019). Phenolic acids and flavonoids profiles of commercial honey from different floral sources and geographic sources. *International Journal of Food Properties*, 22(1), 290–308. <https://doi.org/10.1080/10942912.2019.1579835>
- Chirife, J., Zamora, M. C., & Motto, A. (2006). The correlation between water activity and% moisture in honey: Fundamental aspects and application to Argentine honeys. *Journal of Food Engineering*, 72(3), 287-292. <https://doi.org/10.1016/j.jfoodeng.2004.12.009>
- Demaku, S., Aliu, A., Sylejmani, D., Ahmetaj, B., & Halili, J. (2023). Determination of Heavy Metals in Bee Honey as a Bioindicator in the Istog, Drenas and Kastriot Regions. *Journal of Ecological Engineering*, 24(5), 191–200. <https://doi.org/10.12911/22998993/161654>
- Dygas, D., Nowak, S., Olszewska, J., Szymańska, M., Mroczyńska-Florczak, M., Berłowska, J., Dziugan, P., & Kręgiel, D. (2021). Ability of yeast metabolic activity to reduce sugars and stabilize betalains in red beet juice. *Fermentation*, 7(3), 1–14. <https://doi.org/10.3390/fermentation7030105>
- Edo, G. I., Onoharigho, F. O., Akpoghelie, P. O., Akpoghelie, E. O., Agbo, J. J., Agoh, E., & Lawal, R. A. (2023). Natural Honey (Raw Honey): Insights on Quality, Composition, Economic and Health Effects: A Comprehensive Review. *Food Science and Engineering*, November, 265–293. <https://doi.org/10.37256/fse.4220232713>
- Erejuwa, O. O., Sulaiman, S. A., & Ab Wahab, M. S. (2012). Honey: A novel antioxidant. *Molecules*, 17(4), 4400–4423. <https://doi.org/10.3390/molecules17044400>
- Evahelda, E., Pratama, F., & Santoso, B. (2017). The changes of moisture content, pH, and total sugar content of honey originated from the flowers of Bangka rubber tree during storage. *Int. J. Sci. Eng. Res.*, 5(5), 33-36.
- Food Review Indonesia. (2020). *Potensi Madu Indonesia*. Food Review Indonesia. Retrieved from <https://foodreview.co.id/blog-5670113-Potensi-Madu-Indonesia.html>
- Gašić, U. M., Milojković-Opšenić, D. M., & Tešić, Ž. L. (2017). Polyphenols as possible markers of botanical origin of honey. *Journal of AOAC International*, 100(4), 852–861. <https://doi.org/10.5740/jaoacint.17-0144>
- Harborne, J. B., & Williams, C. A. (2000). Advances in flavonoid research since 1992. *Phytochemistry*, 55(6), 481–504. [https://doi.org/10.1016/s0031-9422\(00\)00235-1](https://doi.org/10.1016/s0031-9422(00)00235-1)
- Hasanah, S., Kiromah, N. Z. W., & Fitriyati, L. (2023). Uji Angka Lempeng Total (ALT) Dan Angka Kapang Khamir (AKK) Pada Jamu Gendong Di Pasar Tradisional Wonokriyo Kecamatan Gombong Kabupaten Kebumen. *Jurnal Farmasi Sains Dan Terapan*, 10(1), 51–56. <https://doi.org/10.33508/jfst.v10i1.4195>
- Hassanpour, S. H., & Doroudi, A. (2023). Review of the antioxidant potential of flavonoids as a subgroup of polyphenols and partial substitute for synthetic antioxidants. *Avicenna Journal of Phytomedicine*, 13(4), 354–376. <https://doi.org/10.22038/AJP.2023.21774>
- Ichsan, D. S., Hafidzah, T. S., Putri, S. B., Aurene, S. V., & Nurdin, I. (2022). Deteksi Madu

- Palsu Dan Kualitas Madu Dengan Enzim Diastase. *Poltekita : Jurnal Ilmu Kesehatan*, 16(3), 278–283. <https://doi.org/10.33860/jik.v16i3.1685>
- Karabagias, I. K., Vavoura, M. V., Nikolaou, C., Badeka, A. V., Kontakos, S., & Kontominas, M. G. (2014). Floral authentication of Greek unifloral honeys based on the combination of phenolic compounds, physicochemical parameters and chemometrics. *Food Research International*, 62, 753–760. <https://doi.org/10.1016/j.foodres.2014.04.015>
- Manoochehri, H., Hosseini, N. F., Saidijam, M., Taheri, M., Rezaee, H., & Nouri, F. (2020). A review on invertase: Its potentials and applications. *Biocatalysis and Agricultural Biotechnology*, 25(March), 101599. <https://doi.org/10.1016/j.bcab.2020.101599>
- Melina, M., Adawiyah, D. R., & Hunaefi, D. (2023). Indonesian Honey Consumers' Behavior and Sensory Preference for Commercial Trigona Honey. *Jurnal Teknologi Dan Industri Pangan*, 34(1), 86–97. <https://doi.org/10.6066/jtip.2023.34.1.86>
- Olas, B. (2020). Honey and its phenolic compounds as an effective natural medicine for cardiovascular diseases in humans? *Nutrients*, 12(2), 1–14. <https://doi.org/10.3390/nu12020283>
- Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of Nutritional Science*, 5, e47. <https://doi.org/10.1017/jns.2016.41>
- Snowdon, J. A., & Cliver, D. O. (1996). Microorganisms in honey. *International Journal of Food Microbiology*, 31(1), 1–26. [https://doi.org/10.1016/0168-1605\(96\)00970-1](https://doi.org/10.1016/0168-1605(96)00970-1)
- Suhesti, E., & Hadinoto, H. (2015). Hasil Hutan Bukan Kayu Madu Sialang di Kabupaten Kampar (Studi Kasus : Kecamatan Kampar Kiri Tengah). *Wahana Forestra: Jurnal Kehutanan*, 10(2), 16–26. <https://doi.org/10.31849/forestra.v10i2.227>
- Tafere, D. A. (2021). Chemical composition and uses of Honey: A Review. *Journal of Food Science and Nutrition Research*, 4(3), 194-201. <https://doi.org/10.26502/jfsnr.2642-11000072>
- Tomás-Barberán, F. A., Ferreres, F., García-Vignera, C., & Tomás-Lorente, F. (1993). Flavonoids in honey of different geographical origin. *Zeitschrift Für Lebensmittel-Untersuchung Und Forschung*, 196(1), 38–44. <https://doi.org/10.1007/BF01192982>
- Tomás-Barberán, F. A., Martos, I., Ferreres, F., Radovic, B. S., & Anklam, E. (2001). HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. *Journal of the Science of Food and Agriculture*, 81(5), 485–496. <https://doi.org/https://doi.org/10.1002/jsfa.836>
- Ververidis, F., Trantas, E., Douglas, C., Vollmer, G., Kretzschmar, G., & Panopoulos, N. (2007). Biotechnology of flavonoids and other phenylpropanoid-derived natural products. Part I: Chemical diversity, impacts on plant biology and human health. *Biotechnology Journal*, 2(10), 1214–1234. <https://doi.org/https://doi.org/10.1002/biot.200700084>
- Wulandari, D. D. (2017). Kualitas Madu (Keasaman, Kadar Air, dan Kadar Gula Pereduksi) Berdasarkan Perbedaan Suhu Penyimpanan. *Jurnal Kimia Riset*, 2(1), 16–22.
- Yunianto, A. S., & Jannetta, S. (2020). Potensi budidaya lebah madu sebagai harapan di tengah pandemi Covid-19. *Unri Conference Series: Community Engagement*, 2, 192–200. <https://doi.org/10.31258/unricsce.2.192-200>