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RESEARCH Open Access

The Effect of Home Visits on Depression and Anxiety in Pregnant Patients During COVID-19

Elisabeth MF Lalita[1a*], Dian Pratiwi[1b], Amelia Donsu[1c], Nancy Olii[2d]

[1] Department of Midwifery, Health Polytechnic, Ministry of Health, Manado, North Sulawesi, Indonesia
[2] Department of Midwifery, Health Polytechnic, Ministry of Health, Gorontalo, Gorontalo, Indonesia

[a] Email address: emflalita@gmail.com
[b] Email address: pratiwi.dian1826@gmail.com
[c] Email address: ameliyaks.donsu@gmail.com
[d] Email address: oliinancy7@gmail.com

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Abstract

Depression and anxiety during pregnancy can increase the risk of miscarriage, premature birth, low birth weight, low Apgar scores, and fetal death The past COVID-19 pandemic caused significant anxiety and infection fear, causing significant harm to pregnant women's lives. Public access to health services has changed during the COVID-19 pandemic. This study aimed to determine the effectiveness of pregnancy care home visits on depression and anxiety of pregnant women. Efforts should therefore be made to increase visits by pregnant women while maintaining health protocols during the pandemic. The research method is a Quasi-experimental design with one group pretest-posttest design. Sampling was carried out by purposive sampling with the inclusion criteria of pregnant women who did not re-examine at the Health Center for 1 month and pregnant women who were willing to be selected as respondents. obtained 28 pregnant women as respondents. The instruments used are the Edinburgh Postpartum Depression Scale (EPDS) questionnaire and the Hamilton Anxiety Rating Scale (HARS). The data analysis technique used the Paired Simple T-test. The results of the study showed that there was an increase in depression and anxiety during pregnancy during the COVID-19 pandemic, this data was obtained from interviews and pregnancy care with home visits. The depression rate at the initial data of 12.36 dropped to 3.50, and the anxiety rate of 22.46 dropped to 6.61 after being given prenatal care with home visits. In these difficult times, the need for prenatal health care can ensure the provision of protective and safe services and psychological screening of pregnant women at risk for depression to reduce long-term negative outcomes should be carried out.

Keywords: Depression, Anxiety, Home Visit Interventions.

Corresponding Author:

Elisabeth MF Lalita
Department of Midwifery, Health Polytechnic, Ministry of Health, Manado, North Sulawesi, Indonesia
Email: emflalita@gmail.com

1. INTRODUCTION

Depression and anxiety during pregnancy can increase the risk of miscarriage, premature birth, low birth weight, low Apgar scores, and fetal death (NICE, 2014)(Alder et al., 2007). The results of the study by Mizrak Sahin and Kabakci (2021) show that the coronavirus pandemic has great potential to create anxieties, difficulties, and fears that adversely affect pregnant women (Mizrak Sahin & Kabakci, 2021) Other findings report that COVID-19 significantly impacts pregnant mental health (Fan et al., 2021).

The first case report coronavirus disease (COVID-19) from Wuhan described nine pregnant women diagnosed with COVID-19 in the third trimester of pregnancy (Nishiura H et al., 2020)(Chen, YuLi et al., 2020). The ongoing pandemic has caused extreme fear, resulting in an increased need for mental support (Public Health Ontario, 2020)(Ramanathan et al., 2020). Fear of infectious diseases negatively affects the psyche of pregnant women. Pregnancy can make some women more prone to anxiety and depression (Hayakawa et al., 2020).

Public access to health services in the era of the Covid-19 pandemic has changed due to *physical distancing* to prevent the spread of the virus by the community (Goyal et al., 2020)(Nurrizka, Rahmah H., Yuri N., 2021). Efforts should therefore be made to increase visits by pregnant women while maintaining health protocols during the pandemic (Moekroni & Analia., 2016). This study aimed to determine the effectiveness of pregnancy care home visits on depression and anxiety of pregnant women.

2. RESEARCH METHOD

This type of research is *quasi-experimental* with design *a group pretest-posttest design.* Sampling was carried out by purposive sampling with the inclusion criteria of pregnant women who did not re-examine at the Health Center for 1 month and pregnant women who were willing to be selected as respondents. obtained 28 pregnant women as respondents. This research was conducted in Manado City, North Sulawesi Province, and carried out from February to August 2022. The instrument in this study used the *Edinburgh Postpartum Depression Scale* (EPDS) questionnaire. for depression measurement and using the *Hamilton Anxiety Rating Scale* (HARS) for measuring anxiety. The research technique was measured based on EPDS and HARS before and after home visits in providing pregnancy care with 9 points (weight and height, blood pressure, uterine fundus height, Fe tablets, interview/counselors, haemoglobin, urine protein examination, and breast care).

For data analysis for the normality of the data, parametric tests were carried out and checked by applying the Shapiro-Wilk test in the EPDS group and the HARS group, then using the *Paired Simple T-Test test.* The results of the analysis with a probability value of Sig (2-tailed) < 0.05. This research has been approved by the research ethics committee of the Manado Health Polytechnic Ministry of Health with number KEPK.01/07/106/2022.

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 ± 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 ± 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 ±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 ± 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 ± 3,213 with a range of 2 to 12 on the scale (Table 1).

Table 1. Characteristics of Respondents

	Category n (%)	**Mean**	**Standard Deviation**
Maternal age		24.3	5.005
Gestational Age		30.21	3.436
Parity:			
Primi	12 (42.9%)		
Multigravida	15 (53.5%)		
Grandemulti	1 (3.6%)		
Education:			
Elementary-Junior	7 (25%)		
High School	19 (67.9%)		
Bachelor	2 (7.2%)		
Employment			
Household	17 (60.7%)		
Civil servant	11 (39.3%)		
Married Status:			
Yes	25 (89.3%)		
No	3 (10.7%)		
Planning Pregnancy:			
Yes	18 (64.3%)		
No	10 (35.7%)		
Pregnancy Complications:			
Complications of	7 (25%)		
Hypertension	4 (14.35%)		
Diabetes	1 (3.6%)		
Hyperemesis	2 (7.2%)		
Threatened Abortion	0		
No Complications:	21 (75%)		
Level respondent's knowledge about Covid (range 0-10)		7.2 9	1,117
Have a socio-economic effect during pregnancy			
family income,	4(50)		
loss of job	6 (21.4)		
of family members, they are Covid isolated	4 (14.3)		
none	4 (14.3)		
Does Covid have psychological effects such as being			

afraid to leave the house,	17 (60.7)		
afraid to do a pregnancy check	11 (39.3)		
none	0		
EPDS			
Pre Test		12.36	1.9 29
Post Test		3.50	1.552
HARS			
Pre Test		22.46	4.114
PostTest		6.61	3.213

Table 2. Test Results Paired Simple T-Test

Aspects	**t**	**df**	***p***	**mean**	**Std. Deviation**	**Lower limit**	**Upper limit**
Depression of pregnant women	37,836	27	0.001	8.857	1,239	8,377	9,337
The anxiety of pregnant women	56,557	27	0.001	15,857	1,484	15,282	16,432

Table 2 presents that the level of depression based on the EPDS was 12.36 to 3.50, as well as the anxiety level of pregnant women 22.46 decreased to 6.61 on the scale HARS after being given prenatal care. In-person home visits to pregnancy care can provide significant changes that can be addressed by the depression and anxiety that respondents experience. This can be proven by obtaining a *P* of 0.001. This study shows that pregnant women are vulnerable to changes in mental conditions during the COVID-19 pandemic and are entitled to special attention, namely by visiting their homes for prenatal care directly by complying with health protocols according to government rules that have been set. The results of research by Almas, et al. (2021) that three out of every ten pregnant women use antenatal care services, this is still a concern that it is necessary to prioritize maternal health services during COVID-19 and improve the quality of antenatal care services (Almas et al., 2021).

Problems with midwifery services can cause maternal and perinatal morbidity and mortality, especially among the most vulnerable (Roberton et al., 2020)(Kementerian Kesehatan RI, 2019) The pregnancy service program is a way to monitor and ensure the health of pregnant women is in normal condition and can detect complications if they occur Pregnancy care is important to ensure that the natural process of pregnancy is normal. Pregnancy can develop into problems or complications at any time, especially during the COVID-19 pandemic, which can increase the risk that is dangerous for women (Connor et al., 2020). Antenatal care is expected to detect risk factors and prevent and treat complications (Lalita, 2013).

Pregnancy can increase the tendency of anxiety and depression for most women, and changes in mental health status are often associated with fetal development, psychopathology, and maternal health. The COVID-19 pandemic can worsen pregnancy because the COVID-19 pandemic is a very stressful event that causes mental disorders such as depression and anxiety during pregnancy. The results of our study showed that the average pregnant woman experienced depression based on the EPDS assessment of 12.36 ± 1.929 and the level of anxiety based on the HARS was 22.46 ± 4.114 (Table 1). These findings indicate that all pregnant women sampled in this study experienced mild to moderate symptoms of depression and anxiety during the COVID-19 pandemic.

The results of this study are consistent with those of Perzow, et al. (2021). Match found increased symptoms of depression in several pregnant women during the pandemic (Perzow et al., 2021). Another study by Durankuş & Aksu (2020) found that 35.4% of pregnant women scored 13 or higher on the EPDS. These results are based on WHO reports that approximately 10% of pregnant women suffer from mental illness, particularly depression. Based on the results, it can be concluded that during the pandemic mental disorders occurred with a multiple of twice as large as the non-pandemic state (Durankuş & Aksu, 2020). Studies are needed to assess the effects of pandemic anxiety and depression on perinatal and postnatal outcomes, as well as long-term effects on fetal neurobehavior. Interventions should be prioritized to ensure optimal perinatal and infant mental status (Ayaz et al., 2020). Anxiety resulting from pregnant women hurts pregnancy, such as an increased risk of preeclampsia, depression, nausea, and vomiting, and can even lead to miscarriage, more anxiety maternal adverse effects on the newborn, such as low birth weight, growth restriction or low APGAR scores (Abazarnejad T, Ahmadi A, Nouhi E, Mirzaee M, 2019). In the study of Lebel et al., (2021) reported severe symptoms of depression and anxiety raise major concerns about the threat COVID-19 poses to the lives of mothers and babies, as well as unnecessary prenatal care, and relationship stress, It was associated with concerns about social isolation due to the COVID-19 pandemic (Lebel et al., 2021).

The global health crisis due to the Coronavirus (COVID-19) requires government and public action to contain the spread of the virus quickly and appropriately but ultimately has widespread economic and social consequences. The findings in this study are that 50% of respondents experienced a decrease in income due to the Covid-19 pandemic and there were even 21.4% of respondents experienced job losses (Table 1). This finding is like the results of research conducted by Mortazavi, *et al.* (2021) that one of the predictors of concern for pregnant women is low family income. Other researchers have found that difficulties in household finances and unemployment can be risk factors for stress in pregnant women (Mortazavi et al., 2021)(Matsushima & Horiguchi, 2020).

Maternal occupation is one of the risk factors for pregnancy depression (Table 2). This is because most of the respondents play the role of caregiver in the family. Similarly, a study found that pregnant women working from home are at higher risk of developing depressive symptoms during the COVID-19 outbreak in China, therefore they need to balance their careers and family (An et al., 2021). Another risk factor is planning a pregnancy, in the study by Gomez, et al. (2018) reported that 37 out of 50 pregnant women did not plan to become pregnant. When a person is not trying to get pregnant, the client needs contraception from a healthcare provider to prevent unwanted or unwanted pregnancies (Gomez et al., 2018). Pregnancy is an important time for women to be at risk for stress and anxiety disorders. Therefore, with stress and anxiety in mind, all women plan to become pregnant and provide appropriate care to those at risk (Derya, Aksoy et al., 2021). Failure to reduce stress caused by individuals, families, and society can increase the incidence of postpartum depression. Therefore, during the COVID-19 pandemic, it is necessary to identify the sources of maternal stress early and eliminate them promptly.

During the pandemic there was a mental change in most of the respondents, they were afraid to do a pregnancy check-up, so antenatal services were needed to visit their homes directly, hear what their complaints were and provide counseling as a form of support that could overcome depression and anxiety experienced by pregnant women. Mortazavi, et al. (2021) reported that the percentage of women experiencing low welfare conditions is relatively high, this result needs to be considered by health service providers and policymakers (Mortazavi et al., 2021). During the COVID-19 pandemic, care and support for pregnant women, especially those who are disadvantaged, should be a top priority. Home visits are important to carry out pregnancy care so that it can ensure that the natural pregnancy process runs normally, but

pregnancy can become a problem or complication at any time, especially during the COVID-19 pandemic, which can increase the risk of harm for pregnant women. Antenatal care is expected to detect risk factors and prevent and treat complications. According to *Maternal Neonatal Health*, antenatal care or what is known as antenatal care is a routine procedure carried out by midwives which is one of the government programs to reduce maternal and perinatal morbidity and mortality.

The crisis we are facing today if managed properly can be an opportunity, and intervention is needed to overcome mental health. Interventions should be prioritized to ensure the optimal mental health of pregnant women and babies. Midwives can identify problems early and establish a comprehensive care plan for pregnant women in conditions such as extreme stress, emergency and conflict situations, and natural disasters. Therefore, it is necessary to screen or mentally identify pregnant women so that they can prevent the impact of postpartum parenting stress on the baby. Thus, the importance of home visits by healthcare workers during the COVID-19 pandemic helps them get through this particular period with less anxiety and less depression.

4. CONCLUSION

In this study, it was reported that with home visits, health workers providing pregnancy care directly at the patient's home can reduce depression and anxiety during pregnancy during the COVID-19 pandemic. In-person home visits to pregnancy care can provide significant changes that can be addressed by the depression and anxiety that respondents experience. This can be proven by obtaining p of 0.001. Essential health services, including prenatal care, are threatening and can increase maternal and perinatal mortality. In these difficult times, the need for antenatal care services can provide a guarantee of protective and safe services, and psychological screening of pregnant women who are at risk for depression to reduce long-term negative outcomes must be carried out. the need for further research on the long-term impacts of pandemic-related anxiety and depression on mothers and babies.

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RESEARCH **Open Access**

Effect of The Temperature on The Size of Inhibition Zone of the Clindamycin, Levofloxacin, Tetracycline, and Trimethoprim Activity Against *Staphylococcus aureus* ATCC 25923

Sistiyono[1a], Marta Atik Martsiningsih[1b], Menik Kasiyati[1c], Muji Rahayu[1d], Rita Rena Pudyastuti[1e], Suyana[1f], Ully Rahmawati[1g], Rahmad[2h], Zulfikar Husni Faruq[1i], Budi Setiawan[1,3j*]

[1] Department of Medical Laboratory Technology, Poltekkes Kemenkes Yogyakarta, Yogyakarta, Indonesia
[2] UPTD Health Laboratory of East Kalimantan Province, Samarinda, East Kalimantan, Indonesia
[3] Center of Excellent of Innovation Applied Technology on Public Health, Poltekkes Kemenkes Yogyakarta, Yogyakarta, Indonesia

[a] Email address: sistiyono@gmail.com
[b] Email address: atikmartsiningsih@gmail.com
[c] Email address: rifani.2010@gmail.com
[d] Email address: hayuningpuji@gmail.com
[e] Email address: ritapudyastuti1968@gmail.com
[f] Email address: suryaniajeng.2014@gmail.com
[g] Email address: ullya.rahmawati@poltekkesjogja.ac.id
[h] Email address: sky_lights43@yahoo.com
[i] Email address: faruq@poltekkesjogja.ac.id
[j] Email address: budi.setiawan@poltekkesjogja.ac.id

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Abstract
Antibiotic sensitivity should be tested. In the sensitivity test, there are technical factors that influence the formation of the inhibition zone diameter. Based on several research one of the technical factors that affect the diameter of the inhibition in the disc diffusion method is the temperature incubation of the media, this must be examined so that it can be controlled to ensure the validity of the sensitivity test results. This study aims to determine the mean, difference, and analyze the diameter of the inhibition zone of the antibiotics namely Clindamycin, Levofloxacin, Tetracycline, and Trimethoprim against Staphylococcus aureus on Mueller-Hinton agar media with incubation temperatures of 33°C, 34°C, 35°C, 36°C and 37°C for 18 hours. This research is observational, with a cross-sectional design. The data used are primary data with 100 data on the diameter of the antibiotic inhibition zone, obtained by measuring the diameter of the inhibition zone with different incubation temperatures. The selection of antibiotics is based on the mechanism of action of antibiotics inhibiting bacteria namely, the cell wall or membranes that surrounds the bacterial cell; the machineries that make the nucleic acids DNA and RNA and the machinery that produce proteins (the ribosome and associated proteins) with a range of inhibition zones based on Internal Quality Control CLSI. The data will be processed univariately and the Repeated Measure statistical test to determine the significance of the difference in the diameter of the formed inhibition zone using the ANOVA test. The results of the measurement of the inhibition zone diameter on the incubation temperature variation showed a significant difference with p-value 0.000 for Levofloxacin, Tetracycline and Trimethoprim, while for p-value Clindamycin is 0.010. Levofloxacin, Tetracycline, and Trimethoprim antibiotics, the higher the incubation temperature, the average diameter of the inhibition zone is smaller, while for Clindamycin the higher the incubation temperature, the higher the average diameter of the inhibition zone is the same. There is an effect of incubation temperature volume on the diameter of the antibiotic inhibition zone in the disc diffusion method antibiotic sensitivity test. The research indicates that incubation temperature affects the diameter of the antibiotic inhibition zone in disc diffusion tests, underscoring the need for standardized and precise testing conditions to ensure accurate and reliable antibiotic sensitivity results.

Keywords: Incubation Temperature, Inhibition Zone, Sensitivity Test, Disk Diffusion.

Corresponding Author:
Budi Setiawan
Department of Medical Laboratory Technology, Poltekkes Kemenkes Yogyakarta, Yogyakarta, Indonesia
Email: budi.setiawan@poltekkesjogja.ac.id

1. INTRODUCTION

The main health problems that occur in developing countries are infectious diseases. Microorganisms that cause disease in humans are called pathogenic microorganisms, one of which is pathogenic bacteria (Novard et al., 2019). *Staphylococcus aureus* is a Gram-positive commensal bacterium that is an opportunistic pathogen. These bacteria live in about 30% of the healthy adult population and are mostly found on the skin and mucous membranes. Although *Staphylococcus aureus* is a commensal bacterium, it has the potential to cause various diseases with varying degrees of severity (Jenul & Horswill, 2019).

Bacterial infections can be treated using antibiotics that are bactericidal or bacteriostatic. To treat patients appropriately and adequately, data on sensitivity tests of bacteria that cause infection are needed for various antibiotics available on the market (Nadjamuddin, 2023). Antibiotic sensitivity test using disc diffusion method for Staphylococcus sp bacteria can use Mueller-Hinton media with a turbidity standard of 0.5 McFarland. Bacterial growth on the media will be optimal at a temperature of 35°C with ambient air for 16-18 hours of incubation (Zimmer & et all, 2020).

According to several research results on sensitivity testing, technical factors that affect the diameter or size of the inhibition in the disc diffusion method are inoculum density, disc installation time, incubation temperature, incubation time, plate size, and thickness of agar medium and media composition(Lenggu et al., 2020). Research on the effect of incubation temperature on the inhibition zone has also been carried out on bacteria originating from water, which was carried out by incubation at various temperatures and times, including at a temperature of 35°C for 16 hours, 28°C, and 22°C for 24 hours, 22°C. C for 48 hours, and <19°C for >96 hours. The disc method inhibition zone data precision in this study decreased significantly as the incubation temperature decreased and the time increased (Smith et al., 2018).

The selection of antibiotics in this study was based on the function of the mechanism of action of antibiotics in killing or inhibiting the growth of *Staphylococcus aureus* bacteria including, Clindamycin and Tetracycline which work by inhibiting protein synthesis, Levofloxacin which works by inhibiting nucleic acid synthesis, and Trimethoprim which acts as an antimetabolite. In addition, the selection of these four antibiotics was also based on their use as an Internal Quality Control strain of pure bacteria *Staphylococcus aureus* ATCC 25923 (Murray et al., 2021), (Zimmer & et all, 2020).

The main target of laboratory personnel in the field of clinical microbiology is the rapid and accurate identification of pathogenic microorganisms from clinical specimens and providing an accurate interpretation of the results of the sensitivity test of pathogenic bacteria. The accuracy of antibiotic sensitivity test results will help the healing process of patients infected with bacterial diseases (Willey et al., 2017). For this reason, laboratory personnel must continue to carry out internal quality stabilization, one of which is equipment maintenance to ensure the quality of the inspection results.

The microbiological incubator is one of the important instruments in the process of culturing microorganisms or testing for antibiotic sensitivity. This instrument must be periodically tested for performance and calibration so that the quality of the results of laboratory examinations is maintained. However, in reality, there are still laboratory workers who do not perform routine maintenance of microbiological equipment including incubators. Incubators that are not maintained properly will cause high-temperature deviations. the threshold value for electrical safety, the allowable deviation value at the performance output of the maintenance incubator is ±1 C. The easiest way to monitor the incubator temperature is to compare the temperature that appears on the incubator screen with a calibrated thermometer, if the

temperature deviation exceeds 2°C, it is necessary to reset the incubator or calibration (Siregar et al., 2018).

The temperature that is not optimal will affect the formation of bacterial inhibition zones in the bacterial sensitivity test (Nadjamuddin, 2023). Unstable mains voltage, unmonitored equipment performance, frequent opening and closing of the incubator door, the incubator filled with media can cause inappropriate temperature output. The importance of the incubation temperature variable in the antibiotic sensitivity test is the basis for conducting research on the Effect of Incubation Temperature on the Diameter of the Inhibitory Zone in the Antibiotic Sensitivity Test of Staphylococcus aureus.

This study aims to determine the mean, difference and analyze the difference in the diameter of the inhibition zone of the antibiotics Clindamycin, Levofloxacin, Tetracycline, and Trimethoprim against *Staphylococcus aureus* on Mueller-Hinton agar media with incubation temperatures of 33°C, 34°C, 35°C, 36°. C and 37°C for 18 hours.

2. RESEARCH METHOD

The type of research used in this research is observational research with a cross-sectional study. This type of research is a type of research conducted without intervention on the research subject (Notoatmodjo, 2005). This study was conducted to observe whether there was a difference in the diameter of the antibiotic inhibition zone against *Staphylococcus aureus* bacteria incubated at different temperatures. The data collection technique used in this study was to measure the diameter of the inhibition zone in the disc diffusion method of antibiotic sensitivity test. Purity test of *S. aureus* bacteria on blood agar plate media can be seen in figure 1 below.

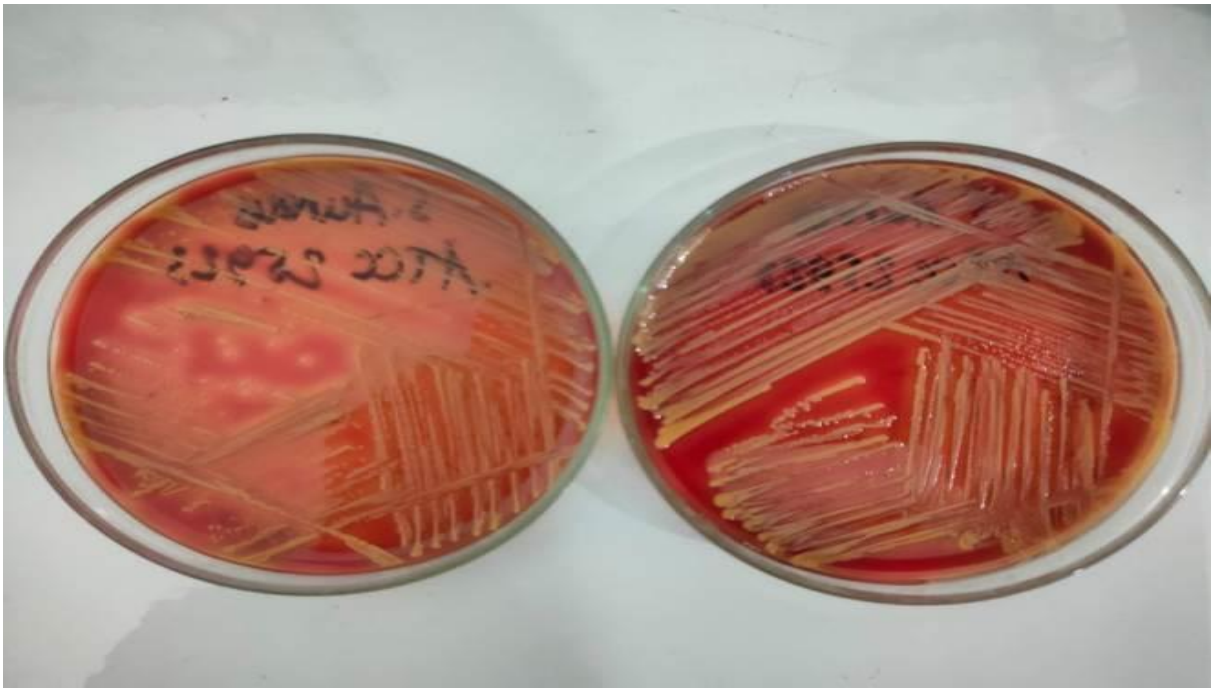


Figure 1. Purity Test of *S. aureus* Bacteria on Blood Agar Plate Media

A pure bacterial strain of *Staphylococcus aureus* ATCC 25923 was obtained from the microbiology laboratory at Health Laboratory East Kalimantan Province. The bacterial strain of *Staphylococcus aureus* ATCC 25923 was enriched for 24 hours in Brain Heart Infusion (BHI) media. The cloudy BHI media indicated bacterial growth, then inoculated on an inclined.

Nutrient Agar medium for antibiotic sensitivity testing, and inoculated on two Blood Agar Plate media to identify the purity of the bacteria. All inoculated media were incubated at

35°C for 24 hours. Identification of *Staphylococcus aureus* bacteria was carried out using an automatic device brand BD Phoenix 50i by reacting a 0.5 McFarland bacterial suspension to the PMIC panel substrate reagent.

The technique of collecting samples with Consecutive sampling is done by selecting and making a standard of bacterial turbidity (0.5 McFarland). The diameter of the inhibition zone on the antibiotics Clindamycin, Levofloxacin, Tetracycline, and Trimethoprim was measured against the pure bacterial strain of *Staphylococcus aureus* ATCC 25923 which was formed after incubation at 33°C, 34°C, 35°C, 36°C, and 37°C. The antibiotic sensitivity test was repeated 5 times, so that the number of plates examined for 5 variations in temperature was 25 times. In one plate can be filled with 4 types of antibiotics so that with 5 repetitions can produce 100 data on the diameter of the antibiotic inhibition zone.

The instrument validity test is carried out by means of periodic maintenance and calibration of the equipment as a form of strengthening the internal quality of the laboratory. One of the tools used in this research is an incubator, this tool is well maintained and has been calibrated regularly every year at the microbiology Laboratory, Health Laboratory of East Kalimantan Province by the Calibration Testing Agency. For other tools such as the Nephelometer, the validity of the instrument can also be done by testing the performance of the tool against the turbidity standard available on the brand of the tool. Other validity tests that were also carried out were the Mueller-Hinton media sterility test and the *Staphylococcus aureus* bacteria purity test. Mueller-Hinton media were prepared according to standard procedures, taken as much as 5% and incubated without inoculation at 35°C for 18-24 hours. After 24 hours of incubation, the colony growth was observed on Mueller-Hinton media. If there is no bacterial growth then all media can be continued for antibiotic sensitivity testing, but if there is bacterial growth then all media in one batch cannot be continued for testing.

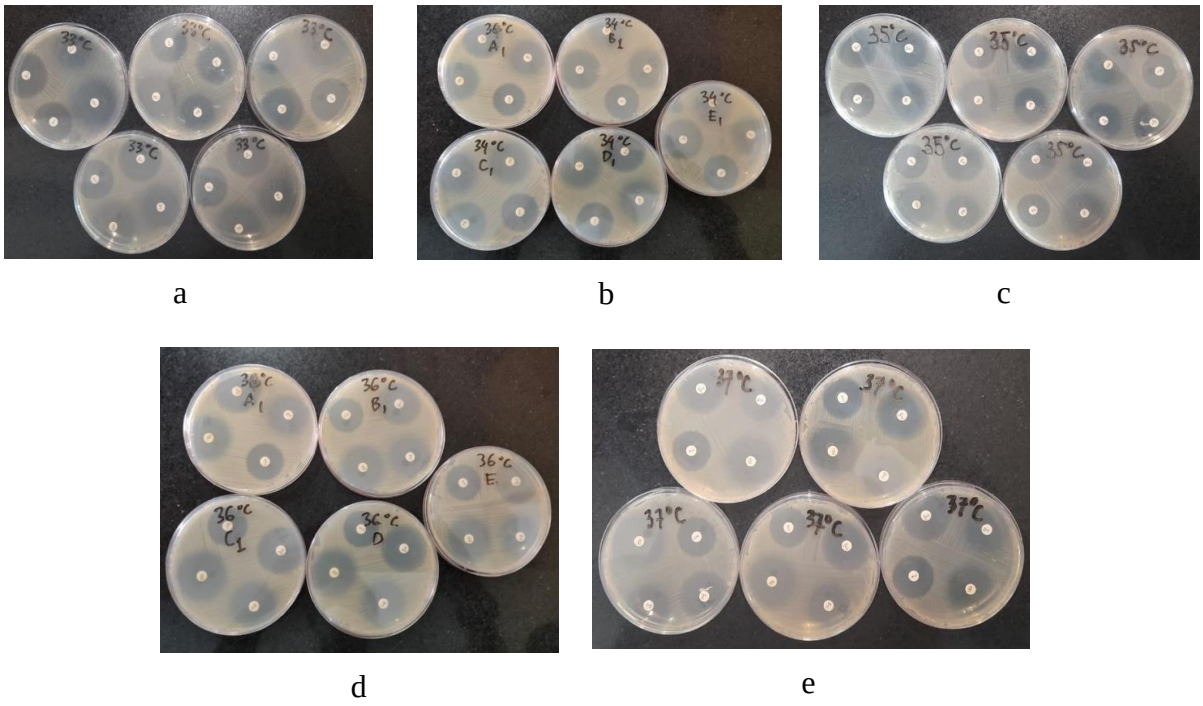
Data were collected by measuring the diameter of the inhibition zone on the antibiotic sensitivity test that had been determined using the disc diffusion method after being incubated at various temperatures. The data collected were analyzed descriptively and statistical analysis using the Repeated Measures ANOVA test with a degree of error (α) of 5%.

3. RESULTS AND DISCUSSION

This study used a pure strain of *Staphylococcus aureus* bacteria. The antibiotics used were Clindamycin 2µg, Levofloxacin 5µg, Tetracycline 30µg, and Trimethoprim 5µg. The results of the study in the form of the diameter of the antibiotic inhibition zone against *Staphylococcus aureus* bacteria were analyzed according to the research objectives, namely, to see the mean and difference in the mean of each independent variable, and to see the significance of the difference in the diameter of the inhibition zone formed after incubation at 33°C, 34°C, 35 °C, 36°C, and 37°C.

Table 1. Average Diameter of Antibiotic Inhibitory Zone against *Staphylococcus aureus* at Variation of Incubation Temperature

Antibiotic	**Average Diameter of Antibiotic Inhibitory Zone (mm)**				
	33°C	34°C	35°C	36°C	37°C
Clindamycin	23.33	25.71	24.28	23.85	23.43
Levofloxacin	28.29	27.41	25.87	26.91	25.30
Tetracycline	28.13	27.58	228.80	25.01	25.23
Trimethoprim	25.77	25.59	23.40	23.26	23.32



a b c

d e

Noted: *a=33℃; b=34℃; c=35℃; d=36℃; e=37℃*

Figure 2. Inhibition Zone at Various Incubation Temperatures.

Table 2. The Difference in Mean Diameter of Antibiotic Inhibitory Zone at Variation of Incubation Temperature to Optimal Temperature

Antibiotic	**Average Inhibitory Zone Diameter at Variation of Incubation Temperature**		
	Temperature Variation	**Optimal Temperature**	**Deviation**
Clindamycin	33°C	35°C	0.95
	34°C		1.43
	36°C		0.43
	37°C		0.85
Levofloxacin	33°C	35°C	2.42
	34°C		1.54
	36°C		1.04
	37°C		0.57
Tetracycline	33°C	35°C	0.67
	34°C		1.22
	36°C		3.79
	37°C		3.57
Trimethoprim	33°C	35°C	2.37
	34°C		219
	36°C		0.14
	37°C		0.08

Table 1 shows the mean diameter of the different inhibition zones of the four types of antibiotics. It can be seen that for Levofloxacin, Tetracycline, and Trimethoprim antibiotics, the higher the incubation temperature, the average diameter of the inhibition zone is smaller, while for Clindamycin the higher the incubation temperature, the higher the average diameter

of the inhibition zone is the same, macroscopically this can be seen in Figure 2. Table 2 shows the difference in the mean diameter of the inhibition zones of each antibiotic. The smallest mean difference is 0.08 (mm) and the largest is 3.79 (mm).

The distribution of the observed data was the diameter of the inhibition zone from each variation of the incubation temperature and the overall data were normally distributed with a significance value of p (0.05). The data then continued with the homogeneity test, all of the data had sig values. (Mauchly's Test of Sphericity s) 0.05 so that all data are homogeneous

Table 3. Statistical Test of Repeated ANOVA

Antibiotic	**Sig. (Sphericity Assumed)**
Clindamycin	0.010
Levofloxacin	0.000
Tetracycline	0.000
Trimethoprim	0.000

Table 4. Pairwise Comparison Test Results of temperature variations to an optimal temperature

Antibiotic	**Incubation Temperature**	**p-value**
Clindamycin	Inhibition zone diameter 33°C	0.137
	Inhibition zone diameter 34°C	0.162
	Inhibition zone diameter 36°C	0.668
	Inhibition zone diameter 37°C	0.219
Levofloxacin	Inhibition zone diameter 33°C	0.005
	Inhibition zone diameter 34°C	0.015
	Inhibition zone diameter 36°C	0.213
	Inhibition zone diameter 37°C	0.322
Tetracycline	Inhibition zone diameter 33°C	0.175
	Inhibition zone diameter 34°C	0.009
	Inhibition zone diameter 36°C	0.000
	Inhibition zone diameter 37°C	0.001
Trimethoprim	Inhibition zone diameter 33°C	0.001
	Inhibition zone diameter 34°C	0.010
	Inhibition zone diameter 36°C	0.735
	Inhibition zone diameter 37°C	0.780

Table 3 comparative test of Repeated ANOVA shows that sig. (Sphericity Assumed) < 0.05, so it can be concluded that H0 is rejected. This means that there is a difference in the diameter of the inhibition zone of each antibiotic (Clindamycin, Levofloxacin, Tetracycline, and Trimethoprim) after incubation at 33°C, 34°C, 35°C, 36°C, and 37°C for 18 hours. While in table 4 it can be observed that the difference in the average variation of incubation temperature to the optimal temperature occurs in Levofloxacin antibiotics at 33°C and 34°C, in Tetracycline antibiotics at 34°C, 36°C, and 37°C, while on the antibiotic Trimethoprim, a significant difference was seen at 33°C and 34°C to the optimal temperature.

DISCUSSION

Microbes can grow everywhere but are still influenced by environmental factors. Temperature is one of the environmental factors that affect microbial growth. Microorganisms can be controlled by an environment, by using various processes or physical means. Bacterial growth depends on chemical reactions and since the rate of these reactions is affected by temperature, bacterial growth is strongly influenced by temperature (Pelczar, 2008).

Every organism has the lowest temperature limit and the highest temperature, microbes need an optimum temperature for their growth and development. In general, bacteria grow at temperatures above 35°C, for each species, there is a maximum and minimum temperature limit for growth. The optimum temperature is closer to the maximum temperature while at the minimum temperature the growth is slower. If the temperature is higher than the maximum temperature, the growth of bacteria will decrease rapidly. This illustrates that temperature mainly affects enzymes, the higher the temperature, the faster the enzyme activity. If the temperature is too high then the enzyme will be denatured so that the cell will then die (Winarwi, 2006).

Staphylococcus aureus is a gram-positive bacterium with a diameter of 0.5-1.0 mm, in the form of a series of grapes, does not form spores, does not move, groups, pairs, and sometimes has short chains. Some strains of this bacterium have a capsule (Karimela et al., 2017). *Staphylococcus aureus* contains polysaccharide antigens and proteins as well as other substances that are important in the cell wall structure. Peptidoglycan, a polysaccharide polymer containing subunits joined together to form a rigid exoskeleton of the cell wall. Peptidoglycan can be damaged by strong acids or exposure to lysozyme. *Staphylococcus aureus* grows well on various bacteriological media under aerobic or microaerophilic conditions. Grows rapidly at 37°C, but the best pigment formation is at room temperature (20-35^{oC}) (Riedel et al., 2019).

The four types of antibiotics used in this study, namely, Clindamycin, Levofloxacin, Tetracycline, and Trimethoprim are types of antibiotics that can be used to monitor the quality and purity of *Staphylococcus aureus* strains (Zimmer & et all, 2020). The selection of these four types of antibiotics is also based on the mechanism of action of antibiotics in inhibiting the growth of *Staphylococcus aureus* bacteria.

Clindamycin is the first lincomycin group of antibiotics to be introduced for clinical use, and its mechanism of action is very similar to that of erythromycin. Clindamycin belongs to a group of narrow-spectrum antibiotics, which are used for anaerobic and aerobic gram-positive bacteria. The mechanism of action of this antibiotic is to inhibit bacterial protein synthesis by reversibly binding to the 50S ribosomal subunit, thereby blocking the transpeptidation reaction or translocation of susceptible bacteria (Fanayoni et al., 2019).

Levofloxacin is a quinolone class of antibiotics that is useful for treating diseases caused by bacterial infections such as pneumonia, sinusitis, prostatitis, conjunctivitis, urinary tract infections, and skin infections. This fluoroquinolones class of antibiotics works by inhibiting the activity of bacterial topoisomerase (DNA gyrase) which is important in the process of bacterial replication (Toy, 2008).

Tetracycline is a broad-spectrum antibiotic used to treat many infectious diseases. This antibiotic works by inhibiting bacterial protein synthesis by inhibiting the binding of aminoacyl-tRNA to the bacterial 30s ribosome (Toy, 2008). Trimethoprim is a class of antibiotics that are bacteriostatic, but trimethoprim has a broad spectrum effect with activity against Gram-positive and Gram-negative organisms. This antibiotic works by binding to dihydrofolate reductase, a process that blocks the reduction of dihydrofolic acid to tetrahydrofolic acid. Interrupting the synthesis of tetrahydrofolic acid is important because these compounds play a central role in the pathway of thymidine synthesis and the synthesis of thymidine, which plays a role in bacterial DNA synthesis. In other words, by binding to dihydrofolate reductase, trimethoprim causes reduced bacterial folate synthesis (Toy, 2008).

The clear zone on the agar layer formed is caused by antimicrobial compounds diffusing into the agar layer and inhibiting the growth of microorganisms (bacteria) and is referred to as the inhibition zone, while the agar layer overgrown with microorganisms will appear cloudy. Antimicrobial compounds work by interacting with bacterial cell walls, resulting in impaired permeability of bacterial cell walls and facilitating antimicrobial compounds to diffuse into

bacterial cells. Diffusion that occurs will result in disruption of a series of growth processes of bacteria to inhibit their growth (bacteriostatic) or provide other effects, namely by killing bacteria (bacteriocidal). In addition, antimicrobial compounds can also penetrate cell membranes and interact with genetic material from bacteria so that bacteria can undergo mutations (Perdana & Setyawati, n.d.).

This study obtained the results that to obtain optimal growth, incubation must be carried out at a temperature of 35°C. Temperatures less than 35°C were found to be able to make most antibiotics form a wider diameter of the inhibition zone. This can be fatal in concluding and determining the choice of antibiotics that can be used for patient therapy. Resistance can be read susceptible if it is incubated at a low temperature, as a result, patient therapy is inadequate and the chances of developing antibiotic resistance are high (Nadjamuddin, 2023). Incubation of the media in the piles can result in the media in the middle of the dish not getting an even temperature distribution and the temperature of the media in the middle of the pile being low below 35°C. The higher the incubation temperature (> 35 ° C) the growth of germs can become fertile. The diameter of the inhibition zone formed is getting smaller, so it could be that antibiotics that should be used for patient therapy are reported to be resistant because the inhibition zone becomes smaller. In addition, high temperatures can also cause the diffusion of antibiotics into the Mueller-Hinton medium to be less good.

The bactericidal activities of ciprofloxacin and levofloxacin against Staphylococcus aureus and epidermidis were significantly reduced when incubated at lower temperatures. Levofloxacin was found to be more effective against both bacteria, suggesting it may be a more suitable option for staphylococcal infections, particularly at skin and soft-tissue sites (Parte & Smith, 1994). Other study found that isoniazid and rifampicin's bactericidal activities decreased at lower temperatures, while TMC207 was immediately bactericidal at 37°C. Pyrazinamide was more bactericidal at lower temperatures and dormant seed cultures. This suggests that low temperatures make bacteria more susceptible to TMC207's blocking of ATP synthesis and hindering the export of pyrazinoic acid (Coleman et al., 2011). Some research examined the impact of temperature on the adhesion and disinfection properties of Ag+-doped BiVO4 coatings. The study found that the monoclinic scheelite phase remained unchanged after annealing at 450-650 °C. The silver-modified samples showed good disinfection activity, with the best adhesion and complete killing of Escherichia coli, Staphylococcus aureus, Shigella, and Salmonella after 2 hours of visible-light irradiation (Zhang et al., 2022).

The inhibition of bacterial growth by antibiotics can be seen from the clear zone formed around the antibiotic disc paper. The inhibition of the growth of bacterial colonies can be caused by damage to the structural components of the bacterial cell membrane. Damage to cell membranes disrupts nutrient transport (compounds and ions) so that bacterial cells experience a lack of nutrients needed for growth (Leboffe & Pierce, 2019). The time to kill *Escherichia coli* inoculum significantly increased after preservative dilution or temperature reduction, with a concentration exponent of 3 and a temperature coefficient of 2.3. (Lusher et al., 1984). The addition of sodium chloride and temperature significantly enhanced the bactericidal activity of spirit vinegar. The time required for a 3 log decrease in viable cell numbers was shortened by 5% sodium chloride and the rise of temperature. The study suggests that vinegar solution containing sodium chloride may be a useful method to prevent food poisoning by reducing the number of viable cells (Lusher et al., 1984).

The study shows that even at a low pH (4.4), ATSSB spores can grow without organic acids, but acetic and lactic acids have significant antibacterial activity. The study also found that a combination of these acids inhibits growth from B. subtilis and B. velezensis, making ambient storage of low-acid pasteurized sauces feasible (Sun et al., 2021). High temperature

aging of copper-bearing 2205 duplex stainless steel (Cu-2205 DSS) improved its corrosion resistance, mechanical properties, and antibacterial activity. Results showed increased γ phase in microstructure and formation of new σ phase and copper-rich precipitates. The mechanical properties were significantly enhanced, and aging increased uniform corrosion resistance but slightly affected pitting resistance. The antibacterial performance was improved due to increased release of copper ions, indicating potential applications in marine engineering (Khan et al., 2022). The antibacterial activity of SPR19, a strain of Brevibacillus sp., using atmospheric and room temperature plasma mutagenesis (ARTP). The 469 mutants were screened and confirmed, with M285 showing the highest activity. M285 was stable and tolerant to various conditions, retaining over 90% of its antibacterial properties. This suggests M285 as a potential antibacterial source (Songnaka et al., 2022). Another study reveals that microbial competition affects the synthesis of antimicrobial compounds by psychrophilic micromycetes, particularly in summer when higher temperatures increase total fungal activity. The most promising strain, Penicillium vulpinum KPB F-290, showed maximum antimicrobial activity against test cultures. It exhibited high antimicrobial activity against opportunistic strains and B. subtilis ATCC 6633, as well as phytopathogenic pectobacteria P. carotovorum and P. savastanoi. The optimal cultivation method for maximum antibiotic production is stationary, with ethyl acetate being the best extractant from the culture liquid (Kuvarina et al., 2022).

A multifunctional temperature-triggered antibacterial hemostatic fluorocopolymer aggregate coating was developed, consisting of fluoropolymer and quaternary ammonium salt. This coating has antibacterial, antibacterial, and hemostatic properties, promoting biocompatibility and anti-adhesion performance. It also has rapid coagulation, low blood loss, minor secondary bleeding, and minimal bacteria infiltration, making it potential for biomedical applications (Li et al., 2022).

In addition to the incubation temperature factor, several other factors can affect the formation of the diameter of the inhibition zone in the antibiotic sensitivity test, including the turbidity of the bacterial suspension, the time of absorption of the bacterial suspension into the MH media, the incubation time, the thickness of the MH media, the distance between the drug discs and the potency of the drug disc. In this study, control of other factors was carried out following standard procedures, such as the bacterial suspension was measured on a nephelometer with a turbidity of 0.5 McF, did not allow bacterial infiltration in MH media to exceed 15 minutes, the incubation time was not more than 18 hours, the thickness of the medium MH is only 4 mm or 20 ml of MH media is poured into the plate, the distance between the discs does not match because only four antibiotics are used which are always stored at -20°C so as not to reduce the quality of the antibiotic potency (Hudzicki, 2009). Another research found the antimicrobial activity of the MgO-NPs increased significantly ($p \leq 0.05$) with increased temperature, pH and NaCl concentration in TSB (Yoon et al., 2022).

The four types of antibiotics that have been selected are based on different mechanisms of action, besides based on the CLSI (2020) the four selected antibiotics namely Clindamycin, Levofloxacin, Tetracycline, and Trimethoprim can be used for Internal Quality Control of *Staphylococcus aureus* ATCC 25923. The yield range of the diameter of the QC inhibition zone for Clindamycin is 24-30 mm, Levofloxacin 25-30 mm, Tetracycline 24-30 mm, and Trimethoprim 19-26 mm (Zimmer & et all, 2020). The synthesized GONs demonstrated significant antibacterial activity against four bacterial strains using the agar well diffusion method. They also showed 60% higher radical scavenging activities (RSA) compared to Gallic acid, a standard used in the study (Goyat et al., 2022).

The results of the average diameter of the inhibition zone at temperatures of 33°C, 34°C, 35°C, 36°C, and 37°C for all tested antibiotics were still within the allowable range, so the results of Quality Control of *Staphylococcus aureus* were acceptable. Appropriate antibiotic sensitivity testing is needed by clinicians to provide antibiotic treatment therapy to patients. To

guarantee sensitivity test results, routine QC testing using pure bacterial strains is very important in clinical microbiology. When selecting Quality Control strains and determining QC ranges, care is required for reproducibility of test results, the objective performance of using QC strains, strain stability, and so on (Hong et al., 2015).

The findings from this study demonstrate that incubation temperature significantly affects the diameter of the antibiotic inhibition zones in disc diffusion tests for Staphylococcus aureus. Specifically, the study observed that the inhibition zone diameters for Clindamycin, Levofloxacin, Tetracycline, and Trimethoprim varied with changes in temperature. For instance, the inhibition zone for Levofloxacin generally decreased as the incubation temperature increased, with significant reductions at 33°C and 34°C compared to the optimal temperature. Tetracycline showed a marked decrease in inhibition zone diameter at higher temperatures, particularly at 36°C and 37°C, indicating a strong temperature dependence. These temperature-related variations were statistically significant, as confirmed by repeated ANOVA tests ($p < 0.05$ for all antibiotics), highlighting the importance of maintaining precise incubation conditions.

The high-temperature requirement A (HtrA) serine protease family is a promising target for antibacterial therapeutics due to their dual functions and diverse oligomerization patterns. A redesigned HtrA production method produces cleaner preparations with high yields by overexpressing and purifying target proteins under denaturing conditions. This method retains proteolytic and chaperone activity, allowing for higher production quantities and application in various protein purification strategies (Ronzetti et al., 2022).

The implications of these findings are profound for antibiotic sensitivity testing protocols. To ensure consistent and reliable results, laboratories must standardize incubation temperatures, as deviations can lead to significant differences in the measured effectiveness of antibiotics. This standardization is crucial for accurate diagnosis and appropriate treatment decisions in clinical settings, where inconsistent testing conditions could result in inappropriate antibiotic choices, potentially impacting patient outcomes. Furthermore, these results emphasize the need for rigorous quality control measures and updated guidelines in laboratories to monitor and regulate incubation temperatures meticulously. Ensuring that all personnel are trained on the importance of precise incubation conditions can reduce variability and improve the reliability of antibiotic sensitivity testing across different settings.

4. CONCLUSION

There is a difference in the diameter of the antibiotic inhibition zone at various incubation temperatures. Based on these differences, it can be concluded that there is an effect of incubation temperature on the diameter of the antibiotic inhibition zone in the antibiotic sensitivity test of Staphylococcus aureus by the disc diffusion method. A high incubation temperature (above the optimal temperature) can make the average diameter of the antibiotic inhibition zone in the sensitivity test smaller, while a low incubation temperature (below the optimal temperature) makes the average diameter of the antibiotic inhibition zone in the sensitivity test wider. In addition, the temperature of ± 2°C from the optimal temperature of 35°C did not significantly affect the interpretation of the diameter of the antibiotic inhibition zone for the Internal Quality Control of Staphylococcus aureus. The research is limited by its focus on a single bacterial strain (Staphylococcus aureus) and a narrow range of antibiotics and temperatures, which may not fully represent the variability across different bacteria, antibiotics, and broader temperature ranges, potentially affecting the generalizability and robustness of the findings. Additionally, conducting the study in a single laboratory setting and maintaining a

constant incubation period without exploring the potential interaction with time also limits the comprehensiveness of the results.

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RESEARCH **Open Access**

The Effect of Sub-Acute Inhalation Exposure to Polyethylene and Polyvinyl Chloride Micro-Nano Plastics on the Superoxide Dismutase (SOD) Level and Malondialdehyde (MDA) Level in Rat Ovary

Hikmawan Wahyu Sulistomo[1a*], Laksmitha Janasti[2b], Riana Trinovita Sari[2,3c], Kusworini[4,7d], Safrina Dewi Ratnaningrum[5e], Ihda Dian Kusuma[6f], Nurdiana[6g]

[1] Department of Pharmacology, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia
[2] Master Program of Midwifery, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia
[3] Department of Midwifery, Kemenkes Poltekkes Kalimantan Timur, Samarinda, East Kalimantan, Indonesia
[4] Department of Clinical Pathology, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia
[5] Department of Anatomy Histology, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia
[6] Department of Pathological Anatomy, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia
[7] Dr. Saiful Anwar General Hospital, Malang, East Java, Indonesia

[a] Email address: hikmawan_ws@ub.ac.id
[b] Email address: laksmithaaj234@student.ub.ac.id
[c] Email address: rianats@student.ub.ac.id
[d] Email address: dr.kusworini@gmail.com
[e] Email address: safrina.fk@ub.ac.id
[f] Email address: ihdadk.pa.fkub@ub.ac.id
[g] Email address: nurdianafarmako.fk@ub.ac.id

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Abstract
Plastic is a synthetic or semi-synthetic organic polymer that is widely used in daily life and in industrial production. Microplastics are widespread contaminants and can enter the human body through the consumption of foods containing microplastics, inhalation of microplastics in the air, and skin contact with microplastic particles present in products. Microplastics can enter the ovaries as foreign bodies and can cause inflammation, oxidative stress, and even ovarian granulosa cell death. Polyethylene plastic shards generally have a higher ability to absorb environmental toxins than other types of plastic. Polyvinyl chloride (PVC) is one of the oldest thermoplastic polymers that is often used as water pipes. PVC has carcinogenic monomers and some harmful additives. This study aims to determine the effect of subacute exposure to micro-nanoplastics per inhalation on SOD and MDA levels in rat ovaries. The research method used is a true experimental design with a Randomize Post Test Only Group Design research design. This study used the ovarian organs of female white rats that had been exposed to PVC and PE for 28 days. The number of samples used in this study amounted to 18 female rats. Subacute exposure to micro-nanoplastics per inhalation can lower SOD levels and significantly increase MDA levels in rat ovaries. This study is expected to provide knowledge and an overview for future research on the mechanism of toxicity of micro-nanoplastic exposure that has an impact on female infertility through free radicals in the ovaries.

Keywords: Polyethylene, Polyvinyl Chloride, Oxidative Stress, Rat Ovary.

Corresponding Author:

Hikmawan Wahyu Sulistomo
Department of Pharmacology, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia
Email: hikmawan_ws@ub.ac.id

1. INTRODUCTION

Infertility is a condition in which a couple has difficulty conceiving after having sex without using protection or contraception for a period of 12 months (WHO, 2023). In Indonesia, around 15% or as many as 4-6 million couples out of a total of 39.8 million couples of childbearing age experience infertility problems (Safitriana, 2022). Infertility in women was most commonly identified as being caused by ovulation disorders (25%), endometriosis (15%), pelvic attachment (12%), tubal obstruction (11%), other tube/uterine abnormalities (11%), hyperprolactinemia (7%) (Walker dan Tobler, 2022). Long-term exposure to a variety of endocrine disrupting chemicals in the form of industrial products, *pIasticizers* is associated with decreased fertility by disrupting various hormonal pathways (Bala, Singh and Rajender, 2021). Previous research has shown that reduced or impaired female reproductive function is caused by exposure to microplastics (Yang et al. 2023; Zhou et al. 2023). Continuous and prolonged exposure to microplastics can cause greater exposure to the toxic properties of plastics and interfere with reproduction and cause infertility (Zientika, Amin, and Yoswaty 2021).

Plastics are synthetic or semi-synthetic organic polymers that are widely used in daily life and in industrial production (Cheng et al. 2020). The total plastic waste in Indonesia reaches 3.22 million metric tons per year (Prokić et al. 2019). Plastic pollution has caused widespread concern in the world, especially microplastics (Z. Liu et al. 2022). Microplastics are widespread contaminants and can enter the human body through the consumption of microplastic-containing foods, inhalation of microplastics in the air, and skin contact with microplastic particles present in products (Prata et al. 2020). Microplastics can enter the ovaries as foreign bodies and can cause inflammation, oxidative stress, and even ovarian granulosa cell death (An et al. 2021). Research Wang et al. 2019, found that marine medaka (*Oryzias melastigma*) exposed to polystyrene may cause an increase in *Malondialdehyde* (MDA), a decrease in *superoxide dismutase* (SOD), *catalase* (CAT), *glutathione S-transferase* (GST) and *glutathione peroxidase* (GSH-PX) in the ovaries. This is related to the lack or disability of women's reproductive ability. The study also found that polystyrene can enter the ovarian in rat. In addition, exposure to polyvinyl chloride microplastics in *Daphnia magna* can affect reproductive parameters, such as reducing the number of offspring in the first brood, and interfering with the activity of enzymes such as SOD and CAT (Y. Liu et al. 2022).

Micro-nanoplastics can be classified into several types based on the polymers that make them up such as polyethylene (PE) and polyvinyl chloride (PVC) (Mardiyana and Kristiningsih 2020). PE and PVC are the most widely produced plastics and are potentially hazardous to health (Plastics Europe, 2019). Polyethylene plastic shards generally have a higher ability to absorb environmental toxins than other types of plastic (Prokić et al. 2019). Exposure to polyethylene that occurs continuously for a long time and concentrations that exceed the threshold or overuse can cause adverse side effects because polyethylene can release antimony trioxide compounds that are harmful if they enter the body because they can cause disruption of the reproductive system in women (Dhaka et al. 2022). Polyvinyl chloride (PVC) is one of the oldest thermoplastic polymers that is often used as water pipes. This type of plastic can degrade at relatively low temperatures when exposed to light, releasing hydrogen chloride. PVC has high chemical resistance, as well as resistance to water and various weather conditions (Trivantira, Fitriyah and Ahmad, 2023). PVC has carcinogenic monomers and some harmful additives (Blackburn and Green, 2022). Phthalate and bisphenol A (BPA) are the main raw materials used for PVC synthesis. When BPA is released into the environment and ingested, it destroys amino acid metabolism, steroid metabolis, and energy metabolism pathways and induces oxidative stress (Yang et al. 2022).

The purpose of this study was to determine the effect of subacute exposure to PE and PVC micro-nanoplastics per inhalation on SOD levels and MDA levels in the ovaries of rats of the wistar strain (*Rattus norvegicus*).

2. RESEARCH METHOD

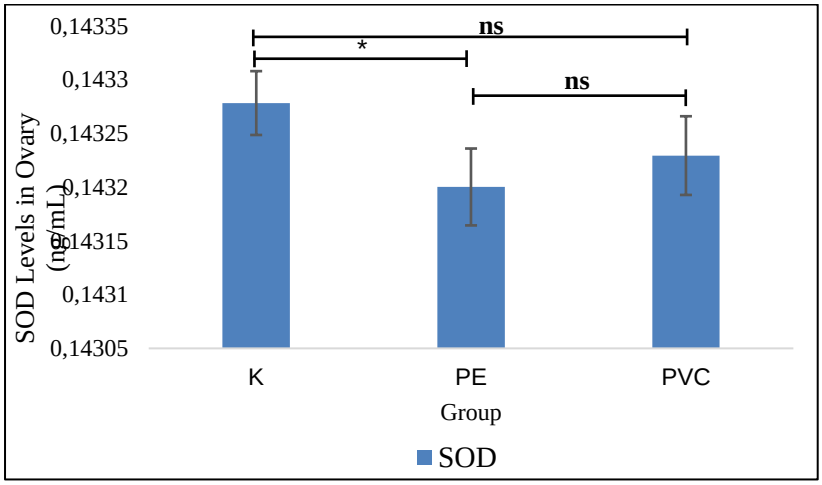
This study is a laboratory experimental research with *a Post Test Only Control Group Design* research design. This study used 18 female wistar rats, confined at a temperature of ±25°C, humidity 40-70%, and fed standard feed and clean water. The mice were divided into three groups consisting of 6 rats each: Group 1, control (not exposed to microplastics); Group 2, PE group (exposed to 15 mg/m3 of PE for 28 days); Group 3, PVC group (exposed to 15 mg/m3 of polyvinyl chloride (PVC) for 28 days). In this study, micro-nanoplastics 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 to humans today.

After 28 days of exposure to microplastics, the animals were then dissected in the proestrus phase and the ovarian organs were isolated. Fresh ovarian organs were homogenized using *phosphate buffer saline* (PBS), then crushed using mortar until smooth and added with 1 ml of Tris KCl 1.17% pH 7.6 buffer. Then, the sample is centrifuged at 4°C, 4000 rpm, for 15 minutes. For every 500 µL of sample, 200 µL of EDTA 100 mM, 100 µL units of NBT 25, 100 µL of xanthin 25 mM, and 100 µL of xanthin oxidase 1 unit are added. The mixture is vortexed and incubated at 37°C for 30 minutes. Then, centrifuge using 4000 rpm for 5 minutes at 4°C and filter. Next, the aquabidest is added before reading using a spectrophotometer with λ580 nm.

This research was conducted at the Biochemistry Laboratory and Pharmacology Laboratory, Faculty of Medicine, Brawijaya University. This research was carried out with the approval of the Health Research Ethics Committee of the Health Research Ethics Committee, Faculty of Medicine, Brawijaya University, number 254/EC/KEPK/08/2023. The data of this study was analyzed by One Way Anova statistics using SPSS. The significance data was shown by $p<0.05$ and further analysis using the Tukey HSD post-hoc test.

3. RESULTS AND DISCUSSION

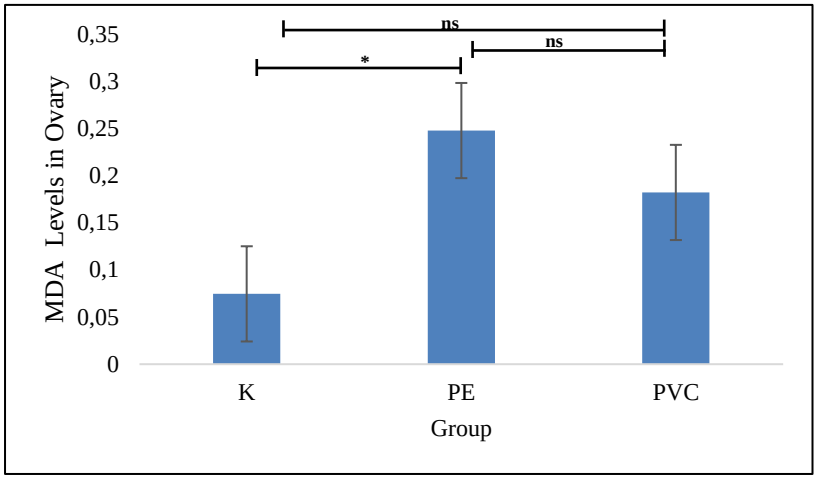
Microplastic exposure caused a decrease in SOD levels in the treatment group compared to the control group ($p < 0.05$). Tukey's HSD test showed that the PE treatment group showed a significant difference compared to the control group ($p < 0.05$), while there was no significant difference in the PVC group compared to the control ($p > 0.05$) and PVC with PE ($p > 0.05$).



Information:ns : *not significant*
(*) : p<0.05
K : Control
PE : Polyethylene
PVC: Polyvinyl Chloride

Figure 1. Graph of Average SOD Levels in Rat Ovary

Microplastic exposure caused an increase in MDA levels in the treatment group compared to the control group ($p < 0.05$). Tukey's HSD test showed that the PE treatment group showed a significant difference compared to the control group ($p < 0.05$), while there was no significant difference in the PVC group compared to the control ($p > 0.05$) and PVC with PE ($p > 0.05$).



Information: ns : *not significant*
(*) : p<0.05
K : Control
PE : Polyethylene
PVC: Polyvinyl chloride

Figure 2. Graph of Average MDA Levels in Rat Ovaries

DISCUSSION

Exposure of Micro-nanoplastics to SOD Levels in the Ovary. This study found that inhalation exposure to polyethylene (PE) and polyvinyl chloride (PVC) micro-nanoplastics at a dose of 15 mg/m³ for 28 days significantly reduced the levels of the antioxidant enzyme superoxide dismutase (SOD) compared to the control group. This result is supported by the research of Hou et al. (2021) and An et al. (2021), which showed a decrease in SOD enzyme activity and an increase in malondialdehyde (MDA) in rat ovaries due to exposure to microplastics. Previous research by Farag et al (2023) of exposure to polyethylene micro-plastics using various doses from low to high is 3.75 mg/kg, PE-MP, 15 mg/kg, and 60 mg/kg for 35 days, showing that there is a significant difference in the reduction of superoxide dismutase (SOD) activity at the highest dose (60mg/kg), while for the lowest dose there are no significant results.

Microplastics are known to have pro-oxidative properties, disrupt the balance of the oxidative-antioxidant system, and increase the production of reactive oxygen species (ROS), causing oxidative stress. This oxidative stress is characterized by an imbalance between oxidative and antioxidant defenses in the body, reducing antioxidant enzymes such as SOD causing mitochondrial dysfunction (Farag et al. 2023). Research conducted by (De Guzman, Chua, and Sedano (2020) on zebrafish causes embriottoxicity and teratogenicity to increase as the concentration of polyethylene is given. Continuous exposure to polyethylene for a long time and concentrations that exceed the threshold can cause adverse side effects because polyethylene can release antimony trioxide compounds that risk disrupting the reproductive system in women (Dhaka et al., 2022).

The results of a study from Ma et al. (2024) show that polyethylene and polyvinyl chloride micro-nanoplastics induce oxidative stress in liver cells triggered by increased intracellular ROS levels and cause negative impacts such as apoptosis, metabolic disorders and mitochondrial dysfunction. SOD activity has an antioxidant capacity that plays an important role in regulating biochemical reactions such as redox reactions and warding off free radicals in the human body (Zheng et al. 2023). The antioxidant system has been activated in the body to fight oxidative damage caused by excessive ROS production. When the impact caused by

micro-nanoplastics exceeds the modulating capacity of antioxidant enzymes, an increase in ROS will lead to a decrease in SOD level activity. Increased ROS (*Reactive Oxygen Species*) in the ovaries can cause apoptosis in granulosa cells and cause follicular atresia in rat which can be a factor in infertility (An et al. 2021).

Exposure of Micro-nanoplastics to MDA Levels in the Ovary. The results of this study showed that subacute exposure to PE micro-nanoplastics per inhalation for 28 days resulted in a significant increase in MDA levels in the ovaries compared to the control group. This proves that exposure to PE micro-nanoplastics can increase ovarian MDA levels which is one of the markers of oxidative stress. Exposure to PVC micro-nanoplastics did not significantly increase ovarian MDA levels compared to the control group. Other studies using PE at higher doses (40 mg/kg/day) for 30 days can cause DNA damage, apoptosis, oxidative stress, and mitochondrial dysfunction in Kunming rat oocytes (Hong, Wu, and Wei 2023). Polyethylene consists of long chains formed by ethylene monomers. It is a stable polymer and an excellent electrical isolator characterized by high strength and flexibility (Baj et al. 2022).

In the study of Sincihu (2022) who used microplastic exposure to LDPE-type PE administered orally for 90 days, showed that the mean expression of MDA and 8-dihydro-2-deoxyguanosine (8-OHdG) metabolites was higher in the microplastic-exposed group. Hippocampal neurons of Wistar rats responded to microplastic by decreasing the synthesis of intracellular antioxidant enzymes. This was due to the consumption of Wistar rats which significantly increased the amount of particles in the blood. Statistically, the large number of microplastics in the blood resulted in lower expression of SOD enzyme. The low level of SOD expression causes membrane damage (seen from the expression of MDA metabolites) and deoxyribonucleic acid (seen from the expression of 8-OHdG metabolites) in hippocampal neurons. Oxidative stress occurs after antioxidants decrease and are no longer able to perform the reduction process. This is one of the body's biological responses to microplastic exposure due to high levels of free radicals. Another study by Cheng et al. (2020) using plastic mulsa film made from polyethylene as exposure to *Eisenia fetida* showed an increase in oxidative stress with a decrease in SOD and CAT gene expression and an increase in MDA and 8-OHdG metabolites. Likewise, the results of this study in rats, which showed a significant increase in ovarian MDA levels following exposure to PE-type microplastics.

In the research of An et al. (2021) who used polystyrene-type microplastics orally at a dose of 1.5 mg/kg/bb for 90 days, MDA levels increased while CAT, SOD, and GSH-PX levels decreased in the exposure group compared to the control group in rat ovaries. The level of ROS increased significantly in granulosa sel given exposure so these results indicate that polystyrene microplastics can induce oxidative stress in the ovaries. Likewise the research of Wang et al. (2019), exposure to polystyrene microplastics in sea bream (*Oryzias melastigma*) can cause an increase in MDA, a decrease in SOD, CAT, glutathione S-transferase (GST) and glutathione peroxidase (GSH-PX) in the ovaries.

Exposure to 4 mm PVC microplastics of 1 gram given to lotus (*Nelumbo nucifera*) after 7 days caused a significant increase in CAT activity. Exposure to PVC caused CAT activity in lotus seedlings to increase by 63% compared to exposure to PET and HDPE PE. GST activity also increased significantly by 82.6% with PVC and 50.3% with PET (Esterhuizen and Kim 2022). In this study, exposure to PVC microplastics increased MDA levels but not significantly.

Plastics are considered to be biochemically inert. In the environment, polymers tend to degrade through biotic and abiotic processes releasing harmful additives. These compounds can penetrate cell membranes and disrupt biochemical reactions resulting in toxic effects PET (Esterhuizen and Kim 2022). Microplastics can enter by inhalation. In the lungs, a very thin tissue barrier smaller than 1 µm separates the alveoli lumen from the bloodstream. Nano-sized

particles have the potential to penetrate the blood vessel system and be distributed throughout the human body. In vitro studies have shown that nanoplastic particles are absorbed by the alveolar epithelium. Nanoplastic particles induce a stronger antioxidant response compared to microplastics. In addition to particle toxicity, micro-nanoplastics can also pose chemical and biologic risks. Plastic has many additives that enhance its properties. There are about 144 hazardous chemicals used as additives. These substances can be released from the plastic matrix inside the organism. The most widely investigated substances are bisphenol A (BPA), vinyl chloride (VC), and benzyl butyl phthalate (BBP) (Baj et al. 2022).

In this study showed a significant difference in MDA activity in rat ovary organs. Malondialdehyde (MDA) is an oxidant or free radical as the final result of lipid peroxide due to the breakage of fatty acid chains which become toxic compounds to cell. Lipid peroxides are formed due to excess reactive oxygen species (ROS) products that attack sel components (lipid membranes and proteins) by involving double fatty acid residues of phospholipids that are very sensitive to oxygen. The imbalance between oxidants in this case ROS and antioxidants will lead to oxidative stress. High MDA concentration indicates the oxidation process in the membrane (Triananda, Primadiamanti, and Angin 2023). Oxidative stress induction is one of the toxic mechanisms of microplastics. An imbalance between ROS products and the anti-oxidation system causes oxidative stress (An et al. 2021). Oxidative stress can induce apoptosis which is considered to be the main route of micro- and nanoplastic toxicity. The source of oxidative stress may come from the large surface area of plastic particles, metals deposited on their surface, and the induction of inflammatory responses. Plastic particles have various functional groups and chemical bonds (such as fenil groups, amino groups) that may be associated with oxidative stress (Baj et al. 2022).

4. CONCLUSION

Subacute exposure to micro-nanoplastics per inhalation can lower SOD levels and significantly increase MDA levels in rat ovaries. Exposure to micro-nanoplastics per inhalation of polyethylene (PE) type further lowered SOD levels and increased MDA levels compared to polyvinyl chloride, but the difference was not significant.

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RESEARCH **Open Access**

Black Garlic Antioxidant Activity Test (*Allium Sativum*) Compound Types with DPPH Method (1,1-*Difenil*-2-*Pikrilhidrazil*)

Sari Wulan Asih[1a*], Tatit Nurseta[2,5b], Kusworini[3,5c], Hendy Setyo Yudhanto[4,5d]

[1] Master Program in Midwifery, Department of Midwifery, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia
[2] Department of Obstetrics and Gynecology, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia
[3] Department of Clinical Pathology, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia
[4] Department of Anatomical Pathology, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia
[5] Dr. Saiful Anwar General Hospital, Malang, East Java, Indonesia

[a] Email address: wulansarikasihsaputra@gmail.com
[b] Email address: tns_obg.fk@ub.ac.id
[c] Email address: dr.kusworini@gmail.com
[d] Email address: hendy.setyo@ub.ac.id

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Abstract
Antioxidants are needed to neutralize Reactive Oxygen Species (ROS) in the body and prevent cardiovascular, cancer, and premature aging as degenerative diseases. Antioxidants will provide resistance to free radicals and prevent damage in normal cells, proteins, and fats, and are able to break chain reactions without disrupting their function. Black Garlic has higher antioxidant properties than regular garlic. S-allyl cysteine (SAC) is a compound from Black Garlic that functions as an antioxidant that can affect the cell cycle in apoptosis, which can provide cell protection from free radical damage. In a study, it was said that a single type of Black Garlic extract obtained IC50 52.055 ppm (µg/mL) which is included in the category of high antioxidant activity. While in the antioxidant activity test, this study used a compound type of black garlic on the grounds that the price is more affordable and easy to find in traditional markets. This study aims to evaluate the potential of compound-type black garlic extract against free radicals by testing antioxidant activity using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The results of the compound type Black Garlic antioxidant test stated to have an IC50 value of 173 ppm (µg/mL) which is classified as moderate antioxidant activity. So researchers assume that the use of single type Black Garlic extract is more effective than compound type Black Garlic.

Keywords: Antioxidant, Black Garlic, *Allium Sativum*.

Corresponding Author:

Sari Wulan Asih
Master Program in Midwifery, Department of Midwifery, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia
Email: wulansarikasihsaputra@gmail.com

1. INTRODUCTION

According to data from the World Health Organization (WHO), one worldwide problem in global health is infertility. The total number of ten percent women experience health issues related to infertility (World Health Organization, 2019). If a couple has not been pregnant in more than two years, they are considered to be infertile in Primary Infertility. However, infertility may also develop if a couple is unable to conceive for more than a year. In this instance, a married couple's attempt to conceive was unsuccessful due to their failure to take contraception. This is called Secondary Infertility (Rahmadiani, 2021). According to a survey, wives account for 64% of infertility and husbands for 36%. From WHO's perspective, women are responsible for 36% of Fallopian tube, 33% of ovulation, 6% of endometriosis, and 40% of other unexplainable or idiopathic conditions. Conversely, Oligozoospermia (16% multifactoral), endocrinologist (20%), and immunologic 2% are from the husband's side (World Health Organization, 2018), subfertility is the term used to describe the effects of lifestyle on exposure to environmental pollution, which often results in abnormal pregnancies. Smoke from cigarettes is one of the major contributing causes of ovulatory disorders.

Ovulatory disorders stand in the second position as infertility disorders owned by women (Irmawati, 2021). An imbalance in hormones caused by the suppression of FSH and LH hormone release is stated to be the cause of this ovulation issue. This hormone exhibits barriers as a result of hypothalamic and pituitary dysfunction. If problems with either hormone's release arise, the follicles' ability to develop will be hampered, which will impact the ovulation process. A variety of intricate chemicals included in cigarette smoke create huge impacts on various reproductive systems (De Angelis et al., 2020). Tobacco elements in smoke that contain nicotine have the potential to alter the body's hormone balance. This is contingent upon the quantity and duration of exposure to cigarette smoke. Reproductive function will be interfered with by any exposure to cigarette smoke. The substances included in cigarettes cause ovarian cell illness which affects estrogen levels. If this occurs, then genetic abnormalities will be more likely to affect female eggs or oocytes (Halimah & Winarni, 2018).

The smoke from cigarettes contains a lot of reactive oxygen species (ROS). Free radicals are defined as less stable molecules that are reactive and able to harm tissue because they can release electrons (Legowo, 2015). Oxidative stress will be impacted in this situation by an imbalance between pro-oxidants and antioxidants. Mitochondrial malfunction results in oxidative stress, which leads to defects in chromosomal segregation, as well as failures in maturation and conception (Ramdiana & Legiran, 2023). Cortisol from the adrenal glands is one of the glucocorticoid hormones produced by the body in response to stress. Gonadotropin-Releasing Hormone (GnRH) hormone released under stress inhibits the Hypothalamic-Pituitary-Gonadal pathway, which in turn impairs reproductive function. The influence of cortisol is a side effect of GnRH secretion inhibition at the pituitary level. It can take advantage of the ovarian level's lowered production of Follicle Stimulating Hormone (FSH) and cortisol, which can obstruct the creation of steroid hormones and cause apoptosis (Setiyono et al., 2015), it has been demonstrated that follicles contain cortisol and Cortisol-Binding Protein (CBP). This stimulates a variety of pathways including oocyte quality and direct steroid genesis effects. The number of follicles that mature from the primordial to the antral stage depends on how they respond to the hormone FSH. Follicles develop and grow in response to FSH stimulation until they reach the mature state. As a result, it may be said that cortisol may disrupt the GnRH pulse, lowering FSH hormone levels and inhibiting the growth of new follicles.

Atomic molecules without an electron pair in their atomic orbit are known as free radicals (Lobo et al., 2010). If free radicals in the body are unbalanced, it can cause a condition called oxidative stress which can eventually disrupt the function of organs in the body and cause disease (Nurkhasanah, 2023). These unbalanced pro-oxidants and antioxidants are said to lead to reproductive diseases such as endometriosis, polycystic ovary syndrome (PCOS), and

unexplained infertility. Weight, lifestyle factors (smoking, alcohol use, drugs), and exposure to environmental pollutants have also been found to trigger the oxidative state and contribute to female infertility (Agarwal et al., 2012). The formation of free radicals or Reactive Oxygen Species (ROS) must be controlled by antioxidants because if not, it will turn into pro-oxidants (Hecht et al., 2016).

Antioxidant is defined as a substance that provides resistance to oxidation activities in the body (Kumar et al., 2012). The antioxidant work system provides one oxidant electron which aims to provide resistance to oxidant activity in cells (Winarsi, 2007). The body needs more antioxidants to balance oxidants and antioxidants. This is especially true if oxidants are present in higher amounts. The female reproductive system is one of the body processes affected by the provision of these antioxidants. Every day, the body will build up its defence system through physical exercise and consuming foods rich in nutrients (Ruder et al., 2008). Antioxidants inhibit free radicals and prevent damage to normal cells, proteins and fats, and can break chain reactions without disrupting their function (Halliwell & Gutteridge, 2007). Free radicals or Reactive Oxygen Species (ROS) trigger an increase in oxidative stress. When oxidative stress increases, there is an inhibition of GnRH (Gonadotropin-Releasing Hormone) secretion which will interfere with the production of FSH (Follicle Stimulate Hormone) and LH (Luteinizing Hormone) in the ovaries causing granulosa cell apoptosis and affecting nutrient supply and oocyte maturity. This affects the number and quality of oocytes and leads to ovulation disorders/infertility. The body is unable to naturally make antioxidants and secondary metabolic compounds so they must be obtained from foods such as spices, fruits, and vegetables. Antioxidants from plants consist of bioactive compounds such as flavonoids, phenolic compounds, tannins, phenolic diterpenes, and vitamins, and have a variety of benefits for the body (Ibroham et al., 2022).

Garlic has long been prescribed by traditional medicine to treat inflammatory and metabolic disorders, including diabetes mellitus, hypertension, cardiovascular diseases, and cancer (Falahatian et al., 2022). Garlic (Allium sativum) is part of a type of plant used for food and health. The development of technology makes the use of garlic not only used as a kitchen spice or eaten directly but can be processed by heating it to produce black garlic (Handayani et al., 2020). Black Garlic has higher antioxidant properties than ordinary garlic (Kimura et al., 2016). Black garlic extract itself is said to increase antioxidant activity and prevent cell damage. S-allyl cysteine (SAC) is a compound from Black Garlic that functions as an antioxidant that can affect the cell cycle in cell apoptosis. This can provide protection to cells from free radical damage (Handayani et al., 2020).

In a study of antioxidant activity tests on garlic compared to three types of garlic, namely compound type local garlic, single type local garlic, and single type imported garlic, it was found that the difference in antioxidant activity values in the three types of garlic varieties (Allium sativum) and the best antioxidant value was found in single type local garlic IC50 10.61 ppm (µg/mL) (Prasonto et al., 2017). While compared to the results of other studies that determine the levels of S-ally cysteine (SAC) by high-performance liquid chromatography (HPLC) on a single type of black garlic extract sample obtained IC50 52.055 ppm (µg/mL) which is included in the category of high antioxidant activity (Romsiah et al., 2022). The results of another study indicate that Black garlic or *Allium sativum*, when fermented in the dichloromethane extract fraction, has the highest total flavonoid content compared to water and n-hexane extracts, measuring at 55.68 mg QE/g. The IC_{50} value of Black garlic in the dichloromethane extract is 361.07 µg/mL, classifying it as a compound with moderate antioxidant activity (Wardhani et al., 2020). The process of heating garlic at very high temperatures can result in black garlic. When processed, garlic turns black in color, has a sweet

and slightly sour taste, and loses the fresh aroma of garlic. S-allyl cysteine is one of the bioactive substances found in black garlic. S-allyl cysteine is the main component of garlic derivatives formed by enzymatic reactions. The presence of heating carried out will form a change in γ-glutamylcysteine to S-allyl cysteine. This makes the S-allyl cysteine content show a higher ratio of five to six times compared to garlic (Handayani et al., 2020).

Previous research on female Wistar rats showed that black garlic extract contains flavonoids that can increase antioxidant activity which is said to reduce oxidative stress and thus inhibit the decline in the number of follicles (Amida et al., 2021). This study aims to evaluate the potential of compound-type black garlic extract against free radicals by testing antioxidant activity using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method.

2. RESEARCH METHOD

The method used to test antioxidant activity in this study utilized the DPPH assay (*1,1-diphenyl-2-picrylhydrazyl*). The extract was dissolved in ethanol p.a and made in various concentrations of 500, 250, 125, 62, and 31 ppm as much as 50 ml each. Into each solution, 1 ml of 0.1 mM DPPH solution was added and incubated for 30 minutes and then measured at a wavelength of 516 nm. As a blank, ethanol p.a and 0.1 mM DPPH containing no test material were used as a positive control of quercetin with concentrations of 1,2,3,4, and 5 µg/mL. DPPH is a type of free radical that remains stable at room temperature and is commonly used to evaluate the antioxidant capability of various compounds or extracts from natural sources. When there is interaction between antioxidants and DPPH, either through electron transfer or hydrogen radical donation, the free radical property of DPPH is neutralized. Several advantages of using this approach include its quick, easy process and the requirement for relatively simple equipment. The lower the IC_{50} value from the antioxidant activity testing using the DPPH method, the higher the antioxidant properties possessed by the substance or material.

This study is a laboratory experimental research with a quantitative experimental research design. The black garlic used in this study was obtained from a producer in Magelang Regency. Compound garlic was processed using a rice cooker at temperatures ranging from 34°C to 38°C for 15 days to produce black garlic. The selection of black garlic was due to its common consumption by the community and by cancer patients.

The inclusion criteria for subjects are: 1) Compound garlic. 2) Fermented black garlic. 3) The part of black garlic used is the bulb of black garlic (suing). The exclusion criteria for subjects are: 1) Rotten and wilted black garlic. The extraction process was conducted at the Herbal Materia Medika Laboratory in Batu Malang.

The materials used are compound black garlic, 96% p.a ethanol, DPPH (*1,1-diphenyl-2-picrylhydrazyl*), and quercetin standard (DPPH solution containing no test material). The equipment used in this experiment includes glassware, knives, rotary evaporator, blender, vortex mixer, magnetic stirrer, oven, analytical balance, filter cloth, and UV-VIS (*Ultraviolet-Visible*) spectrophotometer instrument Genesys.

Black garlic is prepared by peeling and cleaning to remove its skin. Next, the black garlic cloves are thinly sliced lengthwise. These slices are then arranged on a flat baking sheet in a thin layer, approximately 1 cm thick, and dried using an oven at 60°C. The drying process for black garlic is continued until it is completely dried, and then its moisture content is measured.

The black garlic simplisia is extracted using the maceration technique, where 1335 grams of simplisia is soaked in a mixture of 96% ethanol solvent with a total solvent volume of 8000 mL at a ratio of 1:7.5. After macerating for 1 day, the filtrate is filtered and collected in a bottle. The macerated filtrate is then evaporated using a rotary evaporator for 3 hours to remove any remaining solvent, resulting in a concentrated extract called a macerate. Weigh the extracted *Allium Sativum* or Black garlic and store it in the freezer.

The Black garlic extract is mixed with 2 mg of 0.1 mM DPPH powder in a reaction tube containing 96% ethanol solvent. The mixture is then homogenized and incubated. Subsequently, the absorbance of the solution is measured at a predetermined wavelength. The absorbance measurement data is used to determine the percentage of antioxidant activity using the established equation as follows:

$$\% \text{ Antioxidant Activity} = \frac{\text{Absorbance DPPH - Absorbance Sample}}{\text{Absorbance DPPH}} \text{ x } 100\%$$

Afterward, the IC50 value of each extract can be determined using a linear equation with the extract concentration on the x-axis and the percentage of antioxidant activity on the y-axis

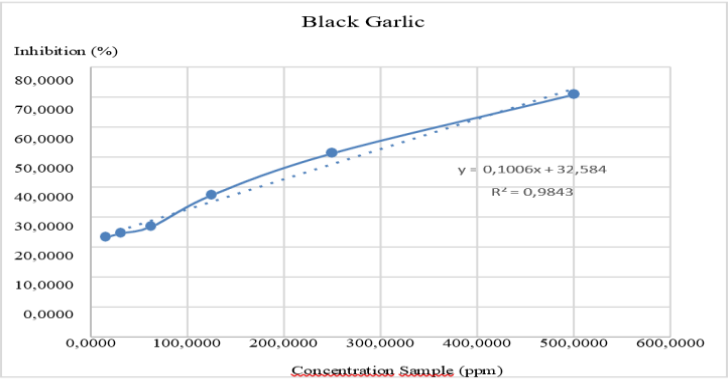
3. RESULTS AND DISCUSSION

This study utilized samples of compound garlic variety black garlic. Black garlic was extracted using the maceration method with 96% ethanol solvent. Following the extraction of the samples, antioxidant activity testing was conducted using the DPPH (*1,1-diphenyl-2-picrylhydrazyl*) method. The parameter used to evaluate antioxidant activity is the inhibition concentration value, commonly referred to as IC_{50}, which is the concentration of an antioxidant substance that causes a 50% loss of radical properties of DPPH or the concentration of an antioxidant substance that provides 50% inhibition. Substances with high antioxidant activity will have low IC_{50} values. Below are the antioxidant test data for black garlic:
Equation: Y = AX + B; A = 0,1006; B = 32,584; R2 = 0,9843

Table 1. Inhibition Percentage Results

Concentration Sample (ppm)	Absorbance Sample	Inhibition	% Inhibition
500,0000	0,1283	0,8084	80,8448
250,0000	0,2591	0,6132	61,3163
125,0000	0,3524	0,4739	47,3867
62,5000	0,4228	0,3688	36,8759
31,2500	0,4374	0,3470	34,6961
15,6250	0,4463	0,3337	33,3674

In this study, an assessment of the antioxidant activity of black garlic was conducted. Graph 1 depicts the relationship between antioxidant concentration and inhibition percentage. The linear equation representing the concentration of antioxidants from the inhibition percentage is y = 0.1006x + 32.584, with a coefficient of correlation (R2) value of 0.9843. This indicates that the method used to assess antioxidant activity yields very good results, with a correlation coefficient value approaching 1 or -1, consistent with previous findings (Pramitha & Sundari, 2020).



Graph 1. The Relationship between Antioxidant Activity and Inhibition

Result: $IC_{50} = \frac{(50 - B)}{} = 173{,}1213$ ppm

UPT Herbal Laboratory Materia Medica Batu conducted the DPPH Black garlic compound type antioxidant test for this investigation, and the findings showed an IC_{50} of 173 ppm. The three categories of antioxidant activity are determined by their IC_{50} value: [1] Strong antioxidant activity, defined as <100 ppm (µg/ml); [2] Moderate antioxidant activity, defined as \100-500 ppm (µg/ml); and [3] Weak antioxidant activity, defined as \>500 ppm (µg/mL) (Wardhani et al., 2020). Through this result, the antioxidant activity of black garlic in this study was categorized as moderate antioxidant group.

A study of black garlic activity using the DPPH method said this method was chosen because it was better than the ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) method in determining antioxidant activity. Where antioxidant activity is expressed in % inhibition to determine the IC50 value which describes the concentration of extracts that can capture free radicals by 50%. The smaller the IC50 value, the higher the antioxidant activity (Wardhani et al., 2020).

Black garlic undergoes a heating process that transforms a number of fresh chemical components into *Amadori/Heyns* compounds, the main substances in the Maillard reaction (Utama et al., 2024). Fresh garlic lacks many beneficial chemical compounds compared to black garlic. Black garlic contains *S-allyl cysteine* (SAC), which exhibits numerous benefits (Woo et al., 2022). According to several studies, black garlic's ability to combat diseases increases with age. Specifically, certain polyphenols, flavonoids, and Maillard reaction intermediates have been identified as potent antioxidants. Additionally, the biological activity of garlic varies geographically; nevertheless, black garlic has significantly higher biological activity, particularly in terms of antioxidant properties, compared to fresh garlic (Kimura et al., 2016). Flavonoids found in extract black garlic have the ability to boost antioxidant activity, which is thought to lower oxidative stress and prevent the loss of follicles (Amida et al., 2021). Consistent with recent research findings indicating that the extract of black garlic (Allium sativum) itself includes polyphenols that support the body's antioxidant defenses (Juniantari & Susanti, 2023).

From the results of this study, it can be concluded that the antioxidant activity of compound type Black Garlic is still less effective in inhibiting the adverse effects of free radicals because the compound type black garlic antioxidant used in this study is classified as a moderate antioxidant with an IC50 value of 173 ppm (µg/mL) where according to Wardhani (2020) the IC50 value range of 100-500 ppm (µg/mL) is categorized as a moderate antioxidant. So the researcher assumes that the use of single-type black garlic extract may be more effective than compound-type black garlic in inhibiting the adverse effects of free radicals.

A single type of black garlic was processed in a rice cooker at a temperature of 340-380 C and incubated for 15 days. The reason the researchers chose this single type of black garlic was because this product was sold and consumed by the public and CA patients, so the researchers applied it to experimental animals. The research literature states that heating black garlic is between 60-700 C because using temperatures above 700 C can damage the structure of reducing sugars, while temperatures below 600 C require a very long time in the process of making black garlic. The optimal heating time is 35 days to prevent free radicals, which produce many flavonoid compounds, tannins, sterols, and saponins (Agustina et al., 2020). Black garlic is generally processed at a temperature range of 30-900 C with relative humidity of 50-90% and an incubation period of 10-80 days. Several aspects can influence the properties and nutrition of Black garlic, including processing technology and processing variables such as time, temperature, humidity, pH, and type of pre-treatment (Ahmed & Wang, 2021).

The limitation of this study is that there is no comparative test between single and compound types of Black Galic extract, therefore it is hoped that further research will be carried

out to analyze the content of compound-type black garlic and single-type black garlic in extract preparations and test their effectiveness against the adverse effects of free radicals.

4. CONCLUSION

The results concluded that the results of the DPPH test of antioxidant activity of compound-type Black Garlic were classified as moderate antioxidants with an IC50 value of 173 ppm (µg/mL) so the use of compound-type Black Garlic was less effective. It is hoped that further research will be conducted to analyze the content of compound-type black garlic and single-type black garlic in extract preparations and test their effectiveness against the adverse effects of free radicals..

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RESEARCH **Open Access**

The Glycemic Index of Gayam (*Inocarpus fagifer* Forst) Cookies as a High Dietary Fiber Food

Agus Wijanarka[1,2a*], Noor Tifauzah[1b], Furaida Khasanah[2,3c], Anisah Nirmala Dewi[4d], Lilis Setyaningsih[1e]

[1.] Department of Nutrition, Politeknik Kesehatan Kementerian Kesehatan Yogyakarta, Yogyakarta, Daerah Istimewa Yogyakarta, Indonesia
[2.] Center of Excellence for Applied Technology Innovation in The Field of Public Health (PUI-NOVAKESMAS), Politeknik Kesehatan Kementerian Kesehatan Yogyakarta, Yogyakarta, Daerah Istimewa Yogyakarta, Indonesia
[3.] Department of Nursing, Politeknik Kesehatan Kementerian Kesehatan Yogyakarta, Yogyakarta, Daerah Istimewa Yogyakarta, Indonesia
[4.] Rumah Sakit Khusus Ibu dan Anak SADEWA, Yogyakarta, Daerah Istimewa Yogyakarta, Indonesia

[a] Email address: agus.wijanarka@poltekkesjogja.ac.id
[b] Email address: noortifauzah@gmail.com
[c] Email address: furaida.khasanah@poltekkesjogja.ac.id
[d] Email address: anisahnirmaladewi@gmail.com
[e] Email address: lilis_setyaningsih@yahoo.com

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Abstract

The type 2 diabetes (T2D) is still one of the world's public health problems. It needs serious handling and prevention efforts in the nutritional aspect. The high prevalence of T2D is related to the low intake of dietary fiber. This type of food has a low glycemic index (GI). Gayam is a high-dietary fiber material that can be used for the manufacture of flour and as processed food material for the high-dietary fiber cookies. The objectives of this research were to study the GI evaluation of cookies from gayam flour. Cookies were tested for chemical composition, and glycemic index. The glycemic index evaluation test used 12 volunteers taken from the healthy group/nondiabetic, normal nutritional status (BMI: 18.5-22.9), and age 19-55 years. The results of this study indicate the dietary fiber content of gayam cookies was 4.57 g/100 g and included in the category of high dietary fiber food. The glycemic index of gayam cookies was 43 and the low glycemic index category. The obtained results show for the first time the potential of gayam cookies in hyperglycaemia management. The conclusion of this study was that gayam cookies has high dietary fiber content and low GI level. Gayam cookies can be used as an alternative snack to control blood glucose levels in the prevention of diabetes mellitus.

Keywords: Blood Glucose, Cookies, Dietary Fiber, Gayam, Glycemic Index.

Corresponding Author:

Agus Wijanarka
Department of Nutrition, Politeknik Kesehatan Kementerian Kesehatan Yogyakarta, Yogyakarta, Daerah Istimewa Yogyakarta, Indonesia
Email: agus.wijanarka@poltekkesjogja.ac.id

1. INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a rapidly growing global health issue, largely due to lifestyle changes, urbanization, and demographic shift(Patil et al., 2023). In 2021, 537 million people worldwide had diabetes, with 90 per cent attributed to T2DM (Khan et al., 2019). The global prevalence rate is 6.1 per cent, with T2DM being the most common form. By 2045, the global diabetes population is projected to reach 783 million, primarily due to obesity, sedentary lifestyles, and population ageing (Lerman Ginzburg, 2023). Low- and middle-income countries are particularly affected, with 80 per cent of the global diabetes population residing in these regions (Ahmad et al., 2022). For this reason, it is very necessary to take this health problem seriously and or prevent it, among others, from the nutritional aspect.

Dietary fibre has a substantial impact on the management and potential reduction of the risk of diabetes mellitus, including type 2 diabetes mellitus (T2DM) and gestational diabetes mellitus (GDM) (Ding, 2024). Consuming dietary fibre has been linked to enhanced glycaemic management, decreased insulin resistance, and improved overall metabolic health(Gao et all., 2022). This response examines the mechanisms and impacts of dietary fibre on the management of diabetes, utilising insights from multiple studies (Fazal et al., 2024). Consumption of dietary fiber between 30-50g/day consistently helps lower blood glucose (Ding, 2024). The importance of dietary fibre in improving insulin sensitivity, a critical element in the management and prevention of metabolic disorders including type 2 diabetes, has been increasingly acknowledged. The research papers offered present a thorough perspective on the influence of dietary fibre on insulin sensitivity through many processes, such as the manipulation of gut microbiota, enhancement of body composition, and regulation of glucose metabolism (Ding, 2024; Gao et al., 2022; Hebbar et al., 2024). The presence of fibre in food has a considerable impact on the glycaemic index (GI), which quantifies the speed at which carbs in diet elevate blood glucose levels (Chiavaroli et al., 2021). Incorporating dietary fibre into carbohydrate-rich diets helps regulate the glycaemic response by retarding the processes of digestion and absorption (Mao et al., 2021). This impact is advantageous for regulating blood glucose levels and mitigating the likelihood of chronic conditions such as diabetes (Zhang et al., 2023). The subsequent sections go into the mechanisms and evidence that substantiate the influence of fibre on the glycaemic index (Ding, 2024). Foods that have a low GI also have the characteristics of causing the process to run slowly so that the rate of stomach emptying is slow (Hebbar et al., 2024; Muhammad Owais Fazal et al., 2024). Consumption of foods with a low glycemic index will result in a lower glycemic response and variability so that insulin response is also not as high as a high glycemic index (Demangeat et al., 2023).

The plant often referred to as "gayam" is formally classified as Inocarpus fagifer (Maulana et al., 2024). It is called by many names in different places and under different circumstances. Gayam (*Inocarfus fagifer* Forst.) is a local food spread throughout Indonesia. This plant holds great significance due to its ecological and therapeutic capabilities, as well as its cultural and economic importance in tropical regions (Wijanarka, Tifauzah, & Wijaningsih., 2020). The subsequent sections delve into the various appellations and circumstances in which gayam is recognised on a global scale (Hany Anastasia et al., 2016). It has a number of commonly used names, e.g. Tahitian chestnut, Polynesian chestnut, Otaheite chestnut, *aila* and *bosua* (Widayati & Umarudin, 2022). Wijanarka (2017) reported that the dietary fiber content of gayam was 21 per cent. However, currently processed gayam products are still limited to being made into boiled or chips even though they are made with flour. The material in the form of flour can be used as a basic ingredient for making cakes which are products that are liked by the public and are durable (Widayati & Umarudin, 2022).

Gayam has exhibited a functional food potential and represents an alternative source of the high dietary fiber (Wijanarka, Tifauzah, & Wijaningsih, 2020). Gayam seeds are rich in carbohydrates, with a content ranging from 76 per cent to 78 per cent, making them a potential

energy source, the seeds also contain significant amounts of protein (11.7%), lipids (8.2%), and ash (3.4%) (Maulana et al., 2024). Consumption of gayam can provide a beneficial effect on human health, associated with indigestible components such as dietary fiber. The high carbohydrate content of gayam flour suggests its suitability for use in various food products, similar to how amaranth flour is utilized in bakery products, pasta, and other functional foods (Gebreil et al., 2020). All of which presented high dietary fiber content, but the glycemic index was not tested. This research was to evaluate the nutrient composition, dietary fiber and glycemic index of gayam cookies. This kind of product can be used as food to prevent diabetics.

2. RESEARCH METHOD

The main ingredient of this research was gayam fruit, which was obtained from Bantul Regency, Special Region of Yogyakarta, Indonesia. The gayam used is yellowish-brown in color, ripe on the tree, 3-4 months old and weighs 75-110 g/seed (medium-large size). The chemicals used for analysis are pro-analytical chemicals. Meanwhile, the enzymes include amylase, amyloglucosidase, and proteases made by SIGMA. Materials for the standard on measuring the blood glucose response of volunteers using pure glucose (glucose anhydrous). Accu-Chek glucometers are essential for diabetes treatment, providing convenience and precision in blood glucose levels testing. Their performance in home and clinical settings has been thoroughly assessed, ensuring accurate measurements for efficient diabetes control and surveillance (Zhao et al., 2024).

The main ingredient is Gayam flour which is pre-gelatinized (Wijanarka, 2017). Gayam fruit has not been peeled off at 100°C for 30 minutes. The Gayam was then peeled and sliced thinly (2-3 mm) and dried in a cabinet dryer at 50-60°C for 48 hours. The dried Gayam was blended and sifted to produce flour with a size of 60 mesh. Gayam cookies use a mixture of wheat flour and gayam flour, respectively, 65 per cent and 35 per cent, while all the main ingredients for control cookies use wheat flour. Additional ingredients for making cookies are butter, margarine, low-calorie sugar, egg yolks, powdered milk, and baking soda. Cookies are made by mixing the ingredients, kneading the dough, and baking at 160°C for 20 minutes.

The glycaemic index (GI) is determined by comparing blood glucose reactions in a test food to a reference food, typically consisting of pure glucose, with equal carbohydrate content. First, a sample (gayam flour) is prepared which will be tested and analyzed for the carbohydrate content and dietary fiber content and calculated total available carbohydrates. Carbohydrate content was calculated by the difference after the sample was analyzed proximately using the laboratory method for analyzing food fiber levels involves incubating samples with enzymes, filtering digestate for IDF determination, precipitating SDFP with alcohol, and quantifying SDFS using liquid chromatography, ensuring accurate measurement of total dietary fiber (McCleary & McLoughlin, 2022). A total of 10 volunteers selected had healthy criteria, had normal nutritional status (body mass index/normal BMI between 18.5-22.9 kg/m2), aged 21-23 years, either male or female, normal blood sugar levels, willing to follow the specified research protocol and have signed informed consent. This study was approved by Politeknik Kesehatan Kementerian Kesehatan Yogyakarta Research Ethics Boards reviewed and approved the study protocol. All volunteers received adequate information about the study and had the opportunity to ask questions.

The volunteers were asked to fast for 10 hours (overnight fasting), except for water, in the morning a 50L blood sample was taken using the finger-prick capillary blood samples method to measure fasting blood glucose levels. Furthermore, volunteers were given the sample food that was tested (steamed gayam flour) and had to be eaten until it was finished. The number of samples was determined based on the carbohydrate content and dietary fiber content so that the sample ate contained total available carbohydrates equivalent to 50 grams of sugar. Half an

hour after the meal was finished, a blood sample was taken again, and the glucose level was measured. Blood samples were taken every half hour until the second hour. The previous activity (point) was carried out on different types of samples on the same volunteers on different days (done every day). From fasting blood glucose levels and after eating (30, 60, 90, and 120 minutes) a glucose response curve was made. The glucose response was also made for the control food i.e., glucose solution. The glucose response curve is made by filling in the results of glucose measurements based on time on the X-axis (measurement time) and Y-axis (blood glucose level). The glycemic index is determined by comparing the area of the sample glucose response curve with the standard glucose response, multiplied by 100.

The composition of the chemical data was analyzed using analysis of variance (one-way ANOVA) with SPSS software for windows. Blood glucose response data were processed to obtain GI values using Microsoft Excel and analyzed descriptively.

3. RESULTS AND DISCUSSION

The results of the analysis of the chemical composition of the Gayam and Control Cookies are presented in Table 1. The moisture, ash, fat, protein, and carbohydrate content of the two cookies did not have a big difference. However, for food content, there is a relatively large difference in the amount. The dietary fiber content in Gayam cookies has a higher dietary fiber content than control cookies. Dietary fibre is crucial for a nutritious diet, categorizing foods into low, medium, and high levels which each contains 2, 4, and 6 grams of dietary fiber per serving, thus Gayam cookies are classified as high in dietary fiber. This helps in understanding and strategizing food consumption for nutritional needs and health goals (El-Habashy, 2017; K et al., 2017).

Table 1. The Chemical Composition of The Gayam Cookies and Wheat Cookies

Samples	Chemical Composition (%wet basis)					
	Moisture	Ash	Fat	Protein	Carbohydrates	Total fiber
Wheat cookies	5.39	1.77	24.62	8.46	59.74	2.38
Gayam cookies	4.69	2.44	25.58	7.88	59.62	4.57

The number of test samples in the form of style cookies given to volunteers is determined based on the total content of available carbohydrates which describes the total carbohydrate content available for the body that is easily digested. The determination of accessible carbs in meals necessitates comprehension of the function of dietary fibre and its influence on the overall carbohydrate content. Available carbohydrates refer to the types of carbohydrates that can be broken down and taken in by the human body, ultimately supplying energy. The computation frequently entails deducting the fibre content from the overall carbs, as fibre is indigestible non the small intestine. This procedure is essential for precise nutritional labelling and dietary planning, particularly for persons with specific dietary requirements like diabetics (Alharbi et al., 2015). In Table 2, the content of KH by difference, total dietary fiber content, and total available carbohydrates (AC), as well as the weight of the cookies samples tested are presented.

Table 2. Carbohydrate Content, Total Dietary Fiber, Available Carbohydrates, and Weight of The Gayam Cookies Consumed Equivalent to 50 Grams of Available Carbohydrates

Samples	Carbohydrates by difference (%wb)	Total fiber (%wb)	Available carbohydrates (%wb)	Weight of sample equivalent to 50 g glucose (g)	Number of cookies (pieces)
Wheat cookies	59.74	2.38	57.36	87.17	9-10
Gayam cookies	59.62	4.57	55.05	90.83	10-11

Notes: **available carbohidrates = carbohydrate by difference – total fiber, wb= wet basis*

In this study, the number of gayam cookies given to volunteers was equivalent to 50 grams of AC in one consumption. The number of samples of Gayam cookies equivalent to 50 grams of AC ranged from 87.83-9.83 grams. The number of samples of cookies given to volunteers weighed between 87 and 91 grams. Samples with these weights were relatively realistic amounts for consumption.

The blood glucose response shown by the volunteers after consuming the food and the standard glucose as presented in Table 3. The glucose response is the blood glucose after we start the meal. In this study, the standard food used a pure glucose solution. The practice of using "pure glucose" as a standard or reference in establishing the GI is widely accepted in the field of nutritional science (Flavel et al., 2021). This technique is essential for establishing a uniform standard against which the glycaemic response of different diets can be evaluated. The utilisation of glucose as a benchmark is endorsed by global protocols and is essential to the methodology of GI testing (Wolever et al., 2019). Nevertheless, there exist discrepancies and obstacles in its implementation that must be resolved in order to achieve more precise and dependable outcomes.

Table 3. Fasting and Postprandial Blood Glucose Concentration (mg/dl) After Consuming Glucose (Reference Food), Wheat Cookies and Gayam Cookies.

Samples	**Time (min)**				
	0	**30**	**60**	**90**	**120**
Glucose	85.25	145.75	124.83	120.50	104.17
Wheat cookies	82.17	100.08	105.08	102.00	96.67
Gayam cookies	80.17	106.17	97.08	93.33	92.67

The glucose response is the blood glucose level after the volunteers studied the test food. In this study, the standard food used a pure glucose solution In Table 3, it can be seen that style cookies and control cookies have a glucose response below the standard glucose. In Figure 1, the blood glucose levels of volunteers are presented after standard glucose, style cookies, and control cookies. The glucose response curve is made by filling in the results of glucose measurements based on time on the X-axis (measurement time) and Y-axis (blood glucose level). The GI is a metric employed to assess the impact of various diets on post-consumption blood glucose levels (On-Nom et al., 2022). Glycaemic index is determined by measuring the rise in blood glucose levels after consuming a test food, in comparison to a standard reference, usually glucose. Understanding this notion is essential for effectively controlling dietary decisions, particularly for persons with diabetes or those who are susceptible to non-communicable diseases. The subsequent sections explore the methods of determining GI, the variability in GI values, and the ramifications of these metrics (Chiavaroli et al., 2021; Flavel et al., 2021). As in the glucose response, it was seen that both cookies had an increase in blood glucose levels below the standard glucose.

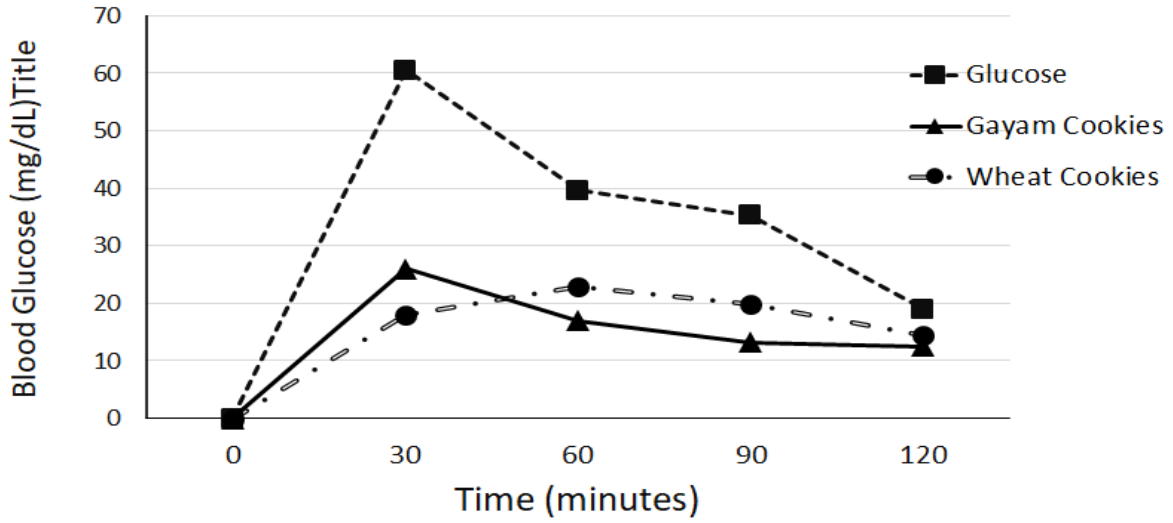

Figure 1. Changes of The Blood Glucose Level of The Volunteers After Consuming Glucose (Reference Food), Wheat Cookies, and Gayam Cookies

The area under the standard glycaemic response curve for glucose or test food is calculated by using blood glucose data obtained from measuring the blood glucose response of volunteers for each food. The data is then plotted on a coordinate axis, with the X-axis representing time (in minutes) and the Y-axis representing blood glucose levels. The GI was determined by measuring the area under the curve for the rise in blood glucose levels following consumption of the glucose-standard food (A) and the tested food (B). The glycaemic index of the food is determined by multiplying the result of the calculation of B/A by 100. Based on Figure 1, it can be calculated the area of the curve for the increase in blood glucose levels after consuming standard glucose foods, style cookies, and control cookies so that the glycemic index can be calculated. The GI is a metric employed to categorize carbohydrates in meals according to their influence on blood glucose levels(Simões et al., 2021). The glycaemic index calculation, invented by Wolever et al, (2019) involves measuring the incremental area under the blood glucose response curve (iAUC) after taking a test item and comparing it to a reference food, usually glucose. This method offers a uniform methodology to evaluate the glycaemic impact of different foods, which is essential for dietary control, particularly for those with diabetes (Meng et al., 2017). Using the formula developed by Wolever et al, (2019) curve area (minutes x mg/dL) for standard glucose, cookie style, and control cookies was 4,343.7; 1,870.2, and 2,037.6. The data are presented in Table 4. These figures were used to calculate the GI values.

Table 4. Incremental Area Under Curve (iAUC) and Glycaemic Index (GI) of Glucose (Reference Food), Wheat Cookies, and Gayam Cookies

Samples	**Area under curve (AUC) (mg/dl × min)**	**Glycemic index (GI)**
Glucose	4,343.7	100
Wheat cookies	2,037.6	47
Gayam cookies	1,870.2	43

The results of determining the glycemic index showed that the style of cookies was 43 and the control cookies were 47. According to Wolever, the classification of the glycemic index value of food using pure glucose as a standard food is low GI (< 56), medium GI (55-69), and High GI (>69) (Meng et al., 2017). This shows that Gayam cookies made from Gayam flour have a low GI. This is thought to be due to cookies using pre-gelatinized Gayam flour which

has a low glycemic index. GI values are categorized using pure glucose as a reference food in nutritional research. This method compares a test food's blood glucose reaction with pure glucose, which has a GI value of 100 (Li et al., 2021). Understanding the impact of different foods on blood sugar levels is crucial, especially for managing diseases like diabetes (Wang et al., 2021). However, GI values should consider eating patterns and personal health circumstances when used in practical situations. Combining GI with other nutritional data and personalized dietary guidance can maximize health results (Kalra & Gupta, 2015).

Gayam flour prepared by pre-gelatinization treatment causes the starch in the heat-treated material by boiling to gelatinize. Further treatment with drying in starch will be retrograded. The retrograded starch causes an increase in the content of resistant starch in the Gayam flour so that it has a low GI impact (Wijanarka, Tifauzah, & Wijaningsih., 2020). Several aspects, such as the kind and solubility of dietary fibre, its physiological effects, and its involvement in metabolic health, determine the value of dietary fibre in food, notably its impact on the GI. Dietary fibre, encompassing both soluble and insoluble forms, plays a vital function in regulating the GI of foods, which quantifies the speed at which carbohydrates in diet elevate blood glucose levels. This response is crucial for the management of diseases such as diabetes and obesity (Chiavaroli et al., 2021; Hebbar et al., 2024; Mao et al., 2021). In this study, the possibility that affects the GI value is the content of dietary fiber. This is supported by data on food fiber content in Gayam cookies, including the high category.

Until now, there is no research on the GI of gayam cookies, so the results of this study cannot be compared with the same type of food. Flakes produced by combining mangrove fruit flour, porang, and mocaf flour have demonstrated low GI values (Jariyah et al., 2022). Adding pulse components such as pea and lentil flour to cereal-based products can reduce their GI. For example, pasta and bread made with pulse components had lower GI values compared to their counterparts made with wheat flour, rendering them healthier choices for managing glycaemic control. Roti produced with Caryota urens and Cycas circinalis flours are classified as low GI foods, having GI values of 57 and 66, respectively. These conventional flours have the potential to be used as alternatives to wheat and rice flours with higher GI (RamyaBai et al., 2019). Although low-GI foods are advantageous for regulating blood glucose levels, it is crucial to take into account the complete nutritional composition and dietary circumstances. For example, foods that have a low GI may yet have a high calorie or fat content, which can affect both weight control and overall well-being. Furthermore, the GI of a dish can differ depending on how it is prepared and how an individual's metabolism responds to it. This indicates that while GI is important, it should be considered alongside other aspects when planning a diet. Moreover, whereas in vitro techniques offer preliminary understanding, in vivo experimentation is essential for precise gastrointestinal (GI) assessment, as evidenced by inconsistencies between projected and observed GI values in certain investigations (Augustin et al., 2020). However, the GI of the two foods used the standard food of white bread, so to compare the GI of gayam cookies in this study had to be multiplied by 0.7 because the standard food used is glucose. On the other hand, if the glucose standard is used, it must be multiplied by 1.4 when compared with the standard white bread.

This condition is thought to be due to the higher dietary fiber content of gayam cookies than control cookies. The same thing was also reported by Wijanarka (2017) who reported that the content of dietary fiber in Gayam was 21 per cent. Consuming foods that are rich in fibre can effectively regulate appetite and enhance the feeling of fullness, so facilitating the control of food consumption and potentially assisting in weight management (Patil et al., 2023). The cause of this action can be traced to a variety of physiological pathways that are triggered in the body by dietary fibres (Zhang et al., 2023; Akhlaghi, 2024). Research has demonstrated that consuming diets rich in fibre can result in decreased energy consumption and body weight,

along with enhanced feelings of fullness and decreased appetite. Nevertheless, the degree of weight reduction varies, and several studies indicate just minimal alteration (Borkoles et al., 2022). Dietary fibres can impact hunger by exerting an effect on the gastrointestinal tract. They induce stomach distention and hinder gastric emptying, hence extending the sensation of satiety (Ehret et al., 2023). In addition, dietary fibres enhance the quantity of undigested nutrients that reach the ileum, thereby prompting enteroendocrine cells to release hormones such as cholecystokinin, glucagon-like peptide-1 (GLP-1), and peptide YY. These hormones are recognised for their ability to decrease appetite and extend the feeling of fullness. The gut ferments fibres to generate short-chain fatty acids (SCFAs), including acetate, butyrate, and propionate. The short-chain fatty acids (SCFAs) are assimilated and have the ability to stimulate the brain to produce satiety signals, resulting in decreased appetite and a diminished need for high-calorie foods (Eley, 2022). Cookies made from Gayam flour make the product high in dietary fiber, glycemic index, and high satiety. Cookies are also cookies which are popular and durable products. Gayam cookies can be used as food to prevent type 2 diabetes (Wijanarka, Tifauzah, & Wijaningsih, 2020).

4. CONCLUSION

The Gayam cookies have a glycaemic index of 43, placing them in the low glycaemic index category. Gayam cookies are a suitable snack option for managing blood glucose levels and reducing the risk of diabetes due to their high dietary fibre content and low glycaemic index. Further clinical research on patients with type 2 prediabetes is necessary to investigate the hypoglycemic effect of gayam cookies.

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RESEARCH Open Access

Therapeutic Effects of Garlic (*Allium Sativum*) Compounds from Different Pretreatment Processes on Women's Reproductive Health: A Narrative Review

Dear Ayu Permata[1a*], Tatit Nurseta[2b], Novida Ariani[3c]

[1.] Master Program of Midwifery, Brawijaya University, Malang, East Java, Indonesia
[2.] Department of Obstetrics and Gynecology, Brawijaya University, Malang, East Java, Indonesia
[3.] Department of Midwifery, Brawijaya University, Malang, East Java, Indonesia

[a] Email address: dearayu26@gmail.com
[b] Email address: tns_obg.fk@ub.ac.id
[c] Email address: novidaariani@ub.ac.id

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Abstract

Women's reproductive health disorders occur due to oxidative stress and can be treated with antioxidant intake. One of them is by utilizing the phytochemical content of garlic (Allium sativum). The semi-systematic review method helps authors to identify the pretreatment of garlic compound S-allyl cysteine (SAC) on women's reproductive health. The literature collection was adjusted to the study of the treatment of garlic compounds with search keywords used "Garlic", "Reproductive health", "S-allyl cysteine", and "Women". The sources used come from online publications from 2010 to 2024. The form of research in the literature review was carried out on test animals, test cells, and test treatments on humans. The selection of information in the reviewed article refers to the year of publication, test products, sample objects, research methods, results, and discussion. The interpretation of the results will be explained descriptively based on the review analysis. Ten study articles have a positive effect of garlic compounds on women's reproductive health. The pretreatment carried out was garlic extract, garlic powder, hexane extract of aged black garlic, dried garlic powder, and aged garlic (black) extract. This review shows that garlic has a main compound S-allyl cysteine (SAC). The bioactive components of garlic can stimulate antioxidant activity and increase the fertility of reproductive organs because it increases the metabolism of oocytes, hormonal regulation, and the maturation of the endometrium. The results of garlic pretreatment showed that aged garlic (black garlic) extract contained S-allyl cysteine (SAC) with more compounds, was more stable, soluble in water, and had minimal toxic content. It allows black garlic (SAC) to have prophylactic properties at the clinical level, so it is possible to develop research on black garlic's effects on women's reproductive health.

Keywords: Garlic, Reproductive Health, S-allyl cysteine, Women.

Corresponding Author:

Dear Ayu Permata
Master Program of Midwifery, Brawijaya University, Malang, East Java, Indonesia
Email: dearayu26@gmail.com

1. INTRODUCTION

Women's reproductive health overall health condition includes physical, mental, and social life related to reproductive tools, functions, and processes functions and processes (Akbar, et al., 2021). Problems in reproductive health are a challenge that has a significant impact on life. The women's reproductive system is a complex organ system and plays a vital role in the reproductive process (Jafari, et al., 2023). Reproductive health disorders are often caused by oxidative stress, such as oocyte maturation disorders, ovarian steroidogenesis, ovulation, blastocyst formation and luteal maintenance in pregnancy. Regeneration from the menstrual cycle can also be disrupted due to low estrogen in the body, leading to implantation disorders (Ajiningrum, et al., 2020). Oxidative stress can be prevented and reduced by adding adequate and optimal antioxidant intake in the body. Antioxidants can inhibit or prevent the oxidation process in substrates by binding to free radicals and reactive molecules (Yoga and Komalasari, 2022). Naturally, antioxidants are formed in the body due to the physiological process of the emergence of free radicals called prooxidants balanced with endogenous defence. In certain circumstances, prooxidants and antioxidants will be in balance, and if there is an imbalance, oxidative stress conditions will be formed (Yusliani, 2018).

Garlic (*Allium sativum*) is the main ingredient that contains many phytochemicals. Several bioactive molecules have an essential role in maintaining human physiology and have the potential to reduce various diseases. Based on scientific research, garlic contains 65% water, 30% carbohydrates, and 5% other bioactive groups, especially sulfur-containing compounds (Suleria, et al., 2012). Therefore, Garlic is one of the herbal plants that is the prima donna in the health sector. The most beneficial benefits of garlic are its antioxidant, antimutagenic, antiproliferative, antimicrobial or immunomodulatory properties (Krumm, et al., 2012). Apart from being a therapeutic ingredient, organosulfur and phenolic compounds acting as antioxidants in garlic are important role holders in preventing cell and organ damage from oxidation (Prasonto, et al., 2017). Garlic contains γ-glutamyl-S-allylcysteine, which can be hydrolyzed and oxidised to form alliin. Alliin will be converted to allicin by the alliinase enzyme after crushing, cutting, chewing or heating. Warming causes the change of GSAC (γ-Glutamyl-S-allyl cysteine) to SAC (S-allyl cysteine) (Azhar and Yuliawati, 2021).

Meanwhile, as an antimicrobial, garlic significantly prevents various diseases, ranging from organ infections to heart disorders. Adding garlic powder to the treatment showed an increase in the hormone progesterone. Progesterone and oestradiol are hormones that control sexual and reproductive maturity (Hagag, et al., 2023). In addition, garlic extract (allicin) has been shown to increase the secretion of gonadotropins and hormones produced by the ovaries by activating the pituitary gland (Hajiuon, 2014).

Some studies show that garlic can be a beneficial compound in health. However, reviews regarding the use of garlic in women's reproductive health are still rare. Therefore, this review summarises and describes how the therapeutic effects of several garlic compounds (Allium sativum) produced from various garlic pretreatment processes for women's reproductive health. Thus, it can provide a broader picture of the use of garlic on women's reproductive health and can be developed in further research.

2. RESEARCH METHOD

The research method used in this study is the semi-systematic literature review method. However, this method could be a good map of theoretical approaches or themes and help identify knowledge gaps within the literature (Snyder, 2019). Data Collection This review article carried out a literature search for relevant studies on the effect of garlic compound extract on women's reproductive health with search keywords used “Garlic”, “Reproductive health”, “*S-allyl cysteine*”, and “Women”. The literature was found in national and international

journals and published in 2010-2024. The data search was carried out using an online platform. When writing this review article, the primary references were cited from trusted sources such as Google Scholar, Scopus, PubMed, and Science Direct. This study was chosen because an analysis was carried out on garlic's therapeutic effect on women's reproductive health in the form of research on test animals, test cells, and test treatments on humans. Total articles were selected ten articles from 1.039 articles. The selection of information in the reviewed article refers to the year of publication, test products, sample objects, research methods, results, and discussion. The interpretation of results is carried out descriptively based on the analysis of the review results (Jafari, et al., 2023).

3. RESULTS AND DISCUSSION

Semi-systematic reviews are conducted to provide a description and evaluate existing research. The study results are from a review and analysis of several studies regarding the therapeutic effects of garlic compounds (*Allium sativum*) from the difference in pretreatment on women's reproductive health. This semi-systematic review shows that garlic contains several compounds that have a role in the female reproductive system—a total of 10 research articles that meet the desired characteristics of review articles according to the method. An overview of the characteristics of the study can be seen in Table 1.

Table 1. Characteristics of included research in the review research

Author (Year)	Product Treatment	Component of the Research Method	Outcome
Kim, et al (2013)	Hexane extract of aged black garlic (raw garlic incubating at 75 dC and 70% relative humidity for two weeks)	• The type of research design is a clinical research study • Human Endometrial Stromal Cells (HESCs) from endometriotic tissue were obtained from 18 women (aged 25-45 years) with advanced endometriosis. • 5 types doses of HEABG (1, 10, 30, 50,70 µg/ml) • Statistical analysis using a two-tailed student's test	Hexane extract of black garlic inhibits the TNF-α-induced proliferation and cell cycle progression of HESCs by suppressing ERK/MAPK and JNK/MAPK activation. it is indicated that HEABG is effective in the prevention and treatment of endometriosis in humans
Amirsalari, et al (2021)	Supplement form dried powder garlic 400 mg (1100 µg of allicin	• The present study was a randomized placebo-controlled triple-blind clinical trial • 60 patients were aged between 20 and 45 years, being currently married, not being diagnosed	Garlic extract can reduce pelvic and back pain, dysmenorrhea, and dyspareunia, which are important symptoms of endometriosis.

Author (Year)	Product Treatment	Component of the Research Method	Outcome
		with severe physical or mental illness. • Doses of each tablet contain 400 mg of dried garlic powder (1100 μg of allicin). Each group received by garlic tablet per day. • Statistical analysis in pain scores using normality Kolmogorov–Smirnov test, plotting on histograms, and examining skewness. Between groups are measured by ANOVA and ANCOVA.	
Falahatian, et al (2022)	Residu 10 (R 10) from fraction garlic. It contains supernatants and is purified from the Amicon ultrafiltration system	• The type of research design is a clinical research study • 60 female Mice at eight weeks old and 25-30 gram weight, and divided into 5 groups • Treatment was performed by intraperitoneal injection with 20 mg/kg doses • Statistical analysis used by one-way ANOVA test with post hoc Tukey's test	R 10 fraction showed that hormone levels increased, altering the polarizing activity of T cells and increasing IL-4, lowering IL-7 and increasing IFN γ in PCOS patients
Minami, et al (2020)	Garlic powder was extracted in 25 ml of methanol and centrifuged. The supernatant was filtered (*S-allyl*	• The type of research design is a clinical research study • 33 female Wistar rats ten weeks old	garlic extract with S-allyl cysteine showed that it improved neurological symptoms due to ovariectomized in rats that caused menopause without the

Author (Year)	Product Treatment	Component of the Research Method	Outcome
	cysteine and *cycloalliin)*	• Doses of garlic powder is 0.5% (w/w) • Statistical analysis using one-way ANOVA followed by Newman-Keuls multiple comparison test	risk of developing cancer cells due to ERα activation.
Wang, et al (2021)	*S-allyl cysteine* (SAC) extracted from aged garlic	• The type of research design is a clinical research study • Bovine mammary epithelial cell lines (BMECs) were cultured into six-well culture plates for each experiment with modified treatment • Concentration of SAC divided into 8 groups (0, 5, 10, 25, 50, 100, 150 and 200 μM) • Statistical analysis by one-way ANOVA using GraphPad Prism 8.0.1	SAC could protect BMECs from heat stress-induced injury by mediating the pathway, suggesting that SAC could be considered a therapeutic drug for attenuating heat stress-induced Nrf2/HO-1 signalling mammary gland diseases
Falahatin, et al (2024)	Residu 10 (R 10) from fraction garlic	• The type of research is experimental study • Sixty adult female NMRI mice (8 weeks old, 25-30 gr weight) • The use of the R10 fraction dose is 20 mg/kg weight • Statistical Analysis was performed with Prism GraphPad using ANOVA followed by post hoc Tukey's multiple comparison	Treatment R10 showed that garlic has a considerable therapeutic function in relieving the hormone PCOS symptoms. The graafian follicles have significantly increased and, implying reactivation of the normal ovulation process. So, R10 has the potential to modulate the hypothalamic-pituitary-gonadal axis, express crucial genes in oocyte development, and alleviate the histological abnormality of the ovary associated with PCOS.

Author (Year)	**Product Treatment**	**Component of the Research Method**	**Outcome**
Xu, et al (2018)	*S-allyl cysteine* (SAC)	• The type of research design is an experimental study • 6-well plate cultured by human epithelial ovarian cancer cell line A2780 • The concentration of SAC is 0 mmol/L; 2,5 mmol/L; 5 mmol/L; 10 mmol/L • Statistical analysis and data visualization were performed using IBM SPSS with two-tailed Student's test	SAC could inhibit the proliferation of human ovarian cancer A2780 cells and cause cell cycle arrest in the G1/S phase. SAC treatment decreased global DNA methylation levels and DNMT1 expression and reactivated CDKN1A.
Waseem, et al (2015)	Garlic extract	• The type of research design is an experimental study • 30 female BALB/c mice with 25-27 gr weight • The concentration of garlic extract is 500 mg/kg/day in group C for 60 days • Statistical analysis was determined by ANOVA and Post Hoc Tukey test.	Garlic extract plays a protective role in improving uterine tissue. It will be prevention for reducing infertility
Bronowicka-Adamska, et al (2020)	*S-allyl cysteine* (SAC) extracted from aged garlic	• The type of research design is an experimental study • The human breast adenocarcinoma cell line MCF-7 cell culture • SAC concentration is 800 µM, 1000 µM dan 2245 µM	Treatment SAC showed that MCF-7 cells have reduced viability from decreasing MPST and sulfate sulfur level reduction

Author (Year)	Product Treatment	Component of the Research Method	Outcome
		• Statistical analysis using the Mann-Whitney test	
Assayed, et al (2020)	Fresh garlic extract and *L-ascorbic acid* (vit C)	• The type of research design is an experimental study • 18 males and 36 pregnant females Wistar rats • The dose level of fresh garlic is 500 mg/kg body weight in IV • Statistical analysis using ANOVA with data was expressed as arithmetical mean±standar error of the mean (SEM)	Treatment of fresh garlic extract and vitamin C in male and pregnant female rats showed a significant decrease in the percentage of information fetal from the insecticide. it is verified that the treatment decreases reproductive toxicity and teratogenicity of cyprinid toxicity in rats

Table 1 shows that several studies used garlic with different pretreatments as a test of female reproductive therapy. Articles do pretreatment of dried, fresh, and aged (black) garlic to obtain several types of compounds. Different garlic pretreatments will result in different pharmacological properties (Kasuga, et al., 2001). Several studies explain that garlic's biological and pharmacological effects come from sulfur compounds. The sulfur compounds owned include *aliin*, *allicin*, *ajoene*, *allyl-propyl disulfide*, *diallyl trisulfide*, *S-allyl cysteine*, *vinyldithiines*, *S-allylmercaptocystein* (Hajiuon, 2014). Allicin compounds are commonly known as bioactive compounds (Balamurugan, et al., 2014).

Fresh garlic and garlic extract in oil or powder can significantly boost immunity, have anti-tumor properties, and have antioxidant activity to protect the body from free radicals. Black garlic extract contains bioactive compounds in the form of sulfur compounds and saponins; fresh garlic, raw garlic, and garlic oil contain allicin; garlic contains antioxidant compounds; garlic extract has antioxidant and phenolic content (Verma, et al., 2023). Garlic powder can reduce the number of athretic follicles and oxidative stress due to its antioxidant content (Jafari, et al., 2021). Antioxidant activity can be seen from bioactive components in the form of polyphenol compounds. The primary mechanism of antioxidant activity is the regulatory flow of Nrf2-ARE, a transcription of redox-sensitive factors that can simulate the expression of antioxidant genes responsible for antioxidant enzymes and increased antioxidant enzyme activity (Zadoush, et al., 2023).

Garlic is indicated to have immunomodulatory activity. Immunomodulators are known as agents that affect the immune system. It refers to the immune response, which includes stimulation, amplification, expression, or inactivation of several stages of the immune response (Abood, 2017). The activation of immunomodulators that work as inhibitors are *ajoene* compounds and *saponins*. Garlic extract has a fractional protein with a low molecular weight known as R10. In vivo studies, the R10 fraction stimulated macrophage phagocytosis activity (Gamboa-León, et al., 2007). Treatment using the R10 fraction for polycystic ovary syndrome (PCOS) patients showed that there was a decrease in the expression of Gpx3 and Ptx3, which

could describe the presence of regular ovulation and increased fertility rate. In addition, the increase in IFN-γ and IL-17 levels indicates that the polarisation activity of T cells in PCOS occurs (Falahatian, et al., 2022).

Black garlic is known as fresh garlic that is fermented during a specific period with temperature (60-90°C) and humidity (80-90%). This process will change the physicochemical properties of black garlic so that it has higher biological activity than fresh garlic (Kimura, et al 2016). The compounds produced by black garlic have more stable and odorless characteristics, such as *S-allyl cysteine* (SAC), which then decomposes into organosulfur compounds such as *diallyl sulphide* (DAS), *diallyl disulphide* (DADS), *diallyl trisulphide* (DATS), *dithiins*, and *ajoene*. SAC is the dominant active compound produced by black garlic with high antioxidant content (Medina, et al., 2019). Reactive oxygen species (ROS) can cause the emergence of oxidative stress conditions, which then affect the development of obstetric complications. Oxidative stress at increased levels can lead to recurrent miscarriages, preeclampsia, limiting intrauterine growth (IUGR), and premature labour. ROS negatively affects the occurrence of embryonic attachment, the development of endometriosis, and preeclampsia. Therefore, antioxidants are needed to suppress oxidative stress in the body (Zadoush, et al., 2023).

The amount of SAC in black garlic is five to six times higher compared to fresh garlic. The aging and fermentation process of garlic causes *allicin* compounds to change into antioxidant components such as SACs, alkaloids, and flavonoids (Azhar and Yuliawati, 2021). As an antioxidant agent, SAC is a potential compound produced by black garlic that can dissolve in water and have minimal toxic content. It allows SAC to have prophylactic properties at the clinical level (Takemura et al., 2014).

Based on research on the differences in garlic pretreatment, namely raw garlic juice (RGJ), heated garlic juice (HGJ), dehydrated garlic juice (DGP), and aged garlic extract (AGE), showed an increase in NK activity and tumor cell killing. However, AGE can suppress tumor cell growth more than HGJ, RGJ, and DHP. Meanwhile, the antioxidant effect of AGE is an inhibitor of oxidative stress rate. AGE can significantly improve the spermatogenesis process compared to 3 other types of pretreatment (Kasuga, et al., 2001). Black garlic can reach almost 90%, inhibiting radicals and reducing power. In addition, black garlic has a higher antibacterial activity than fresh garlic, which is only around 15-34% (Chua, et al., 2022). It shows that AGE is an effective treatment to get the therapeutic effect of the compound to the maximum.

4. CONCLUSION

Garlic (Allium sativum) with various pretreatments has physicochemical properties that affect female reproductive health. The bioactive components of garlic can stimulate antioxidant activity in the female reproductive organs. Antioxidant activity can increase the fertility of reproductive organs because it increases the metabolism of oocytes, hormonal regulation, and the maturation of the endometrium. Antioxidant imbalances cause increasing oxidative stress and affect ovarian function, which then affects the development of health complications. Black garlic has a more complex compound group than other garlic precessions. The content of compounds in black garlic, such as S-allyl cysteine (SAC), is more obtaining five to six times, soluble in water, and has less toxic content. The production of black garlic compounds has a more stable character, so the effects from the treatment process will be more consistent and maximum. From these results, it is necessary to develop research on black garlic. So, it can be known how effective the treatment of black garlic is on women's reproductive health.

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RESEARCH **Open Access**

Machine Learning-based Prediction Model for Adverse Pregnancy Outcomes: A Systematic Literature Review

Eka Santy Abdurrahman[1,2a], Kemal N. Siregar[3b], Rikawarastuti[4c], Indrajani Sutedja[5d] Narila Mutia Nasir[6e]

[1] Department of Public Health, Faculty of Public Health, Universitas Indonesia, Depok, West Java, Indonesia
[2] Department of Midwifery, Health Polytechnic, Ministry of Health Pontianak, Pontianak, West Kalimantan, Indonesia
[3] Department of Biostatistics, Faculty of Public Health, Universitas Indonesia, Depok, West Java, Indonesia
[4] Department of Dental Nursing, Politeknik Kesehatan Kementerian Kesehatan Jakarta 1, Jakarta, Indonesia
[5] School of Information System, Bina Nusantara University, Jakarta, Indonesia
[6] Department of Public Health, Universitas Islam Negeri Syarif Hidayatullah, Jakarta, Indonesia

[a] Email address: eka.santy06@gmail.com
[b] Email address: nazarudin.kemal51@gmail.com
[c] Email address: rikawarastuti@poltekkesjakarta1.id
[d] Email address: indrajani@binus.ac.id
[e] Email address: narilamutia@uinjkt.ac.id

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Abstract
Most of Adverse Pregnancy Outcomes (APO) are preventable particularly if the health personnel can early detect the risk. This study aimed to review articles on how the machine learning model can predict APO for early detection to prevent neonatal mortality. We conducted a systematic literature review by analyzing seven articles which published between 1 January 2013 and 31 October 2022. The search strategy was the populations are pregnant women, intervention using machine learning for APO prediction, and the outcomes of APO are Low Birth Weight, preterm birth, and stillbirth. We found that the predictors of LBW were demographic, maternal, environmental, fetus characteristics, and obstetric factors. The predictors of preterm birth were demographics and lifestyle. Meanwhile, the predictors of stillbirth were demographic, lifestyle, maternal, obstetric, and fetus characteristics. It was indicated that Random Forest (Accuracy: 91.60; AUC-ROC: 96.80), Extreme Gradient Boosting (Accuracy: 90.80; AUC-ROC: 95.90), logistic regression (accuracy 90.24% and precision 87.6%) can be used to predict the risk of APO. By using a machine learning algorithm, the best APO prediction models that can be used are logistic regression, random forest, and extreme gradient boosting with sensitivity values and AUC of almost 100%. Demographic factors are the main risk factors for APO.

Keywords: Adverse, Pregnancy Outcome, Prediction, Model, Machine Learning.

Corresponding Author:

Eka Santy Abdurrahman
Department of Public Health, Faculty of Public Health, Universitas Indonesia, Depok, West Java, Indonesia
Email: eka.santy06@gmail.com

1. INTRODUCTION

In 2020, 2.4 million neonatal deaths, which are defined as deaths that occur during the first 28 days of life, were recorded globally (WHO, 2022). The high rate of neonatal and under-five mortality has led to the inclusion of this issue into the Sustainable Development Goals (SDGs), where one of its goals is to end the preventable neonatal and under-five mortality in 2030, which countries are expected to be able to decrease neonatal mortality rate to 12 per 1,000 live births in the same year (Bappenas (National Planning Board), 2024).

The first month of life is crucial for a child's survival. Most neonatal deaths during this period are linked to maternal factors, that are preventable, such as preterm birth complications, birth complications (asphyxia), and maternal infections causing stillbirth. A previous study also includes low birth weight as the main predictor of neonatal mortality (Tadese et al., 2022). These causes are parts of the Adverse Pregnancy Outcomes (APO).

Adverse pregnancy outcomes are considered to be the main cause of morbidity and mortality among mothers and babies, affecting both the physical and mental aspects, and particularly occur in low- and middle-income countries in Asia and Africa. In terms of neonatal mortality, Indonesia ranks seventh among countries with high neonatal mortality rates worldwide and ranks first in Southeast Asia. The Indonesian Demography Health Survey (IDHS) demonstrates that the trend of neonatal mortality in Indonesia tends to be stagnant from 2002 to 2017 (National Population and Family Planning Board (BKKBN); Stastistics Central Bureau (BPS); Ministry of Health; USAID, 2017). Low birth weight (34.5%) has been identified as the main cause of neonatal mortality in Indonesia, followed by preterm birth (22.5%), congenital abnormality (11.4%), and infection/sepsis (3.4%) (Indonesia Ministry of Health (Kemenkes RI, 2022).

Some factors may contribute to Adverse Pregnancy Outcomes, including obstetric, maternal, lifestyle, and sociodemographic factors. Since APO is the primary cause of neonatal deaths, it is beneficial to develop procedures that accurately predict the possibility of APO, especially preterm birth, stillbirth, and low birth weight, to avoid neonatal mortality (Mombo-Ngoma et al., 2016; Younger et al., 2022). Several statistical methods and Artificial Intelligence (AI) can predict APO risk factors, one of which is Machine Learning which is used to provide accurate predictions. This research aims to collect and review articles on how machine learning models can predict adverse pregnancy outcomes for early detection to prevent and reduce neonatal mortality.

2. RESEARCH METHOD

This study conducted a Systematic Literature Review (SLR) using Perish for article search, Microsoft Excel (licensed) for inclusion and extraction, and VOSviewer for comparative analysis. The six steps followed were: defining the research question, determining study characteristics, finding relevant articles, choosing articles that meet criteria, synthesizing information, and reporting results. VOSviewer was used to visualize the results of the comparative analysis.

Study Criteria

All articles reviewed in this study discuss the machine learning model used to identify and predict Adverse Pregnancy Outcomes.

Search Strategy

The authors used PICOS to identify articles that meet the inclusion criteria of the study. The topic for PICOS was: P: Pregnant Outcomes OR Low Birth Stillbirth OR Preterm birth, I: Risk Factor, C: Algorithm Machine Learning, O: Accuracy OR ROC OR AUC. We included articles written in English and published between 1st January 2014 and 31st Desember 2023. This was followed by identifying relevant sources using Pubmed, Proquest, Embase, and Scopus databases. The keywords or synonyms used were "Pregnant Outcomes" OR "Low Birth" OR "Stillbirth" OR "Preterm birth" AND "Risk Factor" AND "Machine

Learning" AND "Accuracy" OR "ROC" OR "AUC". PRISMA was then applied to determine whether the articles were relevant to the topic or not.

Study Article Selection

We screened 180 articles for inclusion in the study. After removing duplicates and non-eligible articles, we ended up with seven articles that met the study's inclusion criteria and were included in the analysis.

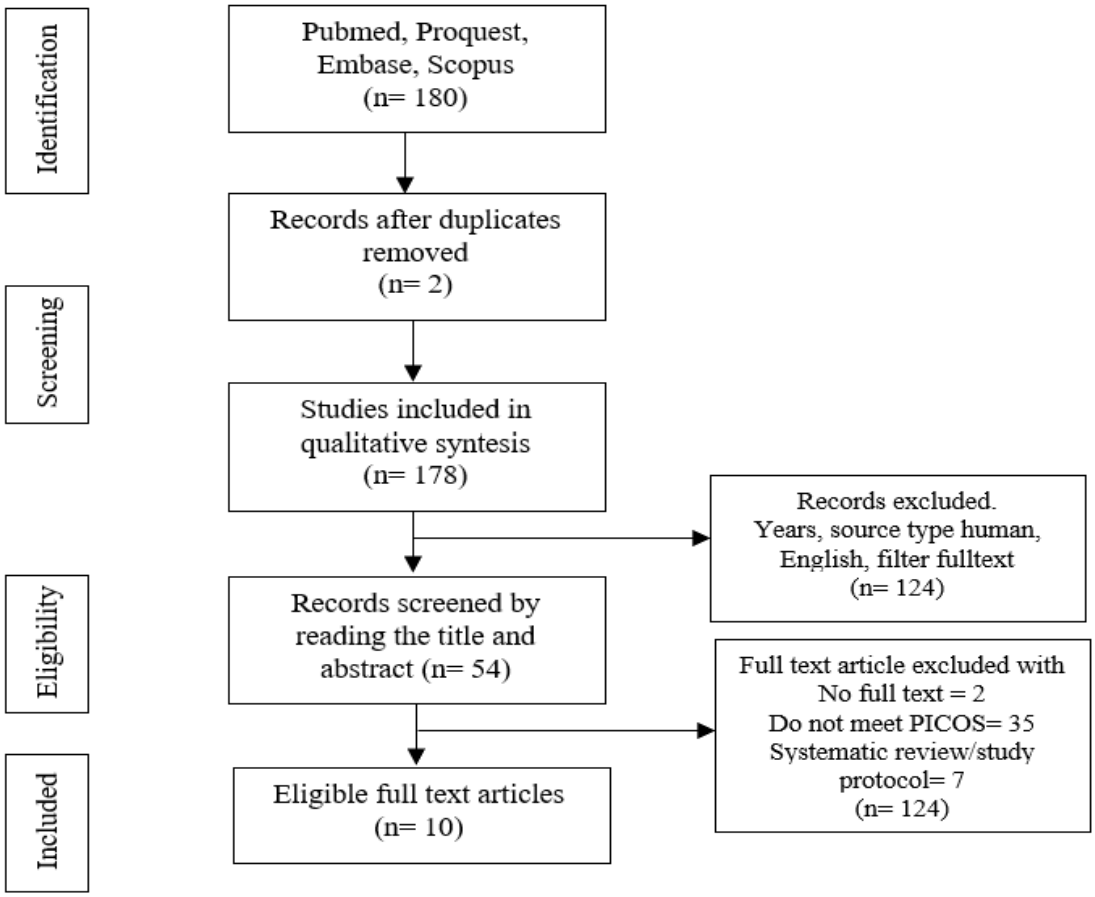

Figure 1. Article selection process using PRISMA SLR

Data Analysis

The authors collected data on respondent characteristics, data type, research design, sample, and results from articles in their study. Adverse Pregnancy Outcome (APO) was classified into three categories: Low birth weight, Stillbirth, and preterm birth. Machine learning techniques such as Random Forest, Decision Tree, Naïve Bayes, Logistic Regression, K-Nearest Neighbor, etc. were used to identify APO risk factors with a focus on ROC and AUC values above 0.7.

Ethical Clearance

As this study used the Systematic Literature Review approach and did not directly affect human, no ethical clearance was needed.

3. RESULTS AND DISCUSSION

Articles included in this study were published in the last 3 years. The duration of study presented in the articles was varied with the shortest being 5 months and the longest being 4 years. The location of the study described in these articles included America, East Asia (China), Australia, South Asia, South Africa, East Africa, and Central Europe. Studies

described in the articles used the prospective and retrospective cohort design with most data sets from data recorded in demographic and national health surveys or hospital data records.

The risk factors for Adverse Pregnancy Outcomes are categorized into six main factors: socio-demographic, general morbidity episodic illness, infections and environment, behavior, history of smoking, infant characteristics, and obstetrics. Among these, socio-demographic factors are identified as the main predictor (Mombo-Ngoma et al., 2016). This categorization provides a clear overview of the main factors influencing the incidence of APO.

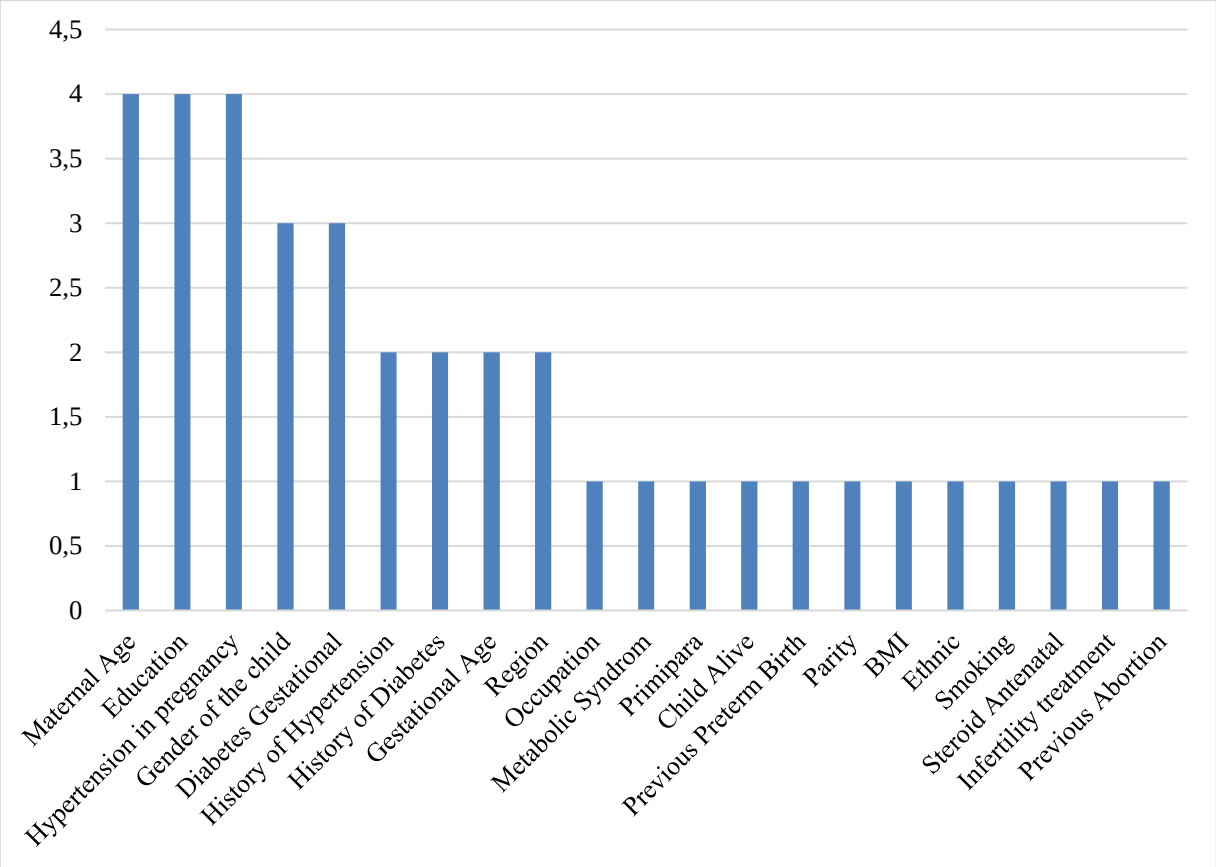

Figure 2. Frequency of Predictors for Adverse Pregnancy Outcomes

Prediction Modeling of Adverse Pregnancy Outcomes Using Machine Learning Algorithm

The research studies used various machine learning models to identify risk factors for Low Birth Weight (LBW) and preterm birth (Bekele, 2022b). Cho et al. and Bekele conducted studies using multiple modeling approaches, such as random forest, decision trees, k-nearest neighbors, support vector machines, Xgboost, and Naïve Bayes (Wang et al., 2016). Pollob et al. and Khan et al. also explored risk factors for LBW using different models (Ashikul Islam Pollob et al., 2022). Belaghi, Beyene, and McDonald used logistic regression, random forest, Artificial Neural Networks, and Decision Tree to predict risk factors for preterm birth (Chen et al., 2023). Zhang et al. used Extreme Gradient Boosting and long short-term memory models for the same purpose.

A similar study was conducted by Koive and Sairanen (2020) using logistic regression modeling (AUC: 0.64), artificial neural network (AUC: 0.66), and gradient boosting decision tree (AUC: 0.67) to determine the risk factors for preterm birth. The same modeling approach was also carried out by Koivu and Sairanen (2020) to determine the risk of early stillbirth with logistic regression (AUC: 0.74), artificial neural network (AUC: 0.74), gradient

boosting decision tree (AUC: 0, 76). As for APO late stillbirth with logistic regression (AUC: 0.61), artificial neural network (AUC: 0.57), and gradient boosting decision tree (AUC: 0.61) (Edwards et al., 2021).

The most frequently used algorithm models of machine learning in the ten articles under study are logistic regression, random forest, extreme gradient boosting, naïve Bayes, and K-Nearest Neighbor. The following visualization was obtained using the keywords adverse, pregnancy outcome, prediction, model, and machine learning (Figure 3).

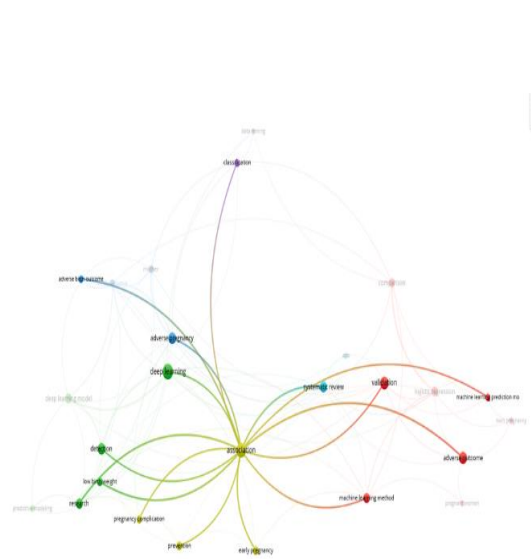

Figure 3. Visualization of keyword

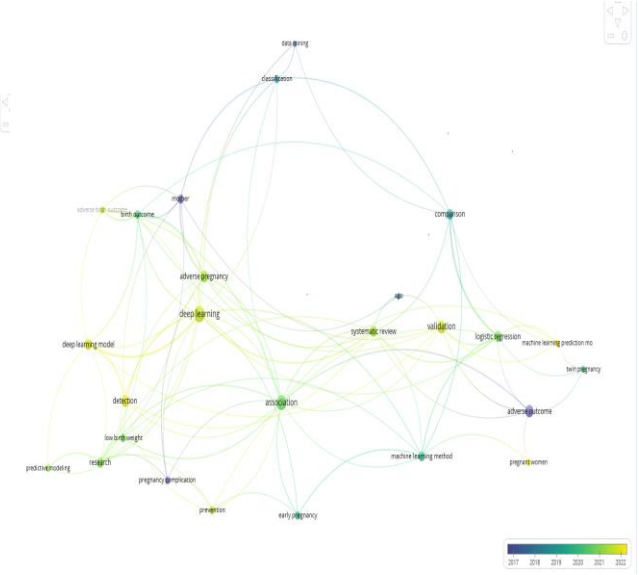

Figure 4. The model used for adverse pregnancy

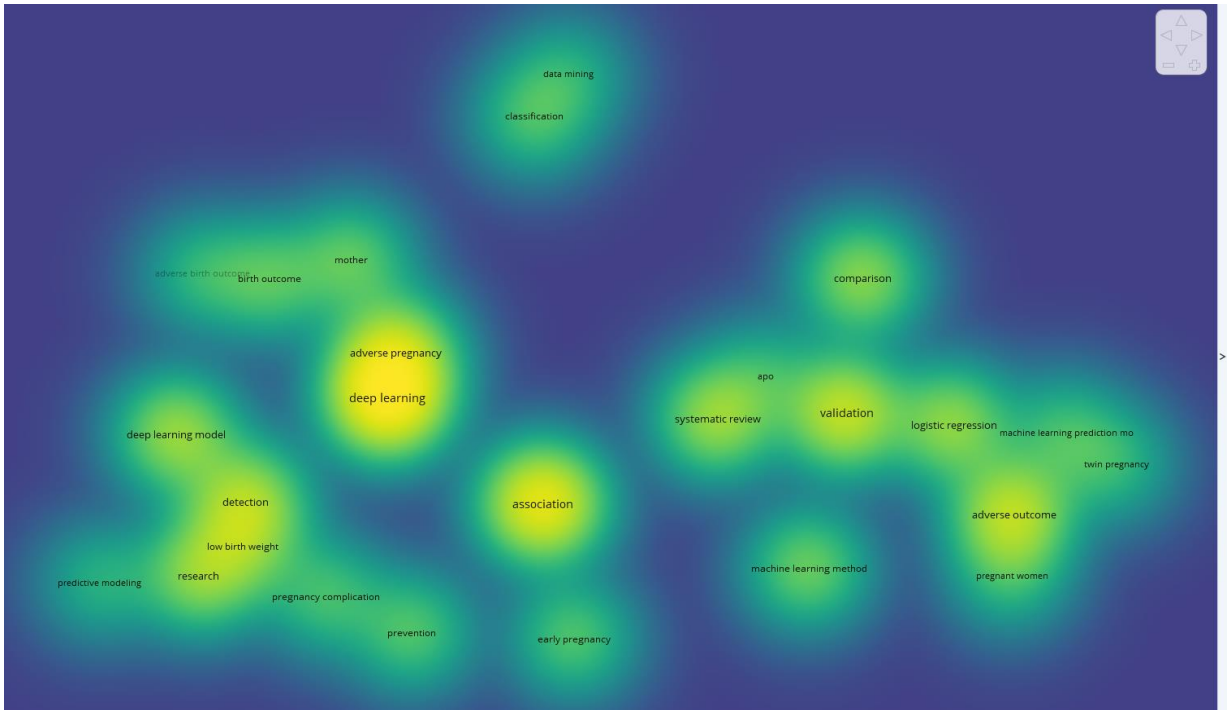

Figure 5. Relationship adverse pregnancy and machine learning method

Adverse Pregnancy is strongly linked to deep learning, machine learning methods, and logistic regression. These methods are frequently used to predict APO (Figure 4). Figure 5 described new study in 2022 discusses the relationship between deep learning, machine learning, and adverse pregnancy. Before 2022, logistic regression was the main model used

for adverse pregnancy, but the shift to machine learning and deep learning has occurred due to the development of machine learning models. Adverse pregnancy also has a strong association with detection, early pregnancy, and low birth weight.

Assessment of Adverse Pregnancy Outcome Predictive Modeling

The text discusses how machine learning algorithms were used in studies to identify predictive factors for adverse pregnancy outcomes such as low birth weight, preterm birth, and stillbirth (Belaghi, Beyene, & McDonald, 2021a; B. Zhang et al., 2019). The studies found that socio-demographic factors such as age, education, place of residence, occupation, primiparous, number of live children, parity, BMI, and maternal race were important risk factors (Ashikul Islam Pollob et al., 2022; Khan, Zaki, Masud, Ahmad, Ali, Ali, et al., 2022; Mangold et al., 2021). The studies suggest that logistic regression and random forest are the best modeling approaches for predicting these risk factors.

A study by Bekele (2022) found that mothers under 18 have a 44.9% higher incidence of low birth weight (LBW) babies. Maternal age is a crucial LBW risk factor, along with area of residence, occupation, education, wealth index, and behavior during pregnancy (Endalamaw et al., 2018). Other risk factors include chorioamnionitis, history of the disease, and pregnancy with steroids (Kassaw et al., 2021). Preterm birth is influenced by metabolic syndromes such as blood pressure, uric acid, blood sugar, and lipids, as well as the history of previous preterm birth, age, education, history of smoking, history of hypertension, and infertility treatment (Belaghi, Beyene, & McDonald, 2021a; Puspitasari et al., 2020). Machine learning models such as logistic regression, artificial neural networks, and extreme gradient boosting show strong predictive capabilities for preterm birth and stillbirth risks (Gao et al., 2019).

Table 2. Machine learning in Predicting Adverse Pregnancy Outcomes

Authors	Adverse Pregnancy Outcomes	Model Analysis	Accuracy, validation, precision, and Sensitivity	Features Included
(Bai et al., 2022)	Gestational age less than 28 weeks, gestational age less than 26 weeks, birth weight less than 1000 g, birth weight less than 750 g, and small-for-gestational-age	Artificial neural network, the decision tree, the logistic regression, Naïve Bayes, the random forest, and the support vector machine were used for predicting preterm birth	The random forest had the best performance (accuracy 0.79, area under the receiver-operating-characteristic curve 0.72). R-Studio 1.3.959	maternal age (0.2131), birth-month (0.0767), PM10 month (0.0656), sex (0.0428), number of fetuses (0.0424), primipara (0.0395), maternal education (0.0352), pregnancy-induced hypertension (0.0347), chorioamnionitis (0.0336) and antenatal steroid (0.0318
(Arayeshgari et al., 2023a; Ashikul Islam Pollob et al., 2022b; W. T. Bekele, 2022b; Khan, Zaki, Masud, Ahmad, Ali, Ali, et al., 2022b)	Low birth weight	Logistic Regression, Decision Tree (Arayeshgari et al., 2023; Bekele, 2022a; Pollob et al., 2022), used too Random Forest (RF) and support vector machine (Arayeshgari et al., 2023; Bekele, 2022c). Artificial neural network (Arayeshgari et al., 2023) Naive Bayes, K-Nearest Neighbor, , Support Vector Machine, Gradient Boosting, and Extreme Gradient Boosting (Bekele, 2022c) and	91.60 persen accuracy, 91.60 persen Recall, 96.80 percent ROC-AUC, 91.60 percent F1 Score, 1.05 percent Hamming loss, and 81.86 percent Jaccard score (Bekele, 2022c). The logistic regression-based classifier performed: with 87.6% accuracy and 0.59 area under the curve for holdout (90:10) cross-validation (Pollob et al., 2022). The accuracy of all models was 87%. Sensitivity 74%,	Gender of the child, marriage to birth interval, mother's occupation, and mother's age (Bekele, 2022c). Region, education, wealth index, weight, height, twin child, child alive, and delivery by CS (Pollob et al., 2022). Gestational age, number of abortions, gravida, consanguinity-ity, maternal age at delivery, and neonatal sex (Arayeshgari et al., 2023) and Diabetes, gestational age, and hypertension

		Absolute error and mean absolute percent error were used for BW estimation (Khan, Zaki, Masud, Ahmad, Ali, & Ahmed, 2022)	specificity 89%, positive likelihood ratio 7.04%,, negative likelihood ratio 29% and ac- curacy 88% (Arayeshgari et al., 2023). The logistic Regression (LR) classifier with 100% oversampling using SMOTE achieved the best classification. performance. accuracy (90.24%), precision (87.6%), recall (90.2%), and F1 (0.89) (Khan, Zaki, Masud, Ahmad, Ali, & Ahmed, 2022).	(Khan, Zaki, Masud, Ahmad, Ali, & Ahmed, 2022)
(Belaghi et al., 2021a) (Zhang et al., 2022) (Sun et al., 2022).	Preterm Birth	Regresi logistic and Machine Learning (Belaghi, Beyene, & McDonald, 2021c). Long short-term memory (LSTM) networks, Time-Series Technology (Y. Zhang et al., 2022). Naive Bayesian (NBM), Support Vector Machine (SVM), Random forest (RF), artificial neural networks (ANN), K-means, and logistic regression (Sun et al., 2022)	AUC increased from 65% (95% CI: 63–66%) to 80% (95% CI: 79–81%) with the inclusion of complications during pregnancy (Belaghi, Beyene, Mcdonald, et al., 2021). LSTM: Accuracy was 0.739, sensitivity was 0.407, specificity was 0.982, and the AUC was 0.651 (Y. Zhang et al., 2022) and RF model was the highest compared with other algorithms (accuracy: 0.816; AUC 0.885, 95% confidence	Abortions (including miscarriages) as the most important predictor of PTB during the first trimester (importance: 28.23 for previous abortions (including miscarriages) vs. 7.79 for diabetes). complications during pregnancy and hypertensive disorders (Belaghi, Beyene, & McDonald, 2021c). Blood pressure, blood glucose, lipids, uric acid, and other metabolic factors (Y. Zhang et al., 2022). Age, magnesium,

			interval (CI): 0.873–0.897) (Sun et al., 2022)	fundal height, serum inorganic phosphorus, mean platelet volume, waist size, total cholesterol, triglycerides, globulins, and total bilirubin (Sun et al., 2022).
(Khatibi et al., 2021)	Stillbirth	decision tree, Gradient boosting classifier, logistics regression, random forest, and support vector machines	Accuracy of 90%, sensitivity of 91%, specificity of 88%. AUC of ±95%, CI of 90.51% ±1.08 and 90% ±1.12	Maternal demographic features, clinical history, fetal properties, delivery descriptors, environmental features, healthcare service provider descriptors, and socio-demographic features
(Koivu & Sairanen, 2020)	Stillbirth and preterm pregnancies	Logistic regression, artificial neural network, and gradient-boosting decision tree	0.76 AUC for early stillbirth, 0.63 for late stillbirth, and 0.64 for preterm birth	Age and BMI, previous pregnancies with adverse effects, various comorbidities, and having an ART pregnancy

4. CONCLUSION

According to the ten articles reviewed in this study, the best APO prediction models that use machine learning algorithms use logistic regression, random forest, and extreme gradient boosting with sensitivity values and AUC of almost 100%. These machine learning models conclude that the risk factors for Adverse Pregnancy Outcomes are mostly socio-demographic, including maternal age, education, occupation, wealth index, area of residence, number of children, and primiparous. Other factors that are also identified as influencing the APO are hypertension, metabolic syndrome (gout, blood pressure, blood sugar, and lipids), diabetes, pregnancy with steroids, history of smoking, history of preterm, multiple pregnancies, weight, and height.

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RESEARCH **Open Access**

The Implementation of Passive Leg Raising in Shocked Patients: Scoping Review

Rotua Rina Verawaty[1a*], Cecep Eli Kosasih[2b], Ristina Mirwanti[2c]

[1] Postgraduate Program, Faculty of Nursing, Universitas Padjadjaran, Bandung, Indonesia
[2] Critical and Emergency Nursing Department, Faculty of Nursing, Universitas Padjadjaran, Bandung, Indonesia

[a] Email address: rotua22001@mail.unpad.ac.id
[b] Email address: cecep.e.kosasih@unpad.ac.id
[c] Email address: ristina.mirwanti@unpad.ac.id

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Abstract

Shock is a life-threatening condition. It is essential to perform hemodynamic support on shocked patients to restore adequate circulation. Passive leg raising (PLR) is a critical act to assess fluid responsiveness which can provide significant information about fluid needs of shocked patients. The present study aimed to identify the implementation of passive leg raising in shocked patients. The scoping review was the method used in the present study. Relevant literatures were obtained from PubMed, CINAHL and Google Scholar Search engines from 2013 to 2023. The articles were manually extracted through tabulation and the data were thematically analyzed with an exploratory descriptive approach. Nine articles were found relevant and hence were included in the present study. The findings of the 9 articles revealed that the implementation of passive leg raising in shocked patients was an early strategy in assessing fluid responsiveness and fluid administration could be decreased after 48 hours of ICU admission. The findings of the current study suggested that the implementation of PLR significantly assessed fluid responsiveness which in the end guided in conducting fluid resuscitation in shocked patients and could reduce fluid administration in shocked patients for the first 48 hours of ICU admission. Therefore, health professionals working in critical care units including critical care nurses should consider performing PLR to shocked patients.

Keywords: Passive Leg Raising, Shock, Fluid Responsiveness, Hemodynamics, Critical Care.

Corresponding Author:

Rotua Rina Verawaty
Postgraduate Program, Faculty of Nursing, Universitas Padjadjaran, Bandung, Indonesia
Email: rotua22001@mail.unpad.ac.id

1. INTRODUCTION

Shock occurs due to an imbalance between the need and supply of oxygen (Dell'Anna et al., 2019). This condition can potentially lead to multi-organ failure and death (Blumlein & Griffiths, 2022). It is crucial to perform hemodynamic support in shocked patients to restore adequate circulation. Fluid resuscitation should be commenced immediately when the shock occurs. It aims to prevent organ dysfunction and organ failure (Vincent & De Backer, 2013).

Fluid resuscitation serves as the foundation for managing patients with acute circulatory failure (Chadi, 2013). Previous studies suggest that when fluid challenge is administered to patients with unstable hemodynamics, only 50% of those patients responded to the volume expansion (Marik & Lemson, 2014). If preload unresponsiveness occurs, the increase in volume in large quantities can lead to hypervolemia which subsequently result in pulmonary and tissue edema, respiratory failure, organ dysfunction, thereby prolongs the length of inpatient care stay at ICU and dependence on mechanical ventilation (Douglas et al., 2020). To prevent interstitial fluid accumulation, it is necessary to consider the addition of inappropriate volume by assessing the fluid responsiveness (Saugel et al., 2013).

Fluid responsiveness is changes in cardiac output or stroke volume ≥10-15% (Xu et al., 2017). Increased stroke volume occurs due to the ability of the left ventricle to respond to the addition of fluid (Hasanin, 2015). An alternative strategy for predicting fluid responsiveness is by performing passive leg raising (PLR) (Rameau et al., 2017). PLR was developed to predict whether volume expansion will increase CO during resuscitation in patients with acute circulatory failure (Monnet et al., 2016).

Passive leg raising is a preload challenge that can be performed repeatedly without administering intravenous fluid (Monnet & Teboul, 2015). The procedure is an approximate amount of 150-350 ml of venous blood moving from the lower extremities translocated to the intrathoracic compartment which can increase the preload of the right ventricle and the left ventricle as a pseudo-fluid challenge (Assadi, 2017). However, the hemodynamic effect is only temporary, reversible autotransfusion in nature (Pickett et al., 2017) that it will quickly return to the initial measurement before PLR is performed (Monnet & Teboul, 2013). Therefore, an indepth review needs to be conducted to examine the implementation of passive leg raising in shocked patients to prevent further complications resulted from the addition of fluid volume. Drawing from the findings of the literature reviews, no reviews that specifically addresses the implementation of passive leg raising has been identified. This scoping review is the first to identify the implementation of passive leg raising in shocked patients. The scoping review aimed to map and identify the implementation of passive leg raising in shocked patients.

2. RESEARCH METHOD

The methodology employed in this review is a scoping review approach, following the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR) (Tricco et al., 2018). The scoping review framework consists of five main stages: finding research questions, finding relevant findings, selecting research, mapping data, compiling, summarizing and reporting findings (Peterson et al., 2017).

a. Eligibility criteria

The present study commenced with a search of literatures on the advantages of passive leg raising, research questions and the eligibility criteria of research articles under PCC (Population, Concept, Context) approach, including: Population: Patient with shock, septic or hypovolemic shock, Concept: Passive leg raise, Context: Implementation of passive leg raising. The inclusion criteria in this review are full-text articles that were accessible in English in the last 10 years since 2020 until 2023 and articles with experimental study research design, case control study, cross-sectional study, longitudinal study, case report, retrospective study and cohort study. Inaccessible and not in English full-text articles, and secondary research papers were excluded.

b. Article Searches and Selection Strategies

Article identification was carried out systematically using 2 main data bases namely CINAHL, Pubmed, and one search engine Google Scholar. The keywords used in gathering relevant articles were Shocked Patient OR septic shock OR hypovolemic shock AND passive leg raise OR passive leg raising test OR passive leg elevation AND hemodynamic.

c. Extraction and Analysis of data

The articles included in this review were extracted manually using table extraction. The results of the search were extracted in a table which outlines the author's name, year, country, research design, population and sample, intervention and research findings. The data were thematically analyzed using an exploratory descriptive approach. The research question in this review is: How is the implementation of passive leg raising in shocked patients?.

3. RESULTS AND DISCUSSION

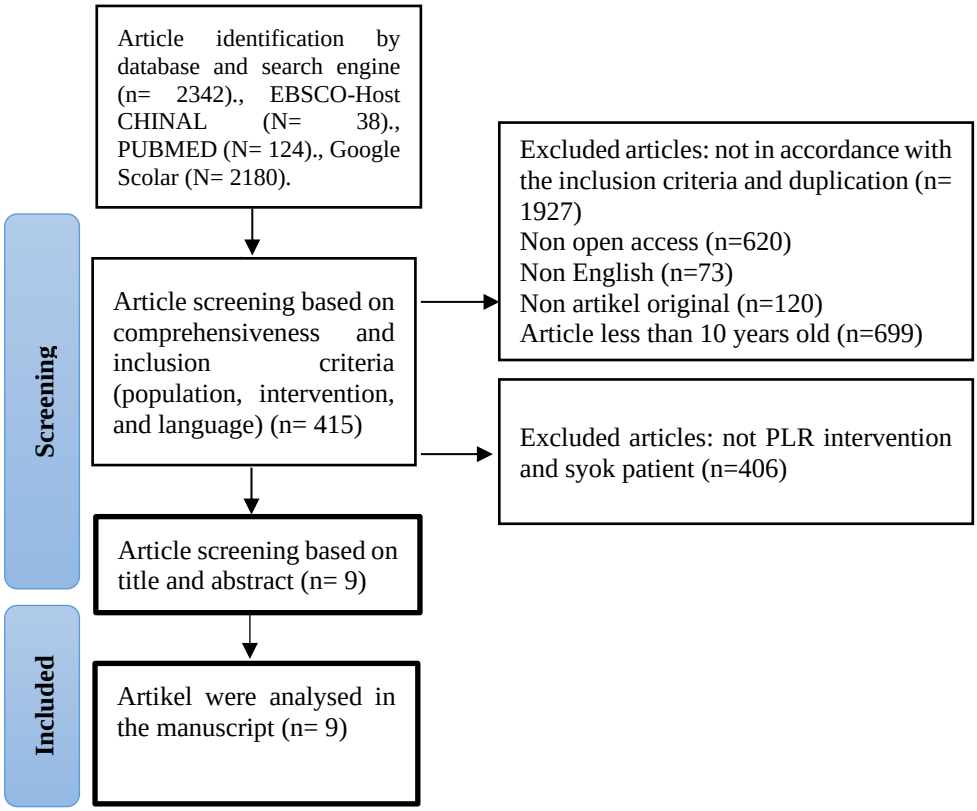

Figure 1. PRISMA Flow Diagram

Nine articles were identified to conform with the criteria set and objective of the present study, viz. to investigate the implementation of passive leg raising of which several studies have proved its effectiveness and ineffectiveness. In general, those articles addressed the implementation and the advantages of passive leg raising. According to the origin of the articles, 1 article was from the Netherlands, 1 article was from the UK, 3 articles were from the USA, 2 articles was from France, 1 article was from Singapore, and 1 article originated from Colorado. All articles under analysis consisted of 3 randomized control trials and 6 prospective observational studies published from 2013 to 2023.

Table 1. Extraction of articles

No.	Author & Country	Population	Research Method	Objective of the Study	Intervention	Result
1	(Rameau et al., 2017) Netherlands	Patients with septic shock aged > 18 years old. Sample: 21 patients	Prospective multi-step intervention study.	To test the effectiveness of PLR to reduce fluid administration.	PLR test on septic shocked patientss	There was a significant and substantial decrease in fluid administration in the first 48 hours of ICU admission and fluid balance was generally significantly reduced.
2	(Elwan et al., 2022) United Kingdom	Patients with sepsis diagnosis Sample: 39 patients	Prospective observational study.	To evaluate the accuracy of PLR in predicting fluid responsiveness	The implementation of PLR for 3 minutes and monitoring using thoracic electric bioimpedance monitor (TEB),	Better predictor PLR tests to assess fluid responsiveness.
3	(Toppen et al., 2020) Los Angeles	Shock patient, patients with persistent vasopresso, secondary hypotensive and conscious patients Sample: 79 patients	Methods: Non-interventional, prospective trial	To evaluate the safety and feasibility of PLR maneuvers.	Maneuver passive leg raising (PLR)	PLR maneuver lead to low incidence of complications in patients with unstable hemodynamics.

No.	Author & Country	Population	Research Method	Objective of the Study	Intervention	Result
4	(Jacquet-Lagrèze et al., 2019) France	Patients with acute circulatory failure Sample: 34	Prospective observational study	To assess ΔCRT-PLR in predicting increased peripheral perfusion	Conducting a PLR and VE test with Ringer Lactate of 500 mL, then performing a capillary refile time measurement	Changes in CRT during PLR predicted CRT responsiveness with good accuracy in acute circulatory failure, with a CRT decrease of 27% during PLR.
5	(Kuan et al., 2016) Singapore	Patients aged > 21 years with septic and serum lactate concentrations greater than or equal to 3.0 mmol/L. Sample: 122 patients	Randomized Controlled Trial (RCT)	To determine the use of noninvasive hemodynamic optimization combined with PLR results in lactate clearance in patients with severe sepsis and septic shock in ICU	The PLR maneuver and intravenous fluid bolus are performed using a noninvasive monitor.	Fluid resuscitation and PLR maneuvers using noninvasive cardiac output monitoring do not provide better results with regular treatment nor does it generate a significant difference in lactate clearance.
6	(Douglas et al., 2020) Colorado	Patients with sepsis or septic shock anticipate ICU admission, refractory hypotension	Randomized Controlled Trial (RCT) .	To guide the amount of intravenous fluids administered to patients with septic shock by assessing fluid responsiveness.	PLR maneuver, immediate administration of crystalloid fluid bolus, and the initiation or the increase in vasopressor titration	Resulted in lower fluid balance and reduced the risk of kidney failure and respiratory failure.

No.	Author & Country	Population	Research Method	Objective of the Study	Intervention	Result
7	(Cronhjort et al., 2017) USA	Septic shocked patients. Sample: 34 patients	Randomized Controlled Trial (RCT)	To determine fluid responsiveness with PLR can reduce fluid accumulation after 3 days of ICU admission and can reduce weight gain	Passive leg raising (PLR) test	The PLR protocol did not generate a significant reduction in weight gain in ICU for septic shocked patientss, thus PLR was not effective in reducing septic shocked patients's body weight.
8	(Mallat et al., 2022) France	Sample: 270 patients	Prospective observational study	To predict fluid responsiveness by understanding changes in pulse pressure (DPPV PLR) induced by PLR in patients with mechanical ventilation	PLR maneuver and volume expansion with 500 ml crystalloid solution administered for 15 minutes	Changes in pulse pressure variation induced by PLR could predict fluid responsiveness in mechanically ventilated patients. Relative and absolute change was a strong predictor of fluid responsiveness,
9	(Marik et al., 2013) USA	Severe septic/septic shock Sample: 34 patients	Observational study	To assess changes in stroke volume index (SVI) induced by PLR to predict volume responsiveness	Bolus 500 mL of NaCl solution is administered for 10 minutes after PLR maneuver.	The PLR maneuver coupled with bioreactance monitoring was an accurate method of determining volume response in critically ill patients.

DISCUSSIONS

1) Implementation of PLR

Passive Leg Raising (PLR) is a temporary action that is reversible autotransfusion in nature to increase preload while (Pickett et al., 2017) passive leg raising test is one of the many strategies to assess fluid responsiveness (Rameau et al., 2017). Fluid responsiveness is an increase in stroke volume or cardiac output by 10% - 15% in response to 500 ml of the crystalloid fluid bolus (Pickett et al., 2017). CO monitoring to assess fluid responsiveness is performed before PLR, during PLR and after PLR (Minini et al., 2020). The peak effect of PLR occurs at 30-90 seconds during leg elevation (He & Liu, 2016). The PLR technique begins by providing a semi-recumbent position for 3 minutes and monitoring cardiac output. Then, the body is lowered in a supine position and the lower leg is passively raised at 45˚ horizontally which is therefore called PLR, leave it for 3 minutes and perform a cardiac output measurement for 30 seconds during leg elevation. Following that, the patient is returned to the initial position for 3 minutes and perform another the cardiac output measurement (Elwan et al., 2022; Toppen et al., 2020).

PLR indication is performed in patients with unstable hemodynamic status or poor tissue perfusion with systolic blood pressure manifestation < 90 mmHg, MAP < 60 mmHg, SVO2 < 65%, Heart rate > 100 x/m, urin output < 0,5 ml/kg/j, acral coldness, respiratory> 20 x/m, capillary refill time > 2, lactate: 2.0 mmol/L (Rameau et al., 2017). Contraindications occurred to patients with abnormal abdominal pressure (increased abdominal pressure) or patients with intra-abdominal hypertension (Beurton et al., 2019).

2) Advantages of PLR

a. PLR is a good predictor of fluid responsiveness.

PLR is a good predictor of fluid responsiveness in shocked patients. This occurs because PLR can increase venous return, resulting in an increase in the *diastolic volume of* the right ventricle followed by an increase in stroke volume of the left ventricle (Elwan et al., 2022). Fluid challenge is a gold standard for diagnosing responsiveness preload but variation in performing it might affect the results. Patients with a negative PLR test will most likely have the negative result if fluid bolus is performed (Elwan et al., 2022). If the patient is not responsive to fluids, then there is no need to administer additional fluids to the patient (Beurton et al., 2019).

Clinically, PLR has been shown to be effective in assessing fluid responsiveness in shocked patients and has been validated by different hemodynamic monitoring methods to measure cardiac output (Toppen et al., 2020). The PLR maneuver using accurate bioreactance monitoring determines volume response. Changes in carotid blood flow after the PLR maneuver is an additional and useful method for determining fluid responsiveness in hemodynamic unstable patients (Marik et al., 2013). An increase of SV > 10% in patients with acute circulatory failure during PLR will also increase peripheral perfusion seen from CRT responsiveness, i.e. a decrease in CRT by 27% during PLR (Jacquet-Lagrèze et al., 2019). Changes in pulse pressure variation induced by PLR can predict fluid responsiveness in patients with mechanical ventilation without cardiac arrhythmias monitored with arterial catheter (Mallat et al., 2022).

However, the effect of Passive Leg Raising (PLR) will be more accurate if the hemodynamic response is monitored directly using invasive CO (cardiac output) measurement than looking at arterial pulse pressure (Monnet et al., 2016). The PLR maneuver does not provide better results when hemodynamic monitoring is performed non-invasively (Kuan et al., 2016). PLR implementation provides relevant information on fluid responsiveness. However, PLR can also lead to reversible complications in patients with unstable hemodynamics, but

these complications are rare. The results of the review suggest that there was a change in heart rate, MAP and atrial fibrillation and desaturation occurred in unconscious patients whereas conscious patients are subject to experiencing discomfort and pain (Toppen et al., 2020).

b. PLR can decrease fluid administration 48 hours after ICU admission

PLR test can be applied to a variety of clinical situations and has the potential to reduce fluid administration, as PLR mechanism is based on reversible autotransfusion. PLR can significantly and substantially reduce fluid administration in the first 48 hours of ICU admission in septic shocked patients. Fluid balance is generally significantly reduced (Rameau et al., 2017). The PLR-guided fluid resuscitation strategy leads to lower fluid balance where fluid volume administered at 72 hours becomes much less, thus, it will improve the function of vital organs that prevent complications of kidney and respiratory dysfunction in the first 72 hours of ICU admission (Douglas et al., 2020). Several study reviews proved that PLR induced fluid responsiveness assessment can reduce fluid needs in shocked patients, however PLR intervention does not significantly reduce body weight in shocked patients (Cronhjort et al., 2017).

Assessment of fluid responsiveness is strongly influenced by the technical and clinical aspects (Alvarado Sánchez et al., 2023). The implementation of PLR must be supported by the compliance of doctors and nurses to correct performance of PLR. In addition, correct hemodynamic measurements are required before, during and after PLR and the correct interpretation is highly crucial. Without the support of changes in therapeutic behavior of doctors and nurses in performing an effective PLR, reduction in fluid administration will not be necessarily achieved (Rameau et al., 2017). In addition, excessive use of PEEP and high dose of norepinephrine use (≥ 0.3 mcg/kg/min) during PLR will affect the assessment results of fluid responsiveness (Alvarado Sánchez et al., 2023).

4. CONCLUSION

The findings of the present study identify the implementation and advantages of PLR. PLR has the practical benefits to assess fluid responsiveness in shocked patients. The assessment of fluid responsiveness may serve as a guide in performing fluid resuscitation in shocked patients. PLR can also decrease fluid administration in septic shocked patients for the first 48 hours of ICU admission. On average, fluid balance is significantly reduced. However, this should be supported by the compliance of doctors and nurses in performing PLR correctly. For that reason, health professionals especially critical care nurses should consider performing passive leg raising to shocked patients. Nonetheless, as the present study only used 2 main data bases, it is recommended that future research to use a larger number of data bases so that a more in-depth results of the implementation of passive leg raising in shocked patients and obstacles in its implementation can be observed.

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RESEARCH **Open Access**

Analysis of The Prevention Needs of Adolescent Girls' Mental Health Disorders: A Qualitative Study

Risza Choirunissa[1a*], Sri Achadi Nugraheni[1b], Cahya Tri Purnami[1c], Nur Endah Wahyuningsih[1d]

[1] Doctoral Program in Public Health, Diponegoro University, Semarang, Central Java, Indonesia

[a] Email address: riszachoirunissa@students.undip.ac.id
[b] Email address: s.a.nugraheni.undip@gmail.com
[c] Email address: cahyatp68@gmail.com
[d] Email address: nurendahwahyuningsih@lecturer.undip.ac.id

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Abstract

Adolescent girls in Jakarta face unique mental health challenges due to cultural, social, and environmental factors. Understanding their prevention needs is critical to inform targeted interventions. This qualitative study aimed to explore the prevention needs of mental health disorders among adolescent girls in Jakarta, shedding light on their experiences and requirements for effective support. We conducted a qualitative study in Jakarta, Indonesia. Participants included 4 counseling guidance teachers consisting of public schools, private schools, Islamic and Christian affiliated schools, 2 parents, 1 coordinator of the School Health Unit, 2 health workers who hold PKPR projects, and the supervisor in charge of the school health unit, selected through purposive sampling. Data were collected through focus group discussion and analyzed using thematic analysis manually. The study reveals that teachers and parents have varying levels of exposure to adolescent mental health information, with some offering counseling but not always tailored to girls' needs. Parents often rely on subtle cues to detect mental health issues in their daughters. There's room for improvement in addressing girls' mental health needs in schools, with specific training for teachers and more open parent-daughter communication. Further research is needed to ensure uniform support for adolescent mental health.

Keywords: Adolescent Girls, Mental Health, Prevention Needs, Qualitative Study, Jakarta.

Corresponding Author:

Risza Choirunissa
Doctoral Program in Public Health, Diponegoro University, Semarang, Central Java, Indonesia
Email: riszachoirunissa@students.undip.ac.id

1. INTRODUCTION

Adolescence is a period of development marked by significant physical, emotional, and psychological changes (Zuhri, 2022). In recent years, there has been growing recognition of the unique mental health challenges faced by adolescent girls (Yoon et al., 2023). Factors such as academic stress, peer pressure, body image concerns, and societal expectations can contribute to the development of mental health disorders during this stage of life (Chok et al., 2023). However, the context-specific experiences of adolescent girls in urban environments, such as Jakarta, Indonesia, have received limited attention. Jakarta, as a bustling metropolis, presents a distinct set of challenges and stressors that may impact the mental well-being of its adolescent female population (Sulistiowati et al., 2019; Suryaputri et al., 2022).

Understanding the prevention needs of mental health disorders among adolescent girls in Jakarta is crucial due to the increasing prevalence of mental health problems in Indonesia, including depression and anxiety (Brooks et al., 2021; Suryaputri et al., 2022). Mental health problems account for approximately one quarter of the disease burden among young people in Indonesia, and suicidality is a substantial concern. Inadequate mental health literacy in adolescents is often identified in low- and middle-income countries like Indonesia, significantly increases the risk of developing moderate-severe depression (Renwick et al., 2024). Adolescents demonstrate a strong preference for peer and family support over professional help-seeking strategies, suggesting that universal mental health literacy programs may have benefits for both primary and secondary disease prevention (Abd El Salam et al., 2023). Schools have several characteristics that make them an ideal place to promote mental health of adolescents, and counselors in primary schools in Indonesia should be equipped with tools to help them screen mental health problems (Pulimeno et al., 2020).

Several qualitative studies in Indonesia explore the prevention of mental health disorders among adolescent girls (Brooks et al., 2021; Renwick et al., 2024; Sarwinanti et al., 2024). The increasing prevalence of mental health issues among adolescents in Indonesia, including depression and anxiety (Sitohang, 2023). These studies emphasize the need for adequate prevention, identification, and early treatment strategies for mental health issues in young persons.

While there have been studies exploring risk factors for mental health disorders in adolescents in general, there is still a lack of research that focuses on young women's unique experiences in urban environments like Jakarta. This study was designed to fill that gap by investigating the specific needs and effectiveness of support felt by adolescent girls, which have not been adequately addressed in the current literature. While previous studies have identified the prevalence and general risk factors of mental health disorders among adolescents, this study specifically addresses the unique experiences and needs of adolescent girls in urban Jakarta. Unlike prior research that broadly covers mental health across various demographics, our focus on this specific population allows for tailored intervention strategies that are more likely to be effective in urban settings. This study aimed to explore how the mental health program was implemented in the school with collaboration between teachers, parents, and primary health care.

2. RESEARCH METHOD

This study used a qualitative design with a phenomenological descriptive approach. With a phenomenological descriptive approach, researchers can discover life experiences. The phenomenological descriptive approach is a qualitative research method that aims to describe the meaning of the lived experiences of individuals about a particular phenomenon.

The data collection was done from 01 to 30 September 2023 at the University of National, South Jakarta. The researchers were the leaders of data collection for every session.

The informants of this study included teachers, staff in primary health care, and female student's mothers. The informants were expected to give the information of mental health program, implementation, collaboration, and evaluation in the household, school, and primary health care. The sample was selected using the purposive sampling method which was selected by inclusion and exclusion criteria. The inclusion criteria were those who knew about mental health counseling programs in school and the primary health care level and had a willingness to participate in the study. The exclusion criteria were those who decided to quit from the data collection and could not communicate to give the information. In total, there were nine informants joined the study.

The measurement of preventing mental health disorders among adolescent girls is an interview guideline. The contents include the experiences of experts during the mental health program. The obstacle they faced and the solution they tried to solve. Additionally, it was also asked about their expectation through the mental health counseling program. The interview guideline was checked by all authors and finalized before data collection started. However, the interview guidelines could elaborate depending on the situation and feedback from the informants. The process, flow, and dynamics of each in-depth interviews were different depends on elaboration of interview guideline and responds from informants. In the interview guideline, there are three different topics discussed including parents, teachers, and health care staff. For the parents, the topics include the way to discuss problems daughters face, motivation book, media used to counsel, bullying, and daughter's safety feeling. For the teachers, the topics include education materials, training in mental health, evaluation of mental health programs, media counseling, effect of zonation program, social media, needs for evaluation, and human resources. For the primary health care staff, the topics include the role, education, information, cadres, expectations of the program, human resources, SDQ (Strength and difficulties questionnaire), and contact with students. The topics for each informant are developed from their responsibility in terms of mental health program for adolescents.

The data collection was led by researchers with recorded after getting permission from the interviewers. Before starting the interview, informants were asked to read the informed consent and sign if they agreed with the condition of the survey. Discussions were audio-recorded and summarized to capture the main points discussed. The data was then manually analyzed to identify recurring themes and patterns, focusing on the specific needs and challenges discussed by the participants. Authors used the Microsoftt Excel to analyze and identify the themes and patterns. This description maintains the accuracy of your process while still providing a clear overview of how the focus group discussions were conducted and analyzed.

Data analysis involves three key concepts: data reduction, data display, and conclusion drawing and verification. Data reduction refers to the process of organizing and meaningfully reducing or reconfiguring the mass of data. Data display involves transforming the reduced data into a format that can be more easily understood and analyzed. Conclusion drawing and verification involves interpreting the data and drawing conclusions based on the analysis. For this study, the interview manuscripts were entered into a Microsoft Excel file. The data was displayed to retrieve the main theme. After that, the data was concluded by all authors.

To ensure the reliability and veracity of the information presented in this study, each statement or data point derived from external sources is explicitly cited. All references come from up-to-date, peer-reviewed journals, which supports the academic integrity of our findings and discussions. This rigorous approach to sourcing underscores our commitment to providing a research output that is not only informative but also dependable and reflective of the latest studies in the field. The instruments, tools, and procedures of this study have been approved by

the Faculty of Public Health, Diponegoro University with number 633/UN7.F9.1/DL/VIII/2023.

3. RESULTS AND DISCUSSION

The informants in this study consisted of nine persons from several backgrounds, education levels, and cohorts. In Table 1 below, the youngest informant was 35 years old and the oldest was 48 years old. According to their sex, the majority of them were female. Based on the educational level, five of the nine informants had bachelor's degrees.

Table 1. The characteristics of the informants

Characteristics	Age	Sex	Education level
Adolescent's parent 1	35	F	Master
Adolescent's parent 2	40	F	Doctoral
Counselling teacher 1	48	F	Bachelor
Counselling teacher 2	46	F	Bachelor
Counselling teacher 3	34	M	Bachelor
Counselling teacher 4	35	F	Bachelor
Counselling teacher 5	45	M	Master
Healthcare staff 1	40	F	Bachelor
Healthcare staff 2	38	F	Diploma

The findings from the in-depth interview revealed some themes related to the implementation of mental health counseling in senior high schools in Jakarta.

Theme 1: Exposure to information about mental health

The information from informants found that some teachers mentioned that there is health counseling in the school but the program is not specifically for mental health issues and not only for female students.

"In schools in particular there is no one to explain, but as a whole, we do it classically. We are teachers who are counseling, and usually, their daughter's students are a bit hesitant with male teachers. Classically we always inform, heartbroken children. Cut the hand incision is already there. Recently, someone has wanted to commit suicide, then called and guided, especially those who just know the opposite sex. There was a child who went on leave because of mental health, but he was able to graduate. Only in this school can choose which teacher to confide in, according to their convenience" (Teacher, P1)

Another teacher mentioned some have not been exposed to information about adolescent mental health at all.

"There is no educational material yet, but there are so many who have problems in families such as broken homes. Disturbed because of his family problems" (Teacher, P3)

The perspective about the program from the parent's side was a bit different. Parents could recognize the kids had a problem by the nonverbal language such as gestures.

"Never, but from the style and gestures can be seen, for example coming home from school does not want to eat directly taking a shower, usually seen and then asked what is the problem. Usually, the problems from schoolwork, lesson pressure, and neighborhood friends are fine" (Parents, P8)

Theme 2: Methods of delivering information about mental health

Some counseling teachers stated that there have been presentations or workshops in schools about the mental health of female students.

"There have been with outsiders, seminars in schools on how those teachers can provide counseling. This August has just been done. Fortunately, counseling guidance has indeed been given training" (Teacher, P1)

"There is information given by primary health care staff about social teenagers. For example, some students rarely enter and are called by the teacher, it turns out that their children have dropped out" (Teacher, P3)

"There is the direct speech in Islamic schools about unhealthy relationships. Additionally, the speech and education were also delivered by Religion Affairs especially encouraging students from sexual activity". (Teacher, P2)

"Education Development forms cadres in schools, elementary schools, small doctors, junior high schools, peer counseling usually approaches per group. Usually explained by peers, taught by children if they don't want to eat, don't give a statement they should, teach more. But if you still can't, then counseling will be referred. Conducting an introduction period to the school environment, usually the school that invited us. This activity is only 3 days. Usually combined with the introduction of drugs" (Primary health care staff, P7)

According to the information above, the methods of delivering mental health information included speech, private counseling, and education.

Theme 3: Adequacy of information about mental health

In terms of the information about mental health, there is more information about how adequate the information was. It was mentioned by teachers that the information is important but the collaboration with parents is not sufficient yet.

"I think this is important in schools because so far most of the screening is only physical. You can also later enter the literacy program, in our school there are tadarus, literacy, literacy on Wednesday. Thursday homeroom coaching, maybe during literacy hours can be inserted for mental health such as video shows. Daughterhood activities can also be interspersed for mental health information" (Teacher, P5)

This statement indicates that although certain programs have been initiated, evaluation and monitoring are still in progress, and parents have also been invited to participate in the monitoring of children's mental health problems.

"There has been counseling, but it may still not be implemented well." (Teacher, P2)

This statement indicates that despite counseling on mental health, there are still shortcomings in the delivery of information or the approach used. This shows an expansion and improvement in mental health counseling in schools.

Theme 4: Effectiveness of media to inform about mental health

After being given examples of booklets, educational videos, and modules by researchers, the respondents said the following statements

"The media used is better like videos if for today, if it's just posters or seminars, children will be bored" (Teacher, P4)

"Now kids love it Tiktok. So maybe the delivery can be through Tiktok." (Teacher, P2)

"The form can be through counseling, but the media is still video" (Parents, P8)

This statement demonstrates an understanding of the importance of using engaging media such as video to convey mental health information to students more effectively.

Theme 5: Counsellor about mental health

In terms of the human resources to work on mental health programs in school, there are some issues with the quantity and quality of the counselors as mentioned by some informants below.

"We recommend teacher guidance counseling but assisted by other teachers because we are only 4 people for all classes" (Teacher, P1)

"There should be a special teacher, our school is honestly lacking, so previously there were counseling teachers because they were sickly so they liked to enter so that the counseling in this school was less running. After I entered, I was active again, the counseling teacher was only me for 3 batches. UKS is also less active because it is combined with 3 units" (Teacher, P3)

"And also, a year ago held a cadre of adolescent health, all every year there is but not yet running" (Teacher, P2)

"Want to realize the formation of adolescent health cadres, in private schools there is no one. Then want to have a daughterhood program for this vocational school"(Teacher, P3)

It was not only mentioned about the counseling teacher but also the cadre. However, there was a lack of information reported about the quality of the counseling teacher and cadre. Cadres can be the students as well as facilitators for their peers.

DISCUSSION

The results of our study shed light on the level of exposure to information about the mental health of adolescent girls in high school. The study aimed to investigate whether educational institutions and parents are adequately addressing the mental health needs of adolescent girls. Based on the responses of teachers and parents, it is evident that there is a mixed level of exposure to mental health information. Some teachers acknowledged that they engage in health counseling, particularly for students dealing with emotional issues, such as heartbreak or suicidal thoughts. However, they also mentioned that these efforts are not specifically tailored to adolescent girls. This finding indicates that while some support is offered, it may not be comprehensive or gender-specific.

On the other hand, some teachers have not been exposed to information about adolescent mental health at all. This suggests a potential gap in teacher training and awareness concerning

the mental health needs of their students. Moreover, parents mentioned that they often rely on subtle cues, such as changes in behavior, to detect mental health issues in their daughters. This indirect approach may not always address the root causes of these problems. The results indicate that there is room for improvement in addressing the mental health needs of adolescent girls in high schools. The lack of specific mental health education and training for teachers may hinder their ability to provide adequate support. Additionally, parents' reliance on indirect cues may delay the identification and intervention of mental health issues. It is crucial to recognize that addressing mental health issues in adolescents requires a comprehensive approach that includes education, awareness, and open communication. In terms of why these findings are significant, they underscore the importance of implementing targeted mental health programs in high schools that focus on the unique challenges faced by adolescent girls. Such programs can equip teachers with the knowledge and tools to address these issues effectively. Moreover, parents should be encouraged to engage in more direct and open conversations with their daughters about their mental well-being.

The findings from our study related to the theme of "Effective Informers about the mental health of adolescent girls in high school" hold substantial significance for improving mental health support. The study aimed to identify individuals or groups who play a crucial role in delivering mental health information to adolescent girls. The responses indicate that teachers, parents, and even emerging influencers like TikTok have a pivotal role in this regard. Understanding the importance of these informers is vital, as it underscores the diverse sources from which students can receive valuable mental health information. Recognizing the potential influence of TikTok and other social media platforms highlights the need to adapt to changing adolescent communication trends and preferences. Ultimately, this finding emphasizes the importance of collaboration among these informers to create a comprehensive and effective approach to addressing the mental health needs of adolescent girls in high school.

Mental health counseling in schools is a supporting component for students's well-being. School-based counseling is a common practice in many countries, with England offering it in 6070% of schools, although it is more prevalent in secondary schools compared to primary schools (Finning et al., 2022). School counselors are essential professionals who assist students in their well-being, including social-emotional health, academic progress, and career planning (Xuan Vu, 2023). The role of school counselors is multifunctional and can significantly support children's mental health within school settings (Harrison, 2022). Mental health services in schools are evolving, creating new roles and practice boundaries for counselors, which may require additional competencies across counseling specialties (Mellin, 2009). While many schools have mental health programs, there is a need for more studies demonstrating the effectiveness of psychological counseling, a critical component of school-based mental health programs (Park et al., 2019). Efforts to enhance mental health services in schools include data-based decision-making, school-wide advisory, and computerized identification systems to deliver timely and effective mental health interventions to students (Gruman et al., 2013). The effectiveness of school-based mental health programs in improving adolescents' mental health underscores the importance of having counselors and psychiatrists available to provide interventions for students with mental health issues (Shahraki-Sanavi et al., 2020). Mental health helps identify students in need and connects them with appropriate services, contributing to positive educational outcomes (Weist et al., 2007). Moreover, schools can play a vital role in maintaining students' mental health through guidance and counseling services provided by trained professionals (Wang et al., 2019).

Teachers often being the first point of contact for students. It was found significant to empower teachers through training programs to effectively identify, guide, and refer students facing behavioral and mental health challenges (Afshari et al., 2022). Teachers are not only

responsible for educating students with mental health concerns but also for responding to their needs and promoting positive mental health for all students (Brann et al., 2021). Furthermore, teachers can act as primary providers of basic mental health services in schools, considering the substantial time students spend in educational settings (Pratiwi et al., 2022). Equipping teachers with mental health literacy training and resources to integrate mental health education into the curriculum can enhance both teachers' and students' knowledge and attitudes toward mental health (Gunawardena et al., 2024). Additionally, teachers' counseling competencies can positively influence students' self-esteem and mental well-being (Tisuela & Callo, 2022).

Existing studies highlight the significance of parental involvement in fostering their child's mental well-being through participation in school-based mental health services. Effective communication between school personnel and parents, including home visits and outreach programs, can enhance parents' understanding of their child's mental health needs and facilitate appropriate support (Nadeem et al., 2016). Moreover, parents and school counselors can collaborate to monitor for signs of mental illness in adolescents and ensure that students receive the necessary support (Bignold & Anderson, 2023). Parental involvement is critical for students' mental health, emphasizing the importance of parents in creating a supportive environment for their children (Wang et al., 2019) School counselors now play integral roles in counseling students and parents, providing consultations to both parents and teachers (Nishio et al., 2020). Parents and carers often take on significant responsibilities in managing adolescents' mental health and well-being, highlighting the essential role they play in supporting their children long (Longhurst et al., 2021). Furthermore, parents' perceptions of school-based counseling and their willingness to follow up on referrals can significantly impact students' access to mental health services (Hodges et al., 2021).

The findings, which reveal levels of exposure and methods of delivering mental health information among adolescent girls, show similarities and differences with previous studies. It underscores the importance of information delivery strategies tailored to the specific needs of adolescent girls and emphasizes the need for a more standardized and holistic approach to mental health education in schools. Collaborative efforts between teachers, staff, and parents can be instrumental in providing counseling to both parents and students to address various challenges experienced by students (Hidayah, 2021). By involving parents in mental health education initiatives and counseling programs, schools can create an environment where students feel comfortable seeking help when needed (Zakaria, 2024). In conclusion, parents play a vital role in mental health counseling in schools by actively engaging in their child's mental healthcare, collaborating with school counselors, and supporting mental health education initiatives.

4. CONCLUSION

In conclusion, this study has provided valuable insights into several key themes related to the mental health of female students in high school. In terms of collaboration between parents and teachers, the recommendation targeted comprehensive mental health education in schools and better communication between parents and their daughters regarding mental well-being. To apply this recommendation, schools can implement structured mental health programs integrated into the curriculum, offering workshops or seminars on stress management, emotional regulation, and mental health literacy. These programs should encourage parental involvement through regular meetings, open discussions, and workshops designed to foster communication about mental health issues at home. Furthermore, the role of primary health care is crucial in this collaboration by providing teachers and school counselors with specialized training on identifying early signs of mental health problems and equipping them with appropriate intervention techniques. Routine evaluations of students' mental health through

school-based screenings can be conducted with the support of health professionals to ensure timely intervention and appropriate referrals when needed.

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Determinants of Nurse Compliance in Fall Risk Screening: A Study At X Hospital Pekanbaru

Oktarisa Khairiyah[1a*], Zahroh Shaluhiyah[1b], Cahya Tri Purnami[1c]

[1] Master of Public Health, Faculty of Public Health, Diponegoro University, Semarang, Central Java, Indonesia

[a] Email address: oktarisakhairiyah@yahoo.co.id
[b] Email address: shaluhiyah.zahroh@gmail.com
[c] Email address: cahyatp68@gmail.com

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Abstract

Patient safety is a system that aims to make health services safer, preventing injuries due to errors in carrying out actions or not taking actions that should be taken, by using risk screening for patients. This research was conducted to see the effect of nurses' compliance in completing the complete patient fall risk screening at Hospital X Pekanbaru. This research was a descriptive analytical study with a cross-sectional approach carried out at Hospital X Pekanbaru, using a sample of 133 nurses at Hospital X Pekanbaru. Data was collected using questionnaires and interviews regarding nurses' compliance in filling out the patient fall screening sheet using a Likert scale questionnaire. Several variables were included in the analysis, including age, gender, highest level of education, length of service, work shifts, workload, rewards, knowledge, supervision, attitudes and compliance with the implementation of fall risk screening. All variables are converted into categorical data (nominal or ordinal). The analysis in this study was univariate analysis (frequency and percentage) and bivariate analysis was carried out using the Chi-Square Test statistical method with a p value <0.05. The results showed that length of service had an effect on nurses' compliance in completing the fall risk screening (p = 0.049). However, there is a tendency that those aged <31 years are more likely to be non-compliant, while those aged >31 years are more likely to be compliant in completing the screening requirements. Meanwhile, it was also found that nurses with a working period of <6 years were more likely to be non-compliant, while those with a working period of >6 years were more likely to be compliant in filling out the screening requirements, so it was found that compliance with completing the patient fall risk screening by nurses would increase by 2. 1 time.

Keywords: Risk Factors, Falls, Patient Safety, Compliance.

Corresponding Author:

Oktarisa Khairiyah
Master of Public Health, Faculty of Public Health, Diponegoro University, Semarang, Central Java, Indonesia
Email: oktarisakhairiyah@yahoo.co.id

1. INTRODUCTION

Patient safety is a system that aims to make health services safer, preventing injuries due to errors in carrying out actions or not taking actions that should be taken (Mulyana, 2013). Patient safety standards set by the Joint Commission International (JCI), include: patients identification accuracy, increased effective communication, increased precarious drug safety, intra-operative safety (appropriate location, procedure, and patient), reduced risk of healthcare-related infections, and reduced risk of patient falls (Niken, Kholid, & Wahyuningsih, 2021). The incidence of patients falling in hospitals is the highest incidence with 10 cases at RSU RS X Pekanbaru Padang with 54.54% in elderly patients over 58 years of age (Nurhayati, Rahmadiyanti, and Hapsari 2020).

Patient falls are one of the most troubling incidents and have a negative impact on hospitalized patients. Falls can result in injury, even death (Aziz & Safina, 2016). Patient falls can result in various types of injuries, both physical and psychological damage. The physical impacts include broken bones and soft tissue damage, meanwhile the psychological ones include anxiety, loss of self-confidence, and withdrawal (Sukesi, Kholid, & Wahyuningsih 2021). Patient fall risk screening performed by nurses is one of the recommended efforts. It is also an indicator of JCI in preventing patient falls (Chehab, Salim, and Kathamuthu, 2020).

The research found that there were several incidents at hospitals that were accredited by the Joint Commission International (JCI). A total of 52 incidents were found in 11 hospitals in 5 countries. The highest cases were in Hong Kong with a total of 31% of cases, followed by Australia (25%), India (23%), America (12%), and Canada (10%). The latest data in Indonesia from the Ministry of Health (2021) shows that there were 4,397 cases consisting of 1,508 near-injury incidents (KNC), 1,373 non-injury incidents (KTC), and 1,516 unexpected incidents (KTD). Various side effects were also reported as a result of the incident, including 91 deaths, 36 serious injuries, 296 moderate injuries, 677 minor injuries, and 3,296 no injuries (Nurhayati et al. 2020).

Hospitals should instruct risk of fall screening in an effort to prevent falls. Nurses were asked to complete the screening form when the patient first presented to the hospital and if there was a condition change by the nurse. One of the obstacles in completing the form was that the nurses' workloads were always increasing, which can affect the hospitals' value and quality. Nurses' workloads in hospitals consisted of physical and mental workload. The physical workload includes lifting and bathing patients, helping patients to the bathroom, giving medicine, et cetera (Harsul, Irwan, & Sjattar, 2020).

Mental workload can result from work shifts, work complexity, patient conditions, mental preparation of patients and their families in patients who underwent surgery or were critical, responsibility for patients' recovery, and establishing communication with patients and their families. A nurse will have difficulty aiding many patients, especially if there was an imbalance between the number of nurses and patients. Nurses must be able to provide services to all patients without exception, even though the number of nurses and patients is not equal (Harsul, Irwan & Sjattar, 2018).

The main problem with patient falls stems from the suboptimal implementation of standard operating procedures (SOP) for patient falls, particularly nurses' poor compliance in filling out the patient fall risk screening form (Wardanengsih et al. 2023). There were several types of screening used, such as Humpty-Dumpty for children, Morse Fall Scale for adults, and Ontario Modified Stratify-Sydney Scroing for geriatric patients (Rahmawati et al. 2021). Patients' fall risk screening must be carried out in a form of anticipation, therefore nurses' adherence in filling out the screening form as completely and clear as possible must be performed to establish patient safety (Daryani, Hamranani & Wijaya, 2023).

A preliminary study conducted at X hospital Pekanbaru revealed a problem in fulfilling patients' fall risk screening form, either at baseline or follow-up where only few nurses fulfill the form diligently. This has an impact on interventions for patients at risk of falling. The results of interviews conducted with several nurses showed lack of understanding, problems in assessing risks, and lack of understanding of the SOPs created by the hospital. All of these were obstacles in filling out the fall risk screening sheet.

The researchers were interested in analyzing the factors associated with nurses' compliance in completing the complete patient fall risk screening at Hospital X Pekanbaru.

2. RESEARCH METHOD

This was analytical descriptive research with a cross-sectional approach at X hospital Pekanbaru. Samples included in this research were 133 nurses, who were employees of X hospital Pekanbaru who filled the patients' risk of fall screening and willing to be respondents. This research has received permission from X hospital Pekanbaru. This research was conducted from July to October 2023, from when the researcher requested research permission until analyzing data.

Questionnaires in a Likert scale and interviews regarding nurses' compliance in filling out the patients fall screening sheet were used to collect data. Variables included in the analysis include age, gender, highest level of education, length of service, work shifts, workload, rewards, knowledge, supervision, attitudes and compliance with the implementation of fall risk screening. All variables were converted into categorical data (nominal or ordinal).

Univariate analysis was performed through tables (frequencies and percentages) on the distribution and percentages of the variables workload, rewards, knowledge, supervision and attitudes. Bivariate analysis was carried out using the Chi-Square Test statistical method. Multivariate analysis using logistic regression was conducted on variables with a statistically significant relationship with a p-value <0.05. Multivariate analysis was performed to discover which independent variables had the greatest influence on the dependent variable.

3. RESULTS AND DISCUSSION

The respondents in this study were nurses at the Syafira Pekanbaru Hospital, where the number of respondents was 133 respondents. The characteristics of the respondents were depicted in Table 1.

Table 1. Study respondents' characteristics

Characteristics	Number	
	f	%
Gender		
Male	23	17.3
Female	110	82.7
Age		
20 – 30-year-old	65	48.9
31 – 40-year-old	61	45.9
41 – 50-year-old	6	4.5
>50-year-old	1	0.8
Length of employment		
1 – 5 years	64	48.1
6 – 10 years	37	27.8
11 – 15 years	28	21.1
16 – 20 years	3	2.3
>20 years	1	0.8

Education		
Associate degree in nursing	72	54.1
Ners	44	33.1
Graduate degree in nursing	17	12.8
Work shift		
Night shift	28	21.1
Morning shift	61	45.9
Afternoon shift	44	33.1

The table above showed the respondents' characteristics in several variables, such as gender, age, length of service, education, and work shifts. The majority of respondents were female (82.7%) with an age range of 20 – 30-year-old (48.9%). Moreover, most respondents had worked for 1 – 5 years (48.1%) and had a final education of D3 in nursing (54.1%). Based on work shifts, most study respondents worked in the morning shift (45.9%).

Table 2. The distribution of respondents' responses on workload, reward, knowledge, supervision, attitude, and compliance

Characteristics	**Number**	
	f	**%**
Workload		
Low	63	47.4
High	70	52.6
Reward		
Poor	45	33.8
Good	88	66.2
Knowledge		
Poor	64	48.1
Good	69	51.9
Supervision		
Poor	35	26.3
Good	98	73.7
Attitude		
Poor	53	39.8
Good	80	60.2
Compliance		
Poor	60	45.1
Good	73	54.9

Table 2 revealed that in general, respondents in this study indicated a high workload (52.6%), good rewards (66.2%), good knowledge (51.9%), good supervision (73 .7%), good attitude (60.2%) and good compliance (54.9%). The normality test using the Kolmogorov Smirnov showed a p-value <0.01, therefore the conclusion was the data was not normally distributed. Due to this, Chi-Square was used in bivariate analysis.

Table 3 demonstrated that length of service was associated with nurses' compliance in filling out fall risk screening (p = 0.049). However, there is a tendency that those aged <31 years were more likely to be non-compliant, while those aged >31 were more compliant in filling the screening forms. The percentage of male nurses who did not comply was higher compared to female, while the percentage of female nurses who adhere was higher. Those with

associate degree were commonly had poor compliance, while those with graduate degree/Nursing education had good adherence. Nurses with who worker <6 years were generally did not comply, however those with >6 years of work length were more likely to be compliant in filling out the screening requirements.

Table 3. The association between all variables to compliance of complete fulfilling fall patients screening

Characteristics	Nurse compliance						p-value
	Poor compliance		Good compliance		Total		
	n	%	n	%	n	%	
Age							
<31	34	51.5	32	48.5	66	100.0	0.141
≥31	26	38.8	41	61.2	67	100.0	
Gender							
Male	12	52.2	11	47.8	23	100.0	0.454
Female	48	43.6	62	56.4	110	100.0	
Education							
Associate degree in nursing	34	47.2	38	52.8	72	100.0	0.595
Graduate degree in Nursing/Ners	26	42.6	35	57.4	61	100.0	
Length of employment							
Recent (<6)	39	52.7	35	47.3	74	100.0	0.049
Long (≥6)	21	35.6	38	64.4	59	100.0	
Work shift							
Night shift	16	44.4	20	55.6	36	100.0	0.925
Morning – Afternoon shift	44	45.4	53	54.6	97	100.0	
Workload							
Low	23	51.1	40	48.9	63	100.0	0.059
High	37	42.0	33	58.0	70	100.0	
Reward							
Poor	23	51.1	22	48.9	45	100.0	0.320
Good	37	42.0	51	58.0	88	100.0	
Knowledge							
Poor	25	39.1	39	60.9	64	100.0	0.177
Good	35	50.7	34	49.3	60	100.0	
Supervision							
Poor	18	51.4	17	48.6	35	100.0	0.382
Good	42	42.9	56	57.1	98	100.0	
Attitude							
Poor	26	49.1	27	50.9	53	100.0	0.457
Good	34	42.5	46	57.5	80	100.0	

Nurses with higher workloads mostly had poor compliance compared to nurses with fewer workloads. Nurses with less appreciation generally did not adhere, while nurses with more rewards were likely to comply. Nurses with good knowledge were more likely to not adhere compared to those with lesser knowledge on assessing fall risks screening. Nurses that

were supervised mostly comply, and vice versa. A high percentage of non-compliance was found in nurses with poor attitudes compared to those with good ones.

Table 4. The association between length of work and workload with compliance to completeness of fulfilling fall patients screening by nurse

Independent variables	Significance	Exp (B)	Likelihood	Hosmer and Lemeshow Test (Sig)	Nagelkerke R Square
Work length	0.041	2.104	175.257	0.646	0.077
Workload	0.049	0.491			

The table above revealed the results of the multivariate test on the work period and workload variables simultaneously associated with compliance of completing fall risk screening by nurses by 7%. If the length of service variable was increased, adherence with filling in patient fall risk screening exhaustively by nurses will increase by 2.1 times.

This research showed that length of service had a significant effect on nurses' compliance in filling out patient fall risk screening sheets in hospitals. Age, gender, highest level of education, work shifts, workload, rewards, knowledge, supervision, and attitudes did not have a significant effect. These results were similar with several studies revealing a significant relationship between work experience and compliance with patient safety implementation (Putrina, 2019), (Priambodo, Rosa, & Sundari 2020), (Tunny & Tauran 2023), (Nur, Dharmana, & Santoso, 2017). Nurses with longer work period tend to have good work experience and more compliant in performing their duties (Lestari 2022).

Work period or length of work is an individual's experience that will determine their position increase. The longer a person worked, the higher his/her level of achievement where high achievement originated from good behavior. Someone with lengthier work period had broader insight and more experience in their role in shaping the health workers behavior (Zulkifli & Sureskiarti 2020).

The longer period a nurse spent performing their work, the better their work quality. This was related to adherence in fulfilling patient's fall risk screening. This research also proved that the long hours a nurse worked did not guarantee their compliance with the consistency of her work, for example completing patient fall risk screening. Nurses with more than 10 years of service did not always able to adhere with filling patient's fall risk screening (Pagala, Shaluhiyah & Widjasena, 2017).

According to the theory put forward by Notoatmodjo, the aspect of knowledge, which is very important in shaping human behavior, dominates attitudes of thinking and influences things that influence change. Therefore, having sufficient knowledge leads to compliant behavior towards standard operating procedures. This means less risk of injury from falls. This research has similarities with Jayanti's research which concluded that only a small percentage of nurses' knowledge about the risk of falls was rated as poor and the others were rated as good (Nurhayati et al. 2020).

Our research revealed that the older the nurse, the more obedient they were in nursing care, therefore the tendency of older nurses to had better compliance was acceptable (Manurung et al., 2023). However, Pagala concluded that there was no correlation between age and nurses' compliance behavior in implementing the SOP for the risk of falls in hospital patients (Faridha & Milkhatun 2020). Adherence in completing the fall risk screening depend on dedication and devotion in serving patients, because it was possible that younger nurses were more obedient than older nurses or vice versa (Seguh, Kolibu, & Kawatu, 2019).

This research exhibited no correlation between gender and compliance, even though the nurses who complied in this study were women (Manurung et al. 2023). Psychology theory stated that women were more obedient than men. This research also showed no relationship

between education and compliance with completing patient fall risk screening by nurses, even though nurses who were more compliant had a bachelor's degree in nursing and ners (Amahoru, Said, & Kadar 2022). Formal education alone was not enough for a nurse. They must also take part in training that supported their work (Septiantoro, Triana, & Haniyah 2022).

This research depicted that work shifts were not related to nurses' compliance in completing patient fall risk screening. The different work shifts for nurses, which were morning, afternoon, and night shifts, felt different for each nurse to perform their work. The difference in nurse performance in terms of work shifts meant that on average, nurses with morning and afternoon shifts had higher performance compared to night shift. Nurses with morning shift had enough time to rest in the evening, whereas during the night shift the nurses' physical condition were tired and work concentration was not optimal (Herlinda, 2022).

There was no significant relationship between workload and nurse compliance, although respondents who were non-compliant generally have higher workload. Excessive workloads caused various disadvantages, such as increased cases of patient falls (Nurhayati et al., 2020). Higher workloads also resulted in nurses rushing to do their work, thereby increasing errors due to reduced accuracy. Environmental factors, room quietness, and management also played a role for nurses in implementation of the six correct principles, one of which was the risk of patient falls (Lillah, 2022).

In this study, reward for nurses was not related to compliance in completing patient fall risk screening, although the tendency for those with better appreciation had higher adherence (Astuti et al., 2021). The implementation of a nurse's work should increase through giving awards which will have an impact on a nurse's compliance (Aeni, Virgiani, & Mulyana 2022). Moreover, in this study, knowledge was not related to nurses' adherence in completing the patient fall risk screening at X hospital Pekanbaru.

Knowledge is very crucial in forming a person's actions. Activities that were not based on good knowledge would not have good results. Factors affecting someone's level of adherence include compliance when someone only obeyed the rules due to sanctions, indetification when people embraced the rules only because of scared of damaging good relationships with other people, and internalization when someone felt that the rules must be obeyed and were appropriate with his value.

This research exhibited that good supervision was in line with increased compliance, however this relationship was not statistically significant. Supervision was one of the factors that affect compliance (Nurhayati et al. 2020). Optimal supervision gave optimal impacts such as optimizing prevention of the risk of patient falls. Salma, et al stated that supervision had no effect on nurse adherence. This showed that supervision did not cause someone to be compliant in completing the patient fall risk screening (Faridah, & Winarni, 2022).

The relationship between nurses' attitudes and compliance in completing the patient fall risk screening at X hospital Pekanbaru was not significant. Attitude was a behavior determinant due to its relationship with perception, personality, and motivation. A positive attitude was very important to support the implementation of patient safety in hospitals . Attitude was defined as a reaction or response that existed within a person who was still closed to a stimulus or object. The differences in nurses' attitudes were due to their behavior depended on their knowledge. Attitude was a component of cognition, affection, and conation which interact with each other resulting in understanding, feelings, and behavior towards an object (Faridah, & Winarni, 2022)

The research we conducted had several limitations. First, this study used a cross-sectional research design. Due to this, the researchers were unable to control external factors that can influence the results and interpretation of data. Moreover, the research instruments used can be further developed for further research so that each variable can be explored in more depth.

4. CONCLUSION

The work period had a significant influence on nurses' compliance with the completeness of patient fall risk screening. Age, gender, education, work shifts, workload, rewards, knowledge, supervision, and attitude did not have a statistically significant correlation with nurses' compliance in completing patient fall risk screening. The results showed that length of service had an effect on nurses' compliance in completing the fall risk screening ($p = 0.049$). However, there is a tendency that those aged <31 years are more likely to be non-compliant, while those aged >31 years are more likely to be compliant in completing the screening requirements. Meanwhile, it was also found that nurses with a working period of <6 years were more likely to be non-compliant, while those with a working period of >6 years were more likely to be compliant in filling out the screening requirements, so it was found that compliance with completing the patient fall risk screening by nurses would increase by 2. 1 time.

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RESEARCH **Open Access**

The Effectiveness of Learning Media in Waste Management

Lidia Br Tarigan[1a*], Yuanita C.L.Rogaleli[1b], Olga Mariana Dukabain[1c]

[1] Sanitation Study Program, Poltekkes Kemenkes Kupang, Kupang, East Nusa Tenggara, Indonesia

[a] Email address: lidia.tarigan@gmail.com
[b] Email address: yuanita.rogaleli@gmail.com
[c] Email address: olgadukabain2018@gmail.com

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Abstract
The involvement of the community affects the effectiveness of waste management. By offering suitable media under local conditions, community participation can be increased. This research aims to determine how well educational materials can raise awareness of waste management issues and encourage community involvement. The research method used quasi-experimental. The efficacy of learning media, research variables, respondent knowledge, and waste management follow-up. Representatives from 46 different community groups made up the study subjects. Surveys, pre-and post-tests, and questionnaires were employed to collect data. A paired t-test was utilized to evaluate the data after it had been processed descriptively. The knowledge of the respondents increased by 24%. The results show a significant difference in knowledge between before and after using learning media, according to the results of the paired t-test (p = 0.000). The learning media effectiveness test results showed no difference between book media and leaflet media in increasing respondents' knowledge (p=0.130). Follow-up plans, forming a waste bank (33.3), and sharing the information obtained with other parties (73.8%). The conclusion is there is a significant difference in knowledge before and after using learning media (p = 0.000). There was no difference between book media and leaflet media in increasing respondents' knowledge (p=0.130). The public can utilize books and leaflets to increase their knowledge about waste management.

Keywords: Knowledge, Effectiveness of Learning Media.

Corresponding Author:

Lidia Br Tarigan
Sanitation Study Program, Poltekkes Kemenkes Kupang, Kupang, East Nusa Tenggara, Indonesia
Email: lidia.tarigan@gmail.com

1. INTRODUCTION

There are still issues with waste management in many places (Ardiansah and Oktapani 2023). Understanding that waste is useless and useless items are one factor (Swasono et al. 2020). This comprehension results in a lack of involvement in waste management (Junaidy et al. 2021). Humans and the environment are negatively impacted by unmanaged waste (Sari, Amrina, and Rahmah 2021). Unmanaged waste can become a breeding ground for disease vectors (Jamaluddin and Zarnila 2020), causing floods, affecting the global climate (Ain, Auvaria, and Nurmaningsih 2022). When waste is not managed, it is typically disposed of recklessly or into landfills. According to research findings, a single landfill has the potential to cause adverse health effects and increase global warming by 6,379,506.17 CO2 eq/year (Ula, Prasetya, and Haryanto 2021). The government has made written regulations containing sanctions for violations committed in waste management. (Augia, Akhila, and Fitriyani 2023), However, its implementation has not gone well (Yolanda and Septianda 2023).

Effective waste management (Ni'mattulah, Sjafari, and Riswanda 2022) can reduce the negative impact of waste (Shukor et al. 2011). Effective waste management is related to community involvement because waste comes from community activities (Rosida et al. 2023). Waste disposal, waste sorting, and waste utilization are examples of community involvement in waste management. Understanding that waste is more than just discarded material encompassing items that can be reused is closely associated with the procedure of sorting and using waste (Prayogi and Utama 2022)..

Community characteristics are important in managing waste because by knowing the characteristics of food they are able to identify what information they are willing to understand (Albarracin et al. 2022) and can determine effective methods (Tarigan, Rogaleli, and Waangsir 2020), so as to increase participation (Br Tarigan and Dukabain 2023; Khalil et al. 2022). Participation is related to community motivation (Hayana 2015; Herliani, Humaedi, and Adharai 2018) and learner characteristics in determining learning outcomes (Jennah 2019). Increasing participation can be conducted through various methods, one of which is by increasing understanding of waste management (Rosida et al. 2023). Increasing understanding can be performed through learning media literacy (Saputra, Kusnadi, & Nanna, 2022), where it helps the adoption of behavior (Austin et al. 2021). Increasing public understanding about waste management can be conducted by various learning media such as mass media, online media, books (Daniyati et al. 2023), leaflets and other media sharing (Sakur et al. 2022). Learning media that suits the characteristics of society (Kisworo 2017) will make it easier for the public to absorb the information received (Daniyati et al. 2023). The advantages of using media in the classroom encompass increased clarity, interest, and a positive attitude toward the subject matter and the learning process (Karo-Karo and Rohani 2018). The right learning media has a positive impact on understanding the information provided (Megawati 2013; Suseno, Albab, and Martadireja 2021). This research uses learning media that is prepared according to community conditions related to waste management so that it can increase participation in managing waste.

This research is distinct from previous studies in that the learning materials are created employing data collected from participants during waste management focus groups. Respondents consist of various elements in society who have a role in waste management in an area (Presiden Republik Indonesia, 2012) and serve as a motivator (Affandy, Isnaini, & Yulianti, 2015). These members include housewives, administrators of the Neighborhood Association namely the administrators of the Neighborhood Association (RT)/(RW), dasawisma administrators, Karang Taruna administrators (Darban Astane and Hajilo 2017), and waste management in the RT area. The identification results obtained information about the need for waste management materials. The waste management material is compiled in a book and leaflet for adults (Megawati 2013) which is called learning media in this research. The research aims

to determine the effectiveness of learning media in increasing knowledge and community participation in waste management so that waste problems can be overcome.

2. RESEARCH METHOD

This study employed a qualitative, quasi-experimental research methodology. Mixed methods are the method utilized. The participants in this study included housewives, waste managers in RT 41, administrators of youth organizations, dasawisma, administrators of the RT 41 Association, and other members of the community involved in waste management in Liliba Village. There were 45 individuals in the sample. With a test power of 80% and a significance of 95%, the sample size required to test the hypothesis of the mean difference between two paired groups was determined using the sample size formula (Roflin, & Liberty 2022). The criteria for respondents are being able to read and write and being active in managing waste in their group.

There are four stages to this study. Knowledge of waste management, waste management practices, and the media, which are anticipated to be a source of information on waste management, are all included in the first step of identifying community conditions. Focus groups were utilized to conduct discussions during this phase (FGD). FGDs were carried out on each community element group. The topic discussed was the condition of the community regarding waste management including knowledge, attitudes, waste management practices, and expected learning media. The FGD data was processed using thematic analysis. The results of data analysis become the basis for determining the learning media that is prepared and the content of the learning media, namely reference books, and leaflets. The second step involves creating educational materials, such as books and pamphlets, based on the findings of the focus group discussion. Testing educational materials in the form of books with the ISBN 978-623-5431-47-5 and pamphlets with the copyright 000528694 is the third step. Before this stage, a pre-test is performed to gauge the respondent's level of knowledge. Respondents were given one week to study the books and leaflets provided. After that, a post-test was carried out. The final stage is to identify the waste management follow-up plan that will be carried out by the respondent. After that, the respondents are provided with books and pamphlets to read and utilize for waste management. Then, a post-test was administered and the final stage was to identify follow-up waste management actions performed by respondents.

The data were analyzed qualitatively and tested using the Paired T Test via the SPSS 26 application. The research has proof of research ethics with no. No.LB.02.03/1/0079/2022 published by the Health Research Ethics Committee - Health Polytechnic Ministry of Health Kupang.

3. RESULTS AND DISCUSSION

Table 1 shows that learning media such as books and leaflets are used to raise public awareness about waste management. Respondents' knowledge increased by 24%. Knowledge before using learning media is an average of 54% and after using learning media an average of 79%. Test results with the Paired T-Test demonstrate that there is a significant difference in knowledge before and after using learning media ($p = 0.000$).

Table 1. Pairet test of respondents' knowledge.

Paired Samples Test									
		Paired Differences					T	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Knowledge before using the media - Knowledge after using media	-10.239	10.378	1.530	-13.321	-7.157	-6.692	45	.000

Table 2. Test of the effectiveness of learning media.

Paired Samples Test									
		Paired Differences					T	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Book-leaflet	.283	1.241	.183	-.086	.651	1.544	45	.130

Table 2 shows that a total of 45 respondents were given a learning media of books and leaflets for a week. Respondents stated that both learning media have similarities in increasing knowledge. There are only differences in the appearance of different media. The results of the learning media effectiveness test showed that there was no difference between book media and leaflet media in increasing respondents' knowledge (p=0.130).

Table 3. Description of learning media according to respondents.

Media description	Book		Leaflets	
	Amount	%	Amount	%
Attractive appearance	31	67	26	57
Complete contents	38	83	38	83
Increase knowledge	43	93	39	85
Reference source	42	91	40	87
Gives motivation	45	100	45	98

Table 3 shows that learning media provides motivation (100% books and 98% leaflets), and is a reference source in waste management (91% books and 87% leaflets). Both learning media also increased respondents' knowledge (books 93% and leaflets 85%). Respondents stated that both learning media have similarities in increasing knowledge. The content of the learning media was stated by respondents to be 83% complete, there were only differences in the appearance of the different media.

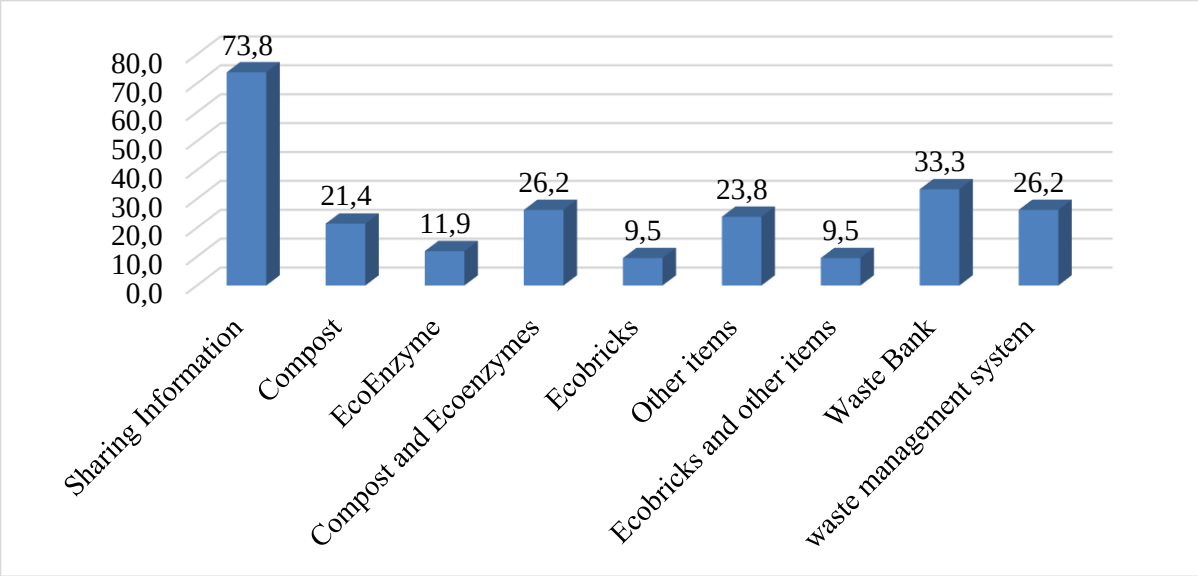

Figure 1. Waste management follow-up plan

Figure 1 shows that the follow-up plan for managing waste by respondents after employing learning media is shown in Figure 1. The highest follow-up was sharing information about waste management at 73.8%, followed by creating a waste bank at 33.3%.

Knowledge about waste management influences a person's attitude in managing waste. Knowledge and waste management are interrelated. In other words, more knowledge means better waste management (Antika, Jumakil, and Nurmaladewi 2023). Known information about waste can change perceptions and actions in managing waste (Magdalena, Tjahyadi, and C. 2023a; Windiari and Salsabiela 2022). There are various sources of information about waste management. One source of information that is easily accessible is books (Firmansyah and Humaidi 2022) and leaflets (Rosadi et al. 2021). The study involved distributing learning media, which are books and pamphlets, to participants, who then owned them. This will make the book exceptionally accessible and allow for repeated reading, which will help with comprehension of the waste management material. According to the study findings, after reading or using the supplied books and pamphlets, respondents' knowledge increased by 24%. This demonstrates how learning resources can expand knowledge (Khalil et al. 2022) community about waste management (Komara et al. 2023).

The results of the study demonstrate that the contributions of book and pamphlet media to the development of public knowledge are comparable. Thus, it has the same effect of increasing public awareness. This demonstrates how well the material is communicated, resulting in no apparent improvement in knowledge development. Statements from respondents describing the two media as equally fascinating and offering comprehensive information serve to support this. These findings also demonstrate that the community can use these two educational resources as informational resources and a source of reference for waste management. In this study, most of the respondents were women, where in managing household waste, women were the main actors (Akhmadi and Amaliyah 2022; Dorris et al. 2022) and creative in processing waste (Hafizah and Hidayat 2023).

Utilizing educational materials also encourages respondents to follow up. Increased community participation in waste management will result from follow-up. The results of the follow-up survey regarding waste management (73.4%) indicate that the information collected is valuable and should be extensively shared. This is an opportunity for more people to become aware of the waste management information discovered in learning media, which could eventually influence how individuals manage their waste. The community can establish a waste

bank because educational materials, particularly books, provide details on the advantages, goals, and procedures involved in accomplishing it. This can encourage respondents to have plans to form a waste bank and change their behavior in managing waste (Leman, Claramita, and Rahayu 2021). The book, which is a medium for information on waste management, also contains information about waste management that has been conducted in the Liliba sub-district, which is RT 41, where the good practices that have been performed can be employed as an example in performing waste management practices (Blaschke and Marin 2020).

Digital applications utilizing community-based research methods may also be employed to provide waste management information to increase community participation in waste management (Saputra et al. 2023). Media designed from the results of FGDs can help increase knowledge and behavior about waste management. This happens because its preparation involves the active participation of users in understanding problems and solutions from their point of view (Sekarningrum et al. 2021). The information presented is also relevant, so it can motivate users to change their behavior positively. Disseminating information through prepared media is one strategy to increase participation in waste management (Wijayanti et al. 2023). Short videos can also be utilized as information media to complement the information obtained through books and leaflets (Al Hanif, Mahdalena, and Handayani 2023). This research can be continued by monitoring changes in community behavior in processing waste (Chaengmongkol et al. 2021) and involving stakeholders (Utami, Nurwati, and Lestari 2024). The information received can change perceptions and attitudes that are more critical towards problems that arise and influence intentions to change behavior (Kasjono et al. 2023; Magdalena et al. 2023b). Efforts to increase education for the community to be more communicative and more flexible have become a planned program that can be achieved and implemented well (Susilawati et al. 2023)

4. CONCLUSION

There is a significant difference in knowledge before and after using learning media. There is no difference between book media and leaflet media in increasing respondents' knowledge. The resulting reference books and leaflets can be used as media to increase people's knowledge and ability to manage waste and overcome problems in waste management.

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