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Original Research Article

A Practice of Supportive Psychotherapy for Borderline Personality Disorder (A Clinical Module Development)

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Abstract

Background: Handling patients with Borderline Personality Disorder (BPD) who are hypersensitive to rejection, have unstable interpersonal relationships, self-image, affect, and behavior is a challenge for a psychiatrist. Supportive psychotherapy is one of the most widely mastered psychotherapy modalities in psychiatric education in Indonesia and is most widely practiced in psychiatric services. There is currently a lack of structured supportive psychotherapy clinical practice modules available for patients with Borderline Personality Disorder in Indonesia.

Objective: To develop and an Indonesian version of structured supportive psychotherapy clinical practice module and to test its clinical practice suitability for patients with Borderline Personality Disorder in Indonesia

Methods: This research was conducted in three stages. Stage 1 was preparing the supportive psychotherapy clinical practice module for 13 weeks. Stage 2 was for the module validation using face validity and content validity by two psychotherapy consultants. Stage 3 was the trials of the developed modules, where 2 interrater therapists applied the modules to treat 5 patients.

Result: The face validity of the two experts for the supportive psychotherapy clinical practice module for Borderline Personality Disorder in Indonesian language was 3.269, meaning that it mostly was done correctly. The results of the content validity of the two experts for the supportive psychotherapy module for Borderline Personality Disorder in Indonesian language was 81.165. The results of the two experts' face validity and content validity scores inferred that the supportive psychotherapy clinical practice module for Borderline Personality Disorder in Indonesian language was suitable for use in services.

Conclusion: The Indonesian version of supportive psychotherapy module for BPD patients has been developed and is suitable for clinical practice.

Keywords: *borderline personality disorder; supportive psychotherapy; psychotherapy*
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INTRODUCTION

Borderline Personality Disorder (BPD) is characterized by hypersensitivity to rejection[1] and resulting in the instability of interpersonal relationships, self-image, affect, and behavior.[2]

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Table 1. Face Validity of the Supportive Psychotherapy Clinical Practice Module in BPD

	Assessment Aspects	Expert 1	Expert 2
1	Professionalism		
	Rapport Building	4	3
	Interview Techniques	3.29	3.14
	Define and clarify the client's chief complaint/problem	3	4
	Therapy contract	4	3
2	Guidelines for the work of supportive psychotherapy in Borderline Personality Disorder	3	3
		3.47	3.067
	AVERAGE TOTAL	3.269	

Interpretation: 0 : data is not graded, 1 : asked a fraction done right, 2 : partially done right, 3 : mostly done right, 4 : almost all done right

Borderline Personality Disorder which causes significant annoyance and distress and is associated with a variety of medical and psychiatric co-morbidities.[3] The surveys estimate that the prevalence of Borderline Personality Disorder was 1.6% in the general population and 20% in the psychiatric and inpatient population.[4]

Psychotherapy is one of the non-pharmacotherapy modalities for psychiatric patients.[5] The results of a survey in America regarding psychiatric services stated that 36% of psychiatrists provided supportive psychotherapy to patients, 19% with insight-oriented psychotherapy, 6% with Cognitive Behavioral Therapy (CBT) and 1% with psychoanalysis.[6] The survey shows Supportive Psychotherapy was the most frequent treatment option given by psychiatrists to patients. This also occurs in psychiatric services in Indonesia, the survey results of the psychotherapy section of Indonesian Psychiatrist Association (*Perhimpunan Dokter Spesialis Kedokteran Jiwa Indonesia*-PDSKJI) stated that the psychiatrists who participated in the survey carried out types of psychotherapy: 154 supportive psychotherapy, 108 CBT, 6 family therapy, 49 marital therapy, 44 dynamic psychotherapy, 24 other psychotherapy and 3 did not do psychotherapy.[7]

Supportive psychotherapy is a basic competency that must be mastered by a psychiatrist and even Markowitz in his research stated that if a psychiatrist does not master supportive psychotherapy then other psychotherapy will not be useful.[8] Supportive psychotherapy is one of the most widely mastered psychotherapy modalities in psychiatric education in Indonesia and is most widely practiced in psychiatric services. There has been no supportive psychotherapy modules available yet in Indonesia.

Grover stated that supportive psychotherapy is flexible and suitable for patients with various diagnoses. Supportive psychotherapy as initial therapy is carried out by psychiatrists before changing forms to other, more complex psychotherapy.[9] Supportive psychotherapy is the heart of all types of psychotherapy. It is important to establish the doctor-patient relationship early in the therapy.[10]

Winston et al. defines supportive psychotherapy as "dyadic treatment that uses direct action to ameliorate symptoms, maintain, restore, or enhance self-esteem, ego functioning, and adaptive skills. Achieving these goals requires examining relationships, real or transferential, and examining past patterns. and currently, emotional or behavioral response."[11] Supportive psychotherapy in terms of technique is based on reflection rather than interpretation or direction.[12] Frequency of Supportive psychotherapy sessions can be conducted at a frequency of less than once a week and supportive psychotherapy can be used for patients who are deemed unsuitable for the requirements of other psychotherapy. [13]

Supportive psychotherapy is expected to help patients with Borderline Personality Disorder who are hypersensitive to rejection, have unstable interpersonal relationships, self-image, affect, and behavior. There has been no supportive psychotherapy clinical practice module for Borderline Personality Disorder patients in Indonesia. This study was aimed to develop of a supportive psychotherapy clinical practice module for Borderline Personality Disorder patients in Indonesia.

MATERIALS AND METHODS

This study had been approved by Ethical committee (Certificate No 976/EC/KEPK-RSDK/202). The study included three stages. Stage 1: Development of supportive psychotherapy skills modules. This study developed a supportive psychotherapy clinical practice module for Borderline Personality Disorder which consists of 8 chapters including Introduction, General Principles of Supportive Psychotherapy, Indications for Individually Supportive Psychotherapy, Therapeutic Contracts, Examination in supportive psychotherapy, Supportive psychotherapy techniques, Supportive psychotherapy techniques for patients with Borderline Personality Disorder and Evaluation of supportive psychotherapy competency.[14]

Table 2. Content Validity of the Supportive Psychotherapy Clinical Practice Module in BPD

Assessment Material	Rated aspect	Expert 1	Expert 2
Language	Follows the rules of Enhanced Spelling	85	80
Manual Outline	The manual is a therapist's guide in carrying out the application of supportive psychotherapy for Borderline Personality Disorder	85	75
Introduction	Contains background problems, competencies to be achieved, criteria for training participants, and learning objectives.	80	80
General Principles of Supportive Psychotherapy	Contains the notion of supportive psychotherapy, and the basic principles of supportive psychotherapy that must be understood.	80	85
Indications for Individual Supportive Psychotherapy	Contains an overview of indications in general and specific indications in specific points.	85	80
Therapeutic Contracts	It contains a brief explanation of the ongoing therapy process, the frequency of meetings, the duration of each meeting, the agreed costs, how to end the therapy contract, how to manage patient non-compliance with the contract, and the ultimate goal to be achieved when the therapy contract ends.	85	75
Examination in supportive psychotherapy.	Contains explanations and steps for collecting symptoms, examination of current problems, examination of therapy history, examination of dynamic psychopathology.	80	80
Basic Techniques of Supportive Psychotherapy	Contains techniques for building therapeutic alliances, building self-esteem, building adaptive behavior skills, supporting ego function.	80	85
Building Therapeutic Alliance	Contains ways of building through expressions of interest, expressions of empathy, expressions of understanding, supportive comments, expressing the reality felt by the therapist, repairing the therapeutic alliance when a rupture occurs in a therapeutic alliance	85	80
Building Self-Esteem	Contains ways to give praise, appeasement (reassurance), normalization, universalization, encouragement and encouragement.	80	85
Development of Adaptive Behavioral Skills	Contains how to give advice, teaching, anticipatory guidance.	80	80
Supporting Ego Function	Using Technique: 1. Reducing and preventing anxiety with conversational styles, sharing agendas, word pads, naming problems, normalizing, reframing, rationalizing. 2. Expanding awareness includes clarification, confrontation, interpretation.	80	80
Supportive psychotherapy techniques in certain conditions in patients with borderline personality	Using Technique: 1. Warning comments on self-injurious behavior. 2. Naming feelings to chronic feelings of emptiness. 3. Normalize on frantic attempts to avoid abandonment. 4. Anticipatory comments or guidance on patterns of unstable interpersonal relationships. 5. Reducing anxiety in affective instability. 6. Offers control over difficulty controlling feelings of anger. 7. Discuss with a cool head on impulsivity. 8. Reveals the psychotherapist's reality to patients with persistent identity disorder	80	80

Table 2. Cont.

Assessment Material	Rated aspect	Expert 1	Expert 2
Evaluation of supportive psychotherapy competency	Contains an assessment of knowledge and attitudes and skills.	85	75
Feasibility of work handbook of Supportive Psychotherapy in Borderline Personality Disorder	The feasibility of work manuals can be used in the education process for psychiatrists and in providing services to patients with Borderline Personality Disorder.	85	80
TOTAL		82.33	80
AVERAGE		81.165	

Interpretation: <25% is only partially done correctly, 25-49% partially done right, 50-74% mostly one right, 75-100% almost all done right

Table 3. Interpersonal Therapist Supportive: Results of 2 expert assessments of 2 Supportive Psychotherapists

Assessment Aspects	Assessment criteria	Expert 1	Expert 2	ICC
1. Supportive interview skills (History taking)	1) Built rapport 2) Inquire effectively and efficiently 3) Systematic interview 4) Get signs and symptoms	79.5	90	0,653 (p=0.165; CI95%:-2334-=,964)
Professionalism	1) Respect the patient 2) Show empathy and compassion 3) Creating trust; 4) Help make the patient comfortable 5) Pay attention to legal aspects 6) Realizing self limitations	84	92.5	
3. Ability to manage patients	1) Planning comprehensive therapy in the biological, psychological, and sociocultural domains. 2) Able to choose rational management according to the diagnosis of the disease. 3) Be able to explain the reasons for choosing pharmacological, social and economic therapy	81	87.5	
Ability to give supportive psychotherapy	1) Explain the reason/basic examination and Supportive psychotherapy to the patient 2) Ask for approval of medical action if necessary from the patient/family (informed consent) 3) Provide education about management, prevention and supportive psychotherapy associated with the disease 4) Be able to determine specific forms of supportive psychotherapy according to the patient's condition	82.5	90	
Organizing/Efficiency	1) Be able to select appropriate sources of information to elicit signs and symptoms 2) Able to determine priorities in conducting interviews 3) Able to adjust to a predetermined time 4) Able to optimize the time to formulate data in the form of a systematic formulation	78.75	90	
TOTAL	The value of the two therapists the difference didn't more than 10 points The ability of the two therapists in providing equivalent supportive psychotherapy	80.75	90	

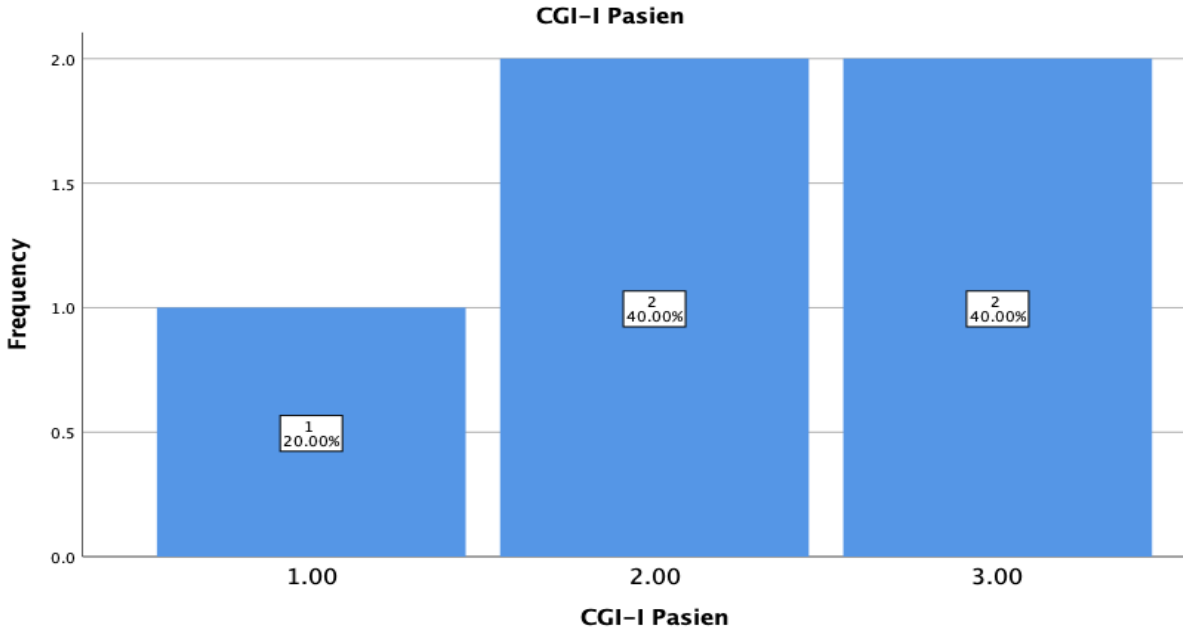

Figure 1. The frequency distribution of patients according to Their CGI-I Score.

Stage 2: Module Validation. In this stage, the module was validated with face validation and content validation by 2 psychotherapy consultants to suit the conditions in Indonesia. Face validity consists of the relevancy to the respondent and evaluates the appearance of the questionnaire in terms of feasibility, readability, consistency of style and formatting, and the clarity of the language used.[15] Content validity involves a formal assessment by subject matter experts, to determine the appropriateness of the content and identify misconceptions or omissions.[16] Content validity is defined as ensuring that the module is comprehensive and relevant and represents the objectives of the module. This can be done by consulting experts in the field of psychotherapy who can provide information, evidence, judgments, and assessments.[17] The content validity of the consultation results of two psychotherapy consultants includes language and an outline of the material provided each week and its eligibility.

Stage 3: Module Trials and Interrater 2 Therapist. The supportive psychotherapy clinical practice moduleon Borderline Personality Disorder in Indonesian language was applied by 2 supportive therapists. The two therapists performed supportive psychotherapy on five patients alternately. The two experts evaluated the supportive psychotherapy given by the two therapists to ensure that the two therapists had a good interrater in providing supportive psychotherapy treatment. There were five assessment points in the interaction between the two therapists, consisting of the ability of supportive interviews, professionalism, the ability to manage patients, the ability to provide supportive psychotherapy and the organization or efficiency of therapy.

RESULTS

Designing and Validation of supportive psychotherapy clinical practice module. The maximum score for the face validity in this study is 4, the results of the two experts face validity for the supportive module are 3.269, which means that most of them are done correctly (Table 1). The maximum score of Content Validity is 100, the result is 81.165 which means almost everything is done right. (Table 2).

Trials of Modules and Interrater 2 Therapists. The score of the two experts for Therapist 1 was 80.75 and for Therapist 2 was 90. This value indicated that the difference in the scores of the two therapists was no more than 10 so that they had equal abilities in providing supportive psychotherapy. The coefficient analysis between classes shows 0.653 which indicates a moderate level of relationship with the results of a weighted kappa interpretation of 100% (Table 3).

The results of the patient's Clinical Global Impression/CGI-Improvement analysis showed that the highest frequency of patients' CGI-I scores were scores of 2 (much better) and 3 (slightly improved), namely 2 people (40%) on each score. A total of 1 (20%) patient was found to have a patient CGI-I score of 1 (greatly improved since initiation of therapy). The mean value indicated that the patient's CGI-I was at a score of 2.2 ± 0.836 with a median of 2 with a minimum value of 1 and a maximum of 3. (Figure 1)

DISCUSSION

This study was aimed to validate the supportive psychotherapy clinical practice module to be used as a guide for psychotherapy for patients with borderline personality disorder (BPD). Testing was carried out on the contents of the module with three stages of testing. Face validity of the two experts for supportive psychotherapy clinical practice module for Borderline personality disorder settings in Indonesian was 3.269, meaning that most of it was done correctly. The result of the content validity of the supportive psychotherapy

clinical practice module for Indonesian Borderline Personality Disorder from the two experts was 81,165. From the results of the face validity and content validity by the two experts, it could be inferred that the supportive psychotherapy clinical practice module for Borderline Personality Disorder in Indonesian language was appropriate for use in psychotherapy services.

Patients with Borderline Personality Disorder often unstable due to periods of acute crisis, aggressive behavior, suicidal attempts and even substance abuse.[2] Providing supportive psychotherapy was considered appropriate for patients with Borderline Personality Disorder because patients who undergo supportive psychotherapy will receive assistance to improve ego function,[18] increase self-esteem and adaptability so that they can function more adaptively.[2]

The supportive psychotherapy clinical practice module explained the details of supportive psychotherapy techniques according to the patient's condition, namely warning comments on self-injury behavior, naming feelings in chronic empty feelings, normalizing panic attempts to avoid abandonment, anticipatory comments or guidance on unstable patterns of interpersonal relationships, reducing anxiety in instability affective, offering control on feelings of anger control difficulties, discussing with a cool head on impulsivity and revealing the reality of psychotherapy to patients with persistent identity disorder.[19] All of the above techniques were deemed appropriate by two experts to be administered to patients with Borderline Personality Disorder.

The therapist needed to gain a clear understanding of the patient's current problems, interpersonal relationship problems, daily functioning and psychological functioning therapeutically, to enhance the therapeutic alliance and encourage the patient to continue therapy.[20] This supported the patient to continue therapy such as contract therapy which supports the success of supportive psychotherapy.

This study had limitations with only small number of samples and the number of psychotherapists was limited to 2 persons.

CONCLUSION

This was a preliminary study on the development of the Indonesian version of supportive psychotherapy clinical practice module for patients with Borderline Personality Disorder. It discussed how to manage self harm behavior, empty feelings, avoid negligence, unstable interpersonal relationship, affective instability, controlling anger, impulsivity and persistent identity disturbance. The module demonstrated good face and content validity, as confirmed by two therapists practicing equivalent supportive psychotherapy. Patients reported feeling significantly improved following the supportive psychotherapy sessions. Thus the module is acceptable and could be applied to help therapist treat patient with Borderline Personality Disorder.

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REFERENCES

1. Chapman J, Jamil RT, Fleisher C. Borderline Personality Disorder. StatPearls Publishing; 2020. PMID: 28613633 Bookshelf ID: NBK430883
2. Zegarra-Valdivia JA, Chino Vilca BN. Social cognition and executive function in borderline personality disorder: evidence of altered cognitive processes. Salud mental. 2019 Mar 8;42(1):33–42. doi:10.17711/SM.0185-3325.2019.005
3. Ruocco AC, Lam J, McMain SF. Subjective cognitive complaints and functional disability in patients with borderline personality disorder and their nonaffected first-degree relatives. Can J Psychiatry. 2014 Jun;59(6):335–44. doi:10.1177/070674371405900607
4. Ten Have M, Verheul R, Kaasenbrood A, van Dorsselaer S, Tuithof M, Kleinjan M, et al. Prevalence rates of borderline personality disorder symptoms: a study based on the Netherlands Mental Health Survey and Incidence Study-2. BMC Psychiatry. 2016 Jul 19;16:249. doi:10.1186/s12888-016-0939-x
5. Elvira DS. Buku Ajar Psikiatri Edisi Kedua, Bab 30 Psikoterapi. Jakarta: Badan penerbit Fakultas Kedokteran Universitas Indonesia; 2013. 428-443 p.
6. Beatson J, Rao S. Psychotherapy for borderline personality disorder. Australasian Psychiatry. 2014 Dec 15;22(6):529–32. doi:10.1177/1039856214555531
7. Division of service and training section of psychotherapy PDSKJI. I. Psychotherapy survey: Challenges of Psychotherapy Services and Adjustment in the Era of New Habit Adaptation. PIT PDSKJI 2020. 2020.
8. Markowitz JC. What is Supportive Psychotherapy? Focus (Madison). 2014 Jan;12(3):285–9. doi: 10.1176/appi.focus.12.3.285
9. Grover S, Avasthi A, Jagiwala M. Clinical Practice Guidelines for Practice of Supportive Psychotherapy. Indian J Psychiatry. 2020 Jan;62(Suppl 2):S173–82. doi: 10.4103/psychiatry.IndianJPsychiatry_768_19
10. Gabbard GO. Psychodynamic psychiatry in clinical practice. Fifth Edition. American Psychiatric Publishing; 2015. 100–135 p.
11. Winston A, Rosenthal RN, Roberts LW. Learning Supportive Psychotherapy. Second Edition, American Psychiatric Association Publishing. 2020
12. Serbanescu I, Walter H, Schnell K, Kessler H, Weber B, Drost S, et al. Combining baseline characteristics to disentangle response differences to disorder-specific versus supportive psychotherapy in patients with persistent depressive disorder. Behaviour Research and Therapy. 2020 Jan;124:103512. doi:10.1016/j.brat.2019.103512

13. Hoeppner SS, Hall MD, Hiranandani M, Greenberg JL, Wilhelm S, Phillips KA. Time to Response in Therapy for Body Dysmorphic Disorder: A Comparison of Cognitive Behavioral Therapy and Supportive Psychotherapy, Behavior Therapy, https://doi.org/10.1016/j. beth.2023.05.006
14. Wardani ND, Widiastuti MI, Sudiyanto A, Lukman PR. Psikoterapi Sportif Pada Gangguan Kepribadian Ambang. UNDIP Press Semarang. 2022
15. Taherdoost H. Validity and Reliability of the Research Instrument; How to Test the Validation of a Questionnaire/Survey in a Research. SSRN Electron J. 2018;5(3):28–36. doi:10.2139/ssrn.3205040
16. Tanner K. Research Methods (Second Edition). Information, Systems, and Contexts. 2018;159–92 p.
17. Yusoff MSB. ABC of Content Validation and Content Validity Index Calculation. Education in Medicine Journal. 2019 Jun 28;11(2):49–54. doi:10.21315/eimj2019.11.2.6
18. da Silva Machado R, Vieira IS, Scaini C, Molina ML, Barbosa LP, da Silva GDG, et al. Ego-defense mechanisms and brief psychotherapies for the management of major depressive disorder in adults: A longitudinal and quasi-experimental study. J Affect Disord. 2023 Jun;330:291–9. doi:10.1016/j.jad.2023.02.141
19. Aviram RB, Hellerstein DJ, Gerson J, Stanley B. Adapting supportive psychotherapy for individuals with Borderline personality disorder who self-injure or attempt suicide. J Psychiatr Pract. 2004 May;10(3):145–55. doi: 10.1097/00131746-200405000-00002
20. Barbisan GK, de Pieri LZ, Gonçalves L, Rebouças CDV, da Rocha NS. Positive Association Between Therapeutic Alliance and Quality of Life in Psychodynamic Psychotherapy, Cognitive Behavior Therapy, and Interpersonal Therapy: The Patient's Perspective. Front Psychiatry. 2022 Jan 25;12. doi:10.3389/fpsyt.2021.613627

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Original Research Article

Neutrophil–Lymphocyte and Platelet–Lymphocyte Ratios are Predictors of Lung Malignancy

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Abstract

Background: Inflammatory cells play an essential role in the neoplastic process by stimulating cancer proliferation, survival, and migration. Neutrophil-lymphocyte and platelet-lymphocyte levels can be used as inflammatory tissue damage markers in cancer patients.

Objective: This study aimed to analyze the increase of neutrophil-lymphocyte ratio (NLR) and platelet–lymphocyte ratio (PLR) as the predictive factors for lung malignancy.

Methods: This study was a diagnostic test to compare NLR and PLR ratios in lung tumor patients at Dr. Moewardi Hospital Surakarta from August to October 2018. The subjects (60) were selected with consecutive sampling who took lung cancer diagnostic tests and divided into two groups of patients with lung tumors (30) and healthy (30) as control. The diagnostic procedures and neutrophil-lymphocyte and platelet–lymphocyte ratio calculation were performed on both groups. The optimum cutoff values for the NLR and PLR were calculated from the receiver operating curve analysis.

Results: The statistical test found a significant difference in the neutrophil–lymphocyte and platelet–lymphocyte ratios between cancer patients and control ($p = 0.0000$). There is a significant difference in the NLR and PLR between healthy and cancer patients. The NLR was calculated and it was 6.25 ± 2.88 in cancer patients and 1.84 ± 0.47 in healthy subjects. Meanwhile, the PLR in cancer patients was 254.93 ± 116.59 and 114.33 ± 27.67 in healthy subjects.

Conclusion: The ROC curve for NLR has an AUC value of 0.941 with a p-value of 0.000 ($P < 0.01$), and for PLR has an AUC value of 0.898 ($p = 0.000$). The increase of NLR and PLR can be used as the predictive factors of lung malignancy.

Keywords: *leukocyte; lymphocyte; lung tumor; malignancy; neutrophil; platelet*
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INTRODUCTION

Lung cancer is the leading cause of cancer morbidity and mortality among men worldwide and the second leading cause of cancer-related deaths among women.[1-5] Lung cancer is also the most common cancer for men in Indonesia, with the rate of incidence of 19.4 per 100.000 men and an average death rate of 10.9 per 100.000 men.[1-6] By contrast, around 200.000 subjects were diagnosed with lung cancer in 2010 in the United States, and nearly 160.000 subjects died of it. The patients diagnosed with lung cancer have an average age of 68–70 years.

The early discovery of lung malignancy is the most encouraging approach to improve the treatment results. Early detection of lung cancer using different serum biomarkers has been tested, but there is no single biomarker that can reliably detect lung cancer.

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Table 1. Characteristics of Study Subjects

Characteristics	Lung malignancy diagnosis		p-value
	Positive (n = 30)	Negative (n = 30)	
Age	54.13 ± 16.50	44.53 ± 7.68	0.005[1]
Gender			
Male	17 (56.7%)	17 (56.7%)	
Female	13 (43.3%)	13 (43.3%)	
Employment			0.005[2]
Not working	19 (63.3%)	28 (93.3%)	
Working	11 (36.7%)	2 (6.7%)	
Education			0.000[3]
Primary school	11 (36.7%)	0 (0.0%)	
Secondary school	8 (26.7%)	5 (16.7%)	
High school	9 (30.0%)	16 (53.3%)	
University	2 (6.7%)	9 (30.0%)	
Brinkman index			0.022[3]
Non-smoker	14 (46.7%)	22 (73.3%)	
Mild smoker	5 (16.7%)	4 (13.3%)	
Moderate smoker	8 (26.7%)	4 (13.3%)	
Heavy smoker	3 (10.0%)	0 (0.0%)	
BMI			0.000[3]
underweight	22 (73.3%)	1 (3.3%)	
normal	5 (16.7%)	8 (26.7%)	
overweight	3 (10.0%)	21 (70.0%)	
Clinical symptom			
cough	14 (46.7%)		
chest pain	7 (23.3%)		
dyspnea	9 (30.0%)		

Notes: BMI = body mass index; [1] *Independent t-test*, [2] Chi-Squared/Fischer's exact test, [3] Mann–Whitney U test

Table 2. The difference between NLR and PLR in patients with lung cancer and healthy subjects

Variable	Lung cancer Diagnosis		p-value
	Positive	Healthy	
Neutrophil	75.47 ± 8.30	56.41 ±5.74	0.000[2]
Lymphocyte	15.20 ± 8.01	31.82 ± 5.11	0.000[2]
Platelet	373.70 ± 109.90	313.40 ± 44.93	0.007[1]
Leukocyte	11693.33 ± 3692.20	9020.00 ± 1558.82	0.003[2]
NLR	6.25 ± 2.88	1.84 ± 0.47	0.000[2]
PLR	254.93 ± 116.59	114.33 ± 27.67	0.000[2]

Note: NLR = neutrophil–lymphocyte ratio; PLR = platelet–lymphocyte ratio; [1]Independent t-test; [2]Mann–Whitney test

Combining the tumor's systemic inflammatory factors could be the primary technique to diagnose lung cancer because inflammation is an essential component in cancer development. Cancer mostly develops at the site of infection, chronic irritation, and inflammation. The tumor microenvironment is largely regulated by inflammatory cells. Moreover, tumor cells produce various cytokines and chemokines that can attract leukocytes. The inflammatory component of neoplasms includes different leukocyte subtypes such as neutrophils, macrophages, and dendritic cells producing inflammatory cytokines and mediators.[7-9]

Platelets are increasing in lung cancer cases. They release angiogenesis factor and attach to micro tumor vessels.[10] The presence of macrophage colony-stimulating factor (M-CSF) and granulocyte colony-stimulating factor (G-CSF) cytokines causes neutrophilia in lung cancer patients.[11] Lung cancer cells release TGF-β and IL-10, causing the suppression of lymphocytes. Neutrophils also secrete endothelial vascular growth factors and pro-angiogenic factors involved in tumor formation, which is also essential for tumor growth and metastase.[10-12]

Inflammation plays a vital role in the neoplastic process by stimulating cancer proliferation, survival, and migration. The neutrophil–lymphocyte ratio (NLR) and the platelet–lymphocyte ratio (PLR) can be used as the inflammatory markers of tissue damage in cancer

patients.[8,9]. Until now, the researchers have not found a similar study conducted in Indonesia. The purpose of this study was to analyze the possibility of patients' NLR and PLR values to predict lung malignancy. The study hypothesizes that the increase of NLR and PLR can be used as the predictive factors of lung malignancy.

MATERIALS AND METHODS

Experimental design

This study was a diagnostic test to compare NLR and PLR ratios. NLR was measured by dividing the number of neutrophils and lymphocytes in the peripheral blood. Meanwhile, PLR was measured by dividing the number of thrombocytes and lymphocytes. The diagnostic value of NLR and PLR was evaluated by calculating the area under the curve (AUC) from receiver operating characteristic (ROC) curves for lung malignancy prediction. The baseline characteristics including age, sex, occupation, education, Brinkman index, body mass index (BMI), and clinical symptoms were collected. This study was ethically reviewed and approved by Dr. Moewardi Hospital Committee for Research on Human Subjects (Medical) (approval number: 705/IX/HREC/2018).

Study subjects

The study was performed at Dr. Moewardi Hospital Surakarta from September 2018 until the number of samples was fulfilled. The study population was 60 subjects, that divided into 2 groups, 30 patients with lung tumors and 30 healthy subjects as control. The inclusion criteria for lung tumor patients were the subjects who had lung tumors and undergone diagnostic procedures at Dr. Moewardi Hospital Surakarta, willing to participate in the study by giving informed consent, aged ≥18 years, and have not undergone any chemotherapy treatment. The inclusion criteria for the control group were healthy subjects without lung tumor, willing to follow the study by signing informed consent, aged ≥ 18 years, and have not undergone any chemotherapy. Lung tumor patients with symptoms of acute infection, COPD, diabetes mellitus, acute or chronic kidney failure, clinical HIV, liver disease, and heart failure and those using corticosteroids were excluded from the study. While the exclusion criteria for the control subject were similar to the lung tumor patients.

NLR and PLR measurements

The subjects were subjected to anamnesis, physical examination, laboratory analysis for blood testing, NLR and PLR values calculation, contrast thoracic CT scanning, and diagnostic procedures. The tests were intended to check the compatibility between diagnostic results with NLR and PLR values.

Data analysis

The data analysis was performed using SPSS 21 (IBM) for Windows. A descriptive univariate analysis was presented with frequency and percentage distribution. The data was analyzed with One Way Anova and Mann-Whitney test. This study used diagnostic procedures based on clinical, radiological, transthoracic needle aspiration (cytology), and bronchoscopy. It determined the point of intersection with a receiver operating characteristic (ROC) curve. Fisher's exact test and Pearson's chi-squared test were used to determining the difference between the baseline data parameters.

RESULTS

Study subjects' characteristics

The subjects' general characteristics include age, sex, occupation, education, degree of smoking, BMI, and complaints were showed in Table 1. The average age of patients with a positive cancer diagnosis was 54.13 ± 16.50 years old while the healthy subjects had an average age of 44.53 ± 7.68 years old. The statistical test obtained a p-value = 0.005 ($p < 0.05$), which means that there were significant differences in patient characteristics based on age.

The majority of patients with a cancer diagnosis were male (56.7%) but the statistical test showed no significant difference in patient characteristics based on gender (p-value = 1.000). As much as 63.3% of cancer patients were working while 93.3% of healthy patients were working, and the statistical test showed significant differences in the characteristics of patients based on work (p-value = 0.005). The majority of the subjects with a cancer diagnosis were elementary school graduates (36.7%) and the majority of healthy subjects were in high school (53.3%). Based on the p-value (0.000), there were significant differences in the education characteristics, where cancer-positive subjects tend to have low education.

The number of smokers in cancer patients was 46.7%, so the majority did not smoke. On the other hand, the majority of healthy subjects also did not smoke (73.3%). The statistical test obtained a p-value = 0.022 ($p < 0.05$), which means that there are significant differences in the subjects' characteristics based on the degree of smoking, where cancer-positive subjects tend to smoke. Most of the cancer patients had low BMI (73.3%) while the majority of healthy subjects with excess nutritional status were 70.0%. The statistical test showed significant differences in the subjects' BMI (p-value = 0.000). As much as 46.7% of complaints were from lung cancer patients while the healthy subjects had no complaint and it was significantly different ($p = 0.000$).

NLR and PLR differences in subjects

The results of NLR and PLR calculation in both groups were listed in Table 2. The average number of neutrophils was significantly lower in healthy patients (p-value = 0.000) with the average number was 75.47 ± 8.30 in cancer patients and 56.41 ± 5.74 in healthy subjects. Interestingly, the average lymphocytes were significantly higher in healthy subjects (p-value = 0.000) with the average number 15.20 ± 8.01 in cancer patients and 31.82 ± 5.11 in healthy subjects. On the other hand, the platelets number was significantly lower in healthy subjects ($p = 0.007$), with an average of 373.70 ± 109.90 in cancer patients and 313.40 ± 44.93 in healthy subjects. The leukocytes were also significantly lower in healthy subjects (p-value = 0.003) with an average number of 11693.33 ± 3692.20 in cancer patients and 9020.00 ± 1558.82 in healthy subjects.

From the blood test results, the NLR was calculated and it was 6.25 ± 2.88 in cancer patients and 1.84 ± 0.47

in healthy subjects. As the p-value = 0.000 ($p < 0.05$) so the difference was significant where healthy subjects have lower NLR. Meanwhile, the PLR in cancer patients was 254.93 ± 116.59 and 114.33 ± 27.67 in healthy subjects. The PLR difference between cancer patients and healthy subjects was significantly different (p-value = 0.000).

The diagnostic test of the neutrophil–lymphocyte ratio

Based on the comparison of diagnostic results from both groups with the NLR results. The ROC curve results were illustrated in Figure 1.

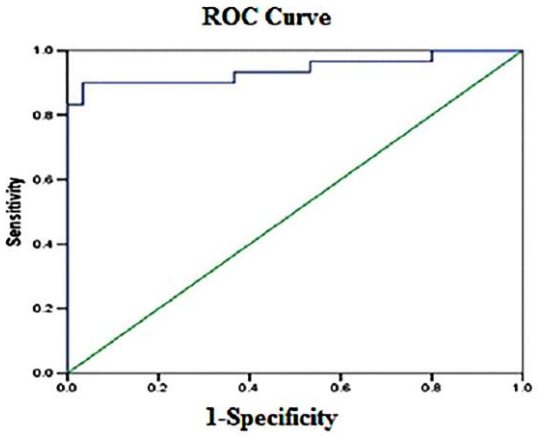

Figure 1. ROC curve test and NLR test

Based on the ROC curve, an AUC value of 0.941 with a p-value of 0.000 ($P < 0.01$) was obtained. The cutoff value for NLR was 2.705 at 90.0% sensitivity and 96.7% specificity. It means that 90.0% of lung cancer patients can be detected by NLR examination. The specificity value of NLR measurements was 96.7%, meaning that it is likely to be healthy, which can be excluded in patients who have NLR > 2.705 of 96.7% (Table 3).

Table 3. NLR and PLR cutoff determination in lung cancer

AUC	Sensitivity	Specificit	Cutoff Value	P
		NLR		
0.941	90.0%	96.7%	2.705	0.000
		PLR		
0.898	83.3%	80.0%	136.63	0.000

The diagnostic test of the platelet–lymphocyte ratio

Similar to NLR measurement, the diagnostic results from both groups were compared with the PLR results to obtain the ROC curve results (Figure 2).

As presented in Table 3, the AUC value of 0.898 ($p = 0.000$) was obtained based on the ROC curve. The cutoff value of PLR was 136.63 at 83.3% sensitivity and 80.0 % specificity. The obtained specificity value suggested that 83.3% of lung cancer patients can be detected by PLR examination. Meanwhile, the obtained specificity value suggested that the probability of a healthy person being excluded in patients who have a PLR > 136.63 is large (80.0%).

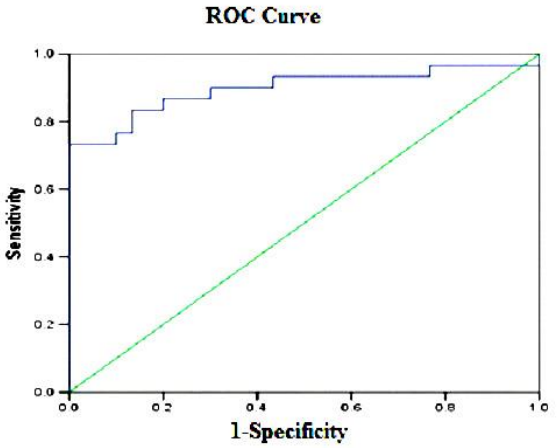

Figure 2. ROC curve for the PLR diagnostic test

DISCUSSION

This study found that the number of males with lung cancer compared with females with lung cancer is in accordance with the study by the World Health Organization. In 2014, lung cancer was the most common type of cancer in Indonesian males. The present study found cancer patients with an average age of 54.13 years[13]. Youlden et al.[14] (2008) reported that in 2002, globally, 5% of patients diagnosed with lung cancer were between the ages of 0 to 44 years; 14% were 45–54 years; 25% were 55–64 years; and 55% among those aged 65 years and older.

The majority of patients with cancer were a worker (63.3%). Also, the majority (53.3%) of patients with a negative diagnosis of cancer have a senior high-school level of education and 36.7% of cancer patients only have a primary school education. Hruba et al.[15] reported that in 2009, there was a 39% increase in the lung cancer risk in unskilled workers compared to office workers and a 31% risk increase for those with lower education compared to those with tertiary education, both after adjusting for the effects of smoking and occupational exposure to carcinogens. The degree of passive smoking by non-smoking healthy patients was 73.3% and 46.7% of the cancer patients were non-smoking. Smoking increases the risk of lung cancer and smokers still show a higher risk than non-smokers because the risk never returns to the baseline or normal. This is consistent with the study of Young et al.[16] in 2009 who reported 85% of lung cancer diagnoses were made in smokers or former smokers. Approximately 50% of lung cancer cases are diagnosed in former smokers. Thus, there are more than 500 million smokers worldwide who are at high risk of lung disease, including lung cancer, and lung cancer occurs in around 15% of smokers.[17]

The majority of patients (73.3%) had a positive diagnosis of cancer and poor nutritional status. In 2018, Zhu et al.[18] reported that high BMI was associated with a low risk of lung cancer. Most of the complaints (46.7%) from patients with lung cancer were the cough. In 2014, Iyer et al.[19] found that the most common symptoms of lung cancer patients were coughing, shortness of breath, and chest pain.

There is a significant difference in neutrophils count between patients with a negative cancer diagnosis and

those with a positive one. Patients with advanced cancer have an increased number of neutrophils in the blood. Tumors inducing neutrophilia can be caused by the production of the granulocyte-macrophage colony-stimulating factor (GM-CSF). Cytokines, such as the granulocyte colony-stimulating factor (G-CSF), interleukin-1 (IL-1), and IL-6, produced by tumors contribute to the increase of neutrophils in the blood. There are significant differences in lymphocytes between healthy subjects and cancer subjects, with a decrease was observed in cancer patients.[20-24] According to Kargl et al.[25] (2008), increased neutrophil count and lymphocyte suppression were obtained in patients with lung cancer.

There is a significant difference in platelet count between healthy and cancer patients. A study conducted by Karagoz et al.[10] found that the number of platelets in lung cancer patients was not different from that in healthy subjects. Another study found thrombocytosis in pulmonary patients was associated with the presence of metastasis.[23]

There is a significant difference in the NLR and PLR between healthy and cancer patients. The relationship between the increased NLR and PLR in various tumors is not yet fully understood, several pathways allow various cancers to grow at the site of infection and inflammation. Inflammation is an essential process in the development and progression of cancer.[24] A study conducted by Kemal et al.[26] supported the predictive value of NLR and PLR markers, reflecting the relationship between cancer and inflammation. This study also supports the results of previous studies in various types of cancer. Nikolic et al. (2016) found a significantly higher NLR and PLR in patients with different histopathological lung cancer subtypes than in the control group.[5] The study also demonstrated that NLR and PLR have a satisfactory diagnostic value in the diagnosis of lung cancer.

The neutrophil–lymphocyte ratio as a predictive factor for lung malignancy

It was observed that the average count of neutrophils was significantly different between groups (p-value = 0.000), with a mean value of 56.41 in healthy controls and 75.47 in cancer patients. It was similar with the lymphocytes number; a significant difference between groups (p-value = 0.000) with the mean value of 31.82 in healthy controls and 15.20 in cancer patients. These results are consistent with Kargl et al.,[25] where the increased neutrophil count and lymphocyte suppression were obtained in patients with lung cancer.

The mean NLR was significantly lower in healthy subjects (p-value = 0.000), with a mean value of 1.84 in healthy controls and 6.25 in patients with a positive diagnosis of cancer. The ROC curve showed the cutoff value of 2.70 for NLR. This is in accordance with a study conducted by Nicolic et al.[5], where there was an increase in the value of NLR in lung cancer patients, which is 2.71. Kemal et al.[26] also showed a significant increase in NLR in patients with lung cancer compared to healthy subjects; 4.42 in lung cancer patients and 2.45 in healthy subjects.

The platelet–lymphocyte ratio as a predictive factor for lung malignancy

The results showed significantly more platelets in cancer subjects (p-value = 0.007), with the mean value of 313.40 in healthy subjects and 373.70 in patients with a positive diagnosis of cancer. However, the lymphocytes were significantly lower in cancer subjects (p-value = 0.000), with an average of 31.82 in healthy controls and 15.20 in patients with a positive cancer diagnosis. Consequently, the result of PLR showed significantly lower values in healthy subjects (p= 0.000) with a mean value of 114.33, while it was 254.33 in patients with a positive cancer diagnosis. In this study, the examination of lung cancer with PLR samples obtained a cutoff value of 136.63 based on the ROC curve. Nicolic et al.[5] found that there is an increase in the PLR value in lung cancer patients, which was 182.31. A study conducted by Kemal et al.[26] also demonstrated a significant PLR increase in cancer patients compared to healthy subjects; 245.1 in lung cancer patients and 148.2 in healthy subjects.

In summary, NLR and PLR are cheap, modest, and readily available biomarkers as predictive information to identify lung malignancy. The limitations of this study are that it cannot distinguish the types of adenocarcinoma lung cancer, squamous cell carcinoma, small cell carcinoma, or large cell carcinoma while helping with the diagnosis. Considering the limitations, the reported results may not give more information for a wide range of lung malignancy types.

CONCLUSION

The increase of NLR and PLR can be used as the predictive factors of lung malignancy. The receiver operating characteristic (ROC) curve for NLR was estimated to have an AUC value of 0.941 ($P < 0.01$), and for PLR, it has an AUC value of 0.898 ($p = 0.000$). The suggestion for further study is the increased number of samples to allow the distinction of lung cancer types.

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REFERENCES

1. Detterbeck FC, Lewis SZ, Diekemper R, Addrizzo-Harris D, Alberts WM. Executive Summary: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest. 2013;143:7S-37S. https://doi.org/10.1378/chest.12-2377.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61: 69-90. https://doi.org/10.3322/caac.20107.
3. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin. 2012; 62: 10-29. https://doi.org/10.3322/caac.20138.
4. Gomes M, Teixeira AL, Coelho A, Araujo A, Medeiros R. Inflammation and lung cancer oxidatives Stress, ROS, and DNA damage. In: Ahmad SI, eds. Reactive Oxygen Species in Biology and Human Health. London: CRC Press; 2016.

5. Nikolić I, Kukulj S, Samaržija M, Jeleč V, Žarak M, Orehovec B, Taradi I, Romic D, Kolak T, Patrlj L. Neutrophil-to-lymphocytes and platelet-to-lymphocytes ratio help identify patients with lung cancer, but don't differentiate between lung cancer subtypes. Croat Med J. 2016; 57: 287–92. https://doi.org/10.3325/cmj.2016.57.287
6. Heron M. Deaths: Leading causes for 2013. Natl Vital Stat Rep 2016; **65** :1-94.
7. Yoon HI, Kwon OR, Kang KN, Shin YS, Shin HS, Yeon EH, Kwon KY, Hwang I, Jeon YK, Kim Y, Kim CW. Diagnostic value of combining tumors and inflammatory markers in lung cancer. J Cancer Prev. 2016;21:187–193. https://doi.org/10.15430/JCP.2016.21.3.187
8. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;19(420):860-867. https://doi.org/10.1038/nature01322
9. Unal D, Eroglu C, Kurtul N, Oguz A, Tasdemir A. Are neutrophils/lymphocytes and platelets / lymphocytes rates in patients with non-small cell lung cancer associated with treatment response and prognosis?. Asian Pac J Cancer Prev. 2013;14:5237–42. https://doi.org/10.7314/apjcp.2013.14.9.5237.
10. Karagöz B, Alacacioğlu A, Bilgi O, Demirci H, Özgün A, Erikçi AA, Sayan O, Yilmaz B, Kandemir EG. Platelet count and platelet distribution width increases in lung cancer patients. Anatol J Clin Investig. 2009;3:32–4.
11. Mikogami T, Kunimura T, Omatsu M, Shiokawa A, Nagai T, Hirota Y, Koji S, Ohike N, Kitami A, Suzuki T. Neutrophil-rich pulmonary carcinoma: Clinicopathological characteristicsand cytokine expression and their relationship with lymph node metastasis. Showa Univ J Med Sci. 2014;26:9-16. https://doi.org/10.15369/sujms.26.9
12. Aldarouish M, Wang C. Trends and advances in tumor immunology and lung cancer immunotherapy. J Exp Clin Cancer Res.2016;35:157. https://doi.org/10.1186/s13046-016-0439-3.
13. Indonesian Ministry of Health. National Guidelines for Medical Services: Lung Cancer. Jakarta: Indonesian Ministry of Health; 2017. p. 1–75. [In Indonesia].
14. Youlden DR, Cramb SM, Baade PD. The International Epidemiology of Lung Cancer: Geographical Distribution and Secular Trends. J Thorac Oncol, 2008;3:819–31. https://doi.org/10.1097/JTO.0b013e31818020eb
15. Hruba F, Fabiánová E, Bencko V, Cassidy A, Lissowska J, Mates D, Rudnai P, Zaridze D, Foretova L, Janout V, Dabrowska NS, Brennan P, Boffrtta P. Socioeconomic indicators and risk of lung cancer in central and eastern Europe. Cent Eur J Public Health. 2009;17: 115–21.
16. Young RP, Hopkins RJ, Christmas T, Black PN, Metcalf P, Gamble GD. COPD prevalence is increased in lung cancer, independent of age, sex and smoking history. Eur Respir J, 2009;34:380-386. https://doi.org/10.1183/09031936.00144208
17. Rahal Z, El Nemr S, Sinjab A, Chami H, Tfayli A, Kadara H. Smoking and lung cancer: A geo-regional perspective. Front Oncol. 2017;**7**:194. https://doi.org/10.3389/fonc.2017.00194.
18. Zhu H, Zhang S. Body mass index and lung cancer risk in never smokers: a meta-analysis. BMC Cancer. 2018;18:635. https://doi.org/10.1186/s12885-018-4543-y.
19. Iyer S, Roughley A, Rider A, Taylor-Stokes G. The symptom burden of non-small cell lung cancer in the USA: A real-world cross-sectional study. Support Care Cancer. 2014;22:181-7. https://doi.org/10.1007/s00520-013-1959-4.
20. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell. 2011;144:646–74. https://doi.org/10.1016/j.cell.2011.02.013.
21. Lechner MG, Liebertz DJ, Epstein AL. Characterization of cytokine-induced myeloid-derived suppressor cells from normal human peripheral blood mononuclear cells. J Immunol. 2010;185:2273–84. https://doi.org/10.4049/jimmunol.1000901.
22. Coffelt SB, Wellenstein MD, de Visser KE. Neutrophils in cancer: neutral no more. Nat Rev Cancer 2016;16:431–46. https://doi.org/10.1038/nrc.2016.52.
23. Zhang X, Ran Y. Prognostic role of elevated platelet count in patients with lung cancer: a systematic review and meta-analysis. Int J Clin Exp Med. 2015;8:5379–87.
24. Peng B, Wang YH, Liu YM, Ma LX. Prognostic significance of the neutrophil to lymphocyte ratio in patients with non-small cell lung cancer: A systemic review and meta-analysis. Int J Clin Exp Med. 2015;8:3098-106.
25. Kargl J, Busch SE, Yang GHY, Kim KH, Hanke ML, Metz HE, Hubbard JJ, Lee SM, Madtes DK, McIntosh MW, Houghton AM. Neutrophils dominate the immune cell composition in non-small cell lung cancer. Nat Commun. 2017;8:14381. https://doi.org/10.21037/jtd.2017.04.55
26. Kemal Y, Yucel I, Ekiz K, Demirag G, Yilmaz B, Teker F, Ozdemir M. Elevated serum neutrophil to lymphocyte and platelet to lymphocyte ratios could be useful in lung cancer diagnosis. Asian Pac J Cancer Prev. 2014;15:2651-2654. https://doi.org/10.7314/apjcp.2014.15.6.2651.

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Original Research Article

The Relationship Between the Duration of Kangaroo Mother Care and Edinburgh Postnatal Depression Scale Outcomes in Mothers with Preterm Infants

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Abstract

Background: Preterm birth has a negative impact on the health of the baby and increases the risk of postpartum depression in mothers. Kangaroo mother care (KMC) is a preterm baby care which is considered to increase bonding between mother and baby, thereby reducing the incidence of postpartum depression.

Objective: To find out the relationship between the duration of KMC and other confounding factors with EPDS outcomes in mothers with preterm infants.

Methods: This study used a quasi-experimental method with non-randomized control group pre-test and post-test design and was conducted on 34 mothers with preterm infants who gave birth at Dr. Kariadi Hospital Semarang. The research subjects were selected using consecutive sampling method and were asked to perform kangaroo mother care for a certain duration. The treatment group was instructed to perform KMC for 120 minutes daily, whereas the control group was instructed to perform KMC 60 minutes daily for 14 days. Evaluation for postpartum depression was carried out using the Edinburgh Postnatal Depression Scale questionnaire, which was completed twice, as a pre-test and post-test. Data analysis was performed using paired sample T-test and independent samples T-test to determine the relationship between variables.

Results: Results showed that there was a significant relationship between the duration of KMC and EPDS outcomes. The difference between the decreased of EPDS scores in the control and treatment groups was significant (p=0.017). The significant decrease of EPDS score was found in the treatment group (-1,398±1,403; p<0.001). The decrease of EPDS score in the control group was not significant (-0,967±1,403; p=0.704). There is a significant relationship between the method of childbirth (p<0,001) and breastfeeding status (p=0,042) with EPDS outcomes in mothers with preterm infants. There is no significant relationship between maternal age and EPDS outcomes (p=0,805).

Conclusion: Increasing duration of KMC lowers the score of EPDS in mothers with preterm infants.

Keywords: *Postpartum depression; Preterm, Kangaroo Mother Care (KMC); Edinburgh Postnatal Depression Scale (EPDS)*
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INTRODUCTION

Preterm birth is defined as a birth that occurs before 37 weeks of gestational age.[1,2] Globally, preterm birth is the leading cause of death among children under the age of five.[1] In 2018, Indonesia ranked fifth among countries with the highest rates of preterm birth in the world, with an incidence of 676,700 per year.[3] According to the *Riset Kesehatan Dasar* 2018 data, 19% deliveries in Central Java occurred at preterm gestational ages.

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Semarang city itself is one of the areas in Central Java that requires special attention due to its higher percentage of preterm birth, which is 31.91%.[4] Although most preterm infants survive, they are at high risk for neurodevelopmental disorders and other complications.[5]

The high risk of complications experienced by preterm infants not only affects the infants themselves, but also has an impact of the psychological well-being of the mothers. Mothers who go through preterm birth are at risk of experiencing postpartum depression, which can affect the mother-infant bonding and the infant's development.[6] Previous research has indicated that 39% women who give birth to preterm infants experience significant symptoms of depression.[7]

Postpartum depression is a major depressive disorder that occurs during the postpartum period, within four weeks of delivery. Postpartum depression screening can be conducted using Edinburgh Postnatal Depression Scale (EPDS) instrument. This instrument consists of 10 short questions about the mother feelings over the past week. The questionnaire has been translated and validated in Indonesian language with a sensitivity of 87.5% and a specificity of 61.6%.[8]

Kangaroo mother care (KMC) is a method of caring for preterm infants that involves direct skin-to-skin contact between the mother and the baby. This method involves placing the baby upright between the mother's breast and positioning the mother's chest against the baby's chest, maintained using a wrap or cloth for at least 60 minutes. This method can be started once the infant is medically stable, and is often done until the baby reaches full-term age, which is around 40 weeks of gestational age, or after the baby reaches a weight of 2500 grams. During those time, baby will start to show signs of discomfort when positioned for KMC.[9] Numerous studies have confirmed that the implementation of KMC not only benefits the growth and development of the baby but also helps improve bonding between the mother and the baby while reducing postpartum anxiety in mothers. KMC allows mother to engage in continuous skin-to-skin contact, which enhances the bond between mother and infant, and promotes increased in positive feeling, which ultimately reduces the risk of postpartum depression.[10,11] However, another study found that prolonged periods of KMC can be a tiring experience, which can have an impact on the mother's psychological well-being.[12]

The differences in previous research findings serve as the basis for conducting a study on the relationship between the duration of KMC and the EPDS outcomes. Time limits of 60 minutes and 120 minutes were used in this study to compare the effectiveness of different durations in reducing EPDS scores in mothers with preterm infants. The selection of these two durations was made to compare both the upper and lower borderline of the recommended time limit, with the hope that babies would still receive optimal benefits from the application of this method.[13,14]

MATERIALS AND METHODS

This study was conducted at the Maternal and Child Care and the Medical Report Unit of Dr. Kariadi Hospital Semarang. Data collection took place over a period of 5 months, specifically from May to September 2023. The method used in this study is a quasi-experimental with non-randomized control group of pre-test and post-test design. The sample size for this study consisted of 34 respondents who met the inclusion and exclusion criteria. Inclusion criteria included mothers who gave birth to their babies at a gestational age of less than 37 weeks, were willing to participate in the study, and had stable baby conditions. Exclusion criteria included mothers with communication limitations, such as not having a cellphone or being unable to make video calls, and experiencing language barriers; mother with education level below junior high school, and mother who has been previously diagnosed with depression. Dropout criteria included subjects who did not perform KMC according to the procedure, or perform KMC with a duration less than the specified guidelines of this study, which is less than 60 minutes per day and/or less than 2 weeks (control group), or less than 120 minutes per day and/or less than 2 seeks (treatment group); and subjects who did not participate in the post-test.

The research began with obtaining ethical research approval from the Health Ethics Commission of the Faculty of Medicine, Universitas Diponegoro with No. 168/EC/KEPK/FK-UNDIP/V/2023. Permission from Dr. Kariadi Hospital Semarang was obtained thereafter. Subsequently, the researcher approached potential participants who met the inclusion criteria to provide an explanation of the research process and inquire about their willingness to participate in this study. Subjects who agreed to participate were asked to sign an informed consent form and complete a demographic questionnaire, including education level, mother's age, baby's age, mother's employment status, gestational age at delivery, delivery method, and breastfeeding status; as well as the EPDS questionnaire. Participants were randomly assigned into control and treatment groups. The control group were asked to perform KMC for 60 minutes per day, while those in the treatment group were asked to perform KMC for 120 minutes per day for 14 days. At the end of the study, all participants were requested to complete the EPDS questionnaire again. The researcher provided support to the participants during the completion of questionnaires and implementation of KMC. Support was conducted in-person while the participants were hospitalized. Following their discharge from the hospital, support was continued via video calls.

This study used independent variable in the form of the duration of KMC, dependent variables in the form of the EPDS outcomes, and confounding variables including delivery method, breastfeeding status, and maternal age during pregnancy. Data obtained were analyzed using SPSS Statistics 27 software. Normality testing of the EPDS scores was conducted using Saphiro-Wilk test. EPDS scores were presented in the form of mean and standard deviation as they followed a normal distribution. Nominal scale data were presented as percentages and frequencies. Hypothesis testing to analyze the relationship between independent and dependent variables was conducted using paired T-tests. Analysis of the outcome differences between the treatment and control groups was performed using independent samples T-tests.

Table 1. Characteristics of the Research Subjects

Variable	Group		*p*
	Control	Treatment	
Mother's employment status			
Employed	10 (29,4%)	12 (35,3%)	0,473*
Not employed	7 (20,6%)	5 (14,7%)	
Infant's age at the start of KMC			
<14 days	7 (20,6%)	8 (23,5%)	0,730*
≥14 days	10 (29,4%)	9 (26,5%)	
Gestational age (median [iqr])	36 (2)	35 (4)	0,454€

Note: * Chi square; € Mann-Whitney Test

Table 2. The Relationship Between the Duration of KMC and EPDS outcomes

Group	EPDS		*p*	Delta
	Pre-test	*Post-test*		
Control	7,076±1,788	6,842±1,683	0,704†	-0,967±1,431
Treatment	6,863±1,673	4,911±1,819	<0,001*†	-1,398±1,403
p	0,872§	0,094§		0,017*§

Note: * Significant (p<0,05); § *Independent samples T-test*; † *Paired T-test*

Table 3. The Relationship Between Confounding Variables and EPDS Outcomes

Variable	n		EPDS (*pre-test)*	*p*
	Control	Treatment		
Method of childbirth				
Normal	7 (20,6%)	4 (11,8%)	4,388±1,613	<0,001£
CS	10 (29,4%)	13 (38,2%)	8,696±1,522	
Breastfeeding status				
Exclusive breastfeeding	12 (35,3%)	14 (41,2%)	6,282±1,685	0,042£
Not exclusive breastfeeding	5 (14,7%)	3 (8,8%)	9,763±1,625	
Maternal Age				
<35 years	12 (35,3%)	15 (44,1%)	6,886±1,767	0,805£
≥35 years	5 (14,7%)	2 (5,9%)	7,2962±1,560	

Note: £ Biserial test

Biserial correlation analysis was used to examine the relationship between confounding variables and the dependent variables.

RESULTS

This research was conducted at Dr. Kariadi Hospital Semarang from May to September 2023. The research subjects involved in this study were mothers with preterm infants who had given birth at Dr. Kariadi Hospital and were still performing KMC. Sampling was carried out using consecutive sampling techniques, using both primary and secondary data. Considering the inclusion and exclusion criteria, the total number of samples obtained was 34 mothers with preterm infants, divided into 17 subjects for the control group and 17 subjects for the treatment group.

Table 1 shows that the majority of employed mothers are from the treatment group, but there is no significant difference. From both groups, it is found that the majority of mothers begin KMC when their baby is 14 days or older, with no significant difference. There is no significant difference in the gestational age of mothers in both the control and treatment groups, but based on the data in the table, it is known that the gestational age in the treatment group is younger than in the control group.

Data in table 2 summarizes the relationship between the duration of KMC and EPDS outcomes. It shows that the mean EPDS scores during the pre-test are lower in the treatment group than in the control group, but the difference is not significant. In the post-test, the treatment group has lower EPDS scores, but the difference remains not significant. From the data obtained, it is found that both the control and treatment groups experienced a reduction in EPDS scores after undergoing KMC for 14 days, with a higher reduction in the treatment group. The reduction in EPDS scores in the control group is not significant, but the reduction in scores in the treatment group is significant. The difference in the magnitude of the reduction of EPDS scores between the control and treatment groups are significant.

Table 3 shows the biserial analysis between confounding variables and EPDS outcomes. Looking at this table, it is known that the majority of mothers who underwent normal delivery are from the control group. Biserial correlation test shows that there is a significant relationship between the childbirth method and EPDS outcomes, where subjects who underwent normal delivery shows lower EPDS score than those who underwent caesarean section. The data also shows that there is a significant relationship between breastfeeding status and EPDS outcomes, where subjects who exclusively breastfeed shows lower EPDS score than

those who do not exclusively breastfeed. Regarding the data on the relationship between maternal age during pregnancy and EPDS outcomes, it is found that there is no significant relationship between these two variables. However, the data shows that EPDS scores are slightly lower in mothers under the age of 35.

DISCUSSION

Data analysis on the characteristics of the research subjects indicates that there is no significant difference in mother's employment status, infant's age at the start of KMC, and gestational age between the control and treatment group. These three variables can influence the EPDS outcomes, so the insignificant differences between two groups reduce bias in the research results. Previous research showed that there is a relationship between employment status and postpartum depression. The risk of postpartum depression was found to be lower in non-employed mothers because they can adapt more easily to their new role as a mother.[15] Referring to the guidelines set by WHO, KMC can only be commenced when the baby's condition is stable, indicated by the ability to breathe spontaneously without the need for oxygen assistance. Babies who can start KMC earlier are those who reach the stable phase sooner.[9] Previous research conducted on mothers with infants admitted in the NICCU revealed that a baby's poor condition can affect the mother's serotonin levels, leading to maternal anxiety and depressive symptoms. The earlier a mother begins KMC, the lower the risk of experiencing postpartum depression.[16] It is known that a lower degree of prematurity would lead to increased levels of anxiety and depressive symptoms in mothers due to the mother's concern about the potentially worse condition of their babies compared to babies born at a more mature gestational age.[17]

Based on the analysis of the relationship between the duration of KMC and EPDS outcomes in mothers with preterm infants in this study, it is evident that performing KMC with a duration of 120 minutes per day for 14 days led to a significant decrease in EPDS scores in mothers with preterm infants. Performing KMC with a duration of 60 minutes per day for 14 days also resulted in a decrease in EPDS scores, but the decrease was not significant. The differences in the reduction of EPDS scores between the two groups was significant. These findings are aligned with the results of a study conducted in Torrecárdenas University Hospital which showed a significant reduction in EPDS scores in mothers who performed KMC for over than 90 minutes per day for 2 weeks.[13] Performing KMC with a longer duration is considered to enhance the bond between the mother and the baby, thereby positively impacting the mother's psychological well-being.[18]

Kangaroo mother care allows mothers to have skin-to-skin contact with their babies. Skin-to-skin contact leads to a continuous significant decrease in cortisol levels and an increase in oxytocin levels in mothers, which in turn contributes to a reduction in anxiety and depression levels.[19]

From a psychological perspective, skin-to-skin contact between mother and her baby can promote increased satisfaction, a sense of peace, heightened energy, tranquility, and relaxation of mind. These effects can help minimize the risk of postpartum depression.[20]

This study found that the method of childbirth is significantly associated with EPDS outcomes in mothers with preterm infants. The pre-test results showed that mothers who underwent a normal delivery had significantly lower average EPDS scores compared to mothers who had a cesarean section (CS). The research findings are consistent with a previous systematic review which states that the incidence of postpartum depression is higher in mothers who have undergone a CS due to their lower satisfaction with the birthing experience and prolonged post-CS pain[21]. Another study also revealed that emergency caesarean section can increase the risk of postpartum depression because most pregnant women generally expect a normal delivery process to experience a more "natural" birthing process. When this process is disrupted by an emergency caesarean section, it is highly likely for a mother to feel disappointed, a sense of failure, and a loss of control; ultimately increasing the risk of postpartum depression.[22]

The analysis of the relationship between breastfeeding status and EPDS outcomes in mothers with preterm infants in this study showed that there is a significant relationship between breastfeeding status and EPDS outcomes in mothers with preterm infants. This is reflected in the pre-test results, which indicate that mothers who exclusively breastfeed have significantly lower average EPDS scores compared to mothers who do not exclusively breastfeed. This finding is consistent with previous research conducted in 2020 which states that exclusive breastfeeding can reduce the incidence of mood disorders.[23] Exclusive breastfeeding also allows mothers to have skin-to-skin contact with their babies, thereby reducing the risk of postpartum depression through the same mechanism as KMC.[19]

This study found that there is no significant relationship between maternal age during pregnancy and EPDS outcomes in mothers with preterm infants. The pre-test results show that mothers under the age of 35 have slightly lower average EPDS scores compared to mothers aged 35 or older, but the difference is not significant. A study conducted by Muraca, et al. states that mothers aged 35 or older have a higher risk of postpartum depression compared to mothers under the age of 35. This is because there is a perception that older women may struggle with the adjustment to motherhood, lack peer support, and have an increased risk of postpartum complications, all of which contribute to the increased risk of postpartum depression. The underlying factor in the role of age in postpartum depression is mainly social support. Older mothers tend to have lower social support due to negative individual perceptions and views about older mothers.[24] This relationship is not always consistent due to variations in the social environment of each subject. When their environment provide sufficient support, older mothers can still maintain lower levels of depression.[25]

This study has several limitations. The first limitation is that the follow-up conducted on patients who were no longer admitted at Dr. Kariadi Hospital was only done using video calls, which could potentially introduce bias. Another limitation is the difference in the timing of EPDS data collection during the pre-test. Some research subjects had already undergone KMC before the pre-test

data collection was conducted.

CONCLUSION

In conclusion, this study shows that there is a relationship between the duration of KMC and EPDS outcomes, where an increase in the duration of KMC reduces the EPDS scores in mothers with preterm infants. This study also shows that there is a significant relationship between the method of childbirth and breastfeeding status with EPDS outcomes in mothers with preterm infants. However, there is no significant relationship between maternal age and EPDS outcomes. Further research which uses direct observation as the follow-up techniques might be needed to obtain more accurate data.

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REFERENCES

1. Walani SR. Global burden of preterm birth. International Journal of Gynecology & Obstetrics. 2020;150(1):31–3. doi:10.1002/ijgo.13195
2. American College of Obstetricians and Gynecologists' Committee on Practice Bulletins—Obstetric. Practice Bulletin No. 171: Management of Preterm Labor. Obstetrics & Gynecology. 2016;128(4):e155–64. doi:10.1097/AOG.0000000000001711
3. Kemenkes RI. Perawatan Bayi Prematur. Direktorat Jenderal Pelayanan Kesehatan. 2022.
4. Kementerian RI. Laporan Nasional RISKESDAS 2018. Jakarta: Badan Penelitian dan Pengembangan Kesehatan ; 2019.
5. Chung EH, Chou J, Brown KA. Neurodevelopmental outcomes of preterm infants: a recent literature review. Transl Pediatr. 2020;9(S1):S3–8. doi:10.21037/tp.2019.09.10
6. Anderson C, Cacola P. Implications of Preterm Birth for Maternal Mental Health and Infant Development. MCN: The American Journal of Maternal/Child Nursing. 2017;42(2):108–14. doi:10.1097/NMC.0000000000000311
7. Handelzalts JE, Krissi H, Levy S, Freund Y, Carmiel N, Ashwal E, et al. Personality, preterm labor contractions, and psychological consequences. Arch Gynecol Obstet. 2016;293(3):575–82. doi:10.1007/s00404-015-3898-4
8. Oktamurdiantri F, Saragih JI. Use of the Edinburgh Postnatal Depression Scale to Detect Postpartum Depression Risk. International Journal of Progressive Sciences and Technologies (IJPSAT). 2020;19(1):31–4. doi:10.52155
9. Department of Reproductive Health and Research WHO. KANGAROO MOTHER CARE: A Practical Guides. World Health Organization. Geneva: World Health Organization; 2003.
10. WHO. Immediate "Kangaroo Mother Care" and Survival of Infants with Low Birth Weight. New England Journal of Medicine. 2021;384(21):2028–38. doi:10.1056/NEJMoa2026486
11. Cong S, Wang R, Fan X, Song X, Sha L, Zhu Z, et al. Skin-to-skin contact to improve premature mothers' anxiety and stress state: A meta-analysis. Matern Child Nutr. 2021;17(4):13245. doi:10.1111/mcn.13245
12. Nyondo-Mipando AL, Kinshella MLW, Hiwa T, Salimu S, Banda M, Vidler M, et al. Mothers' quality of life delivering kangaroo mother care at Malawian hospitals: a qualitative study. Health Qual Life Outcomes. 2021;19(1):186. doi:10.1186/s12955-021-01823-8
13. Cristóbal Cañadas D, Parrón Carreño T, Sánchez Borja C, Bonillo Perales A. Benefits of Kangaroo Mother Care on the Physiological Stress Parameters of Preterm Infants and Mothers in Neonatal Intensive Care. Int J Environ Res Public Health. 2022;19(12):7183. doi:10.3390/ijerph19127183
14. Ionio C, Ciuffo G, Landoni M. Parent–Infant Skin-to-Skin Contact and Stress Regulation: A Systematic Review of the Literature. Int J Environ Res Public Health. 2021 [cited 2023 Mar 31];18(9):4695. doi:10.3390/ijerph18094695
15. Ariyanti R, Nurdiati DS, Astuti DA. Pengaruh Jenis Persalinan terhadap Risiko Depresi Postpartum. STIKES 'Aisyiyah Yogyakarta. 2016;7(2):98–105.
16. Leahy-Warren P, Coleman C, Bradley R, Mulcahy H. The experiences of mothers with preterm infants within the first-year post discharge from NICU: social support, attachment and level of depressive symptoms. BMC Pregnancy Childbirth. 2020;20(1):260. doi:10.1186/s12884-020-02956-2
17. Worrall S, Silverio SA, Fallon VM. The relationship between prematurity and maternal mental health during the first postpartum year. Journal of Neonatal Nursing. 2023;29(3):511–8. doi:10.1016/j.jnn.2022.10.002
18. Mehrpisheh S, Doorandish Z, Farhadi R, Ahmadi M, Moafi M, Elyasi F. The Effectiveness of Kangaroo Mother Care (KMC) on attachment of mothers with premature infants. Eur J Obstet Gynecol Reprod Biol X. 2022;15:100149. doi:10.1016/j.eurox.2022.100149
19. Vittner D, McGrath J, Robinson J, Lawhon G, Cusson R, Eisenfeld L, et al. Increase in Oxytocin From Skin-to-Skin Contact Enhances Development of Parent–Infant Relationship. Biol Res Nurs. 2018;20(1):54–62. doi:10.1177/1099800417735633
20. Herizchi S, Hosseini MB, Ghoreishizadeh M. The Impact of Kangaroo-Mother Care on Postpartum Depression in Mothers of Premature Infants. International Journal of Women's Health and Reproduction Sciences. 2017;5(4):312–7. doi:10.15296/ijwhr.2017.53
21. Safari S, Bahadoran P, Oreizi H. Meta-analysis of the role of delivery mode in postpartum depression (Iran 1997-2011). J Educ Health Promot. 2014;3(1):118. doi:10.4103/2277-9531.145924

22. Tonei V. Mother's mental health after childbirth: Does the delivery method matter? J Health Econ. 2019;63:182–96. doi:10.1016/j.jhealeco.2018.11.006
23. Farías-Antúnez S, Santos IS, Matijasevich A, de Barros AJD. Maternal mood symptoms in pregnancy and postpartum depression: association with exclusive breastfeeding in a population-based birth cohort. Soc Psychiatry Psychiatr Epidemiol. 2020;55(5):635–43. doi:10.1007/s00127-019-01827-2
24. Muraca GM, Joseph KS. The Association Between Maternal Age and Depression. Journal of Obstetrics and Gynaecology Canada. 2014;36(9):803–10. doi:10.1016/S1701-2163(15)30482-5
25. Clark LA, Cuthbert B, Lewis-Fernández R, Narrow WE, Reed GM. Three Approaches to Understanding and Classifying Mental Disorder: *ICD-11*, *DSM-5* , and the National Institute of Mental Health's Research Domain Criteria (RDoC). Psychological Science in the Public Interest. 2017;18(2):72–145. doi:10.1177/1529100617727266

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Original Research Article

The Effect of Sambiloto (*Andrographis Paniculata*) Leaf Extract In Combination With Phosphomycin On The Germ Number, Urine Leukocyte Esterase, And Urine Procalcitonin Levels In Wistar Rats (*Rattus Norvegicus*) Urinary Tract Infection Model

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Abstract

Background: Bacterial resistance to antibiotics is still common due to unwise use. Urinary Tract Infections (UTI), 80% of which are caused by *E. coli* and other bacteria such as *Enterobacter sp, Klebsiella sp, S. aureus*. Fosfomycin is a first-line antibiotic for UTI. Combining natural compounds with antibiotics is one treatment strategy to increase the effectiveness of anti-bacterial therapy. *Andrographis paniculata* has been reported to have strong anti-infective activity. This study aimed to prove the differences in the germ number, leukocyte esterase levels, and urine procalcitonin levels in *Rattus norvegicus* UTI model given the fosfomycin, Sambiloto leaf extract, and Sambiloto leaf extract-fosfomycin combination.

Methods: Thirty *Rattus novergicus* rats were divided into five groups. All groups were induced 50 µl of *E. coli* bacterial inoculum for 7 days, followed by standard feed (negative control), fosfomycin (Monuril®) 54 mg (positive control), Sambiloto leaf extract (S1 [100 mg/BW], S2 [200 mg/BW], Sambiloto leaf extract-fosfomycin combination (FS1 [sambiloto 100 mg/BW and fosfomycin 54 mg], and FS2 [sambiloto 200 mg/BW and fosfomycin 54 mg]) for the next 7 days orally. The germ number, leukocyte esterase, and urine procalcitonin were measured after all rats were given treatment.

Results: The largest average reduction in the germ number, levels of leukocyte esterase, and urinary procalcitonin (4.80 ± 3.70 CFU/ml [$p<0.05$], 3,00 ± 6,71 cells/µL [$p<0.05$], 4,66 ± 1,35 ng/L [$p<0.05$] respectively) was observed in the combination of 200 mg/BW Sambiloto leaf extract-fosfomycin combination group.

Conclusion: A combination of Sambiloto leaf extract and fosfomycin reduced germ number, levels of leukocyte esterase and urinary procalcitonin in rat model of UTI.

Keywords: *Sambiloto (Andrographis paniculata); fosfomycin; E. coli; leukocyte esterase; procalcitonin*

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INTRODUCTION

Urinary Tract Infection (UTI) is marked by bacterial growth and proliferation within the urinary system, extending from the kidneys down to the bladder, often accompanied by notable levels of bacteria in the urine. The prevalence of UTI increases with age, but the peak prevalence is in the 14-24 year age group.[1] The global prevalence rate for uncomplicated UTIs is 11%.[2] The prevalence of healthcare-associated urinary tract infection (HAUTI) is around 24% in several developing countries.[3,4,5]

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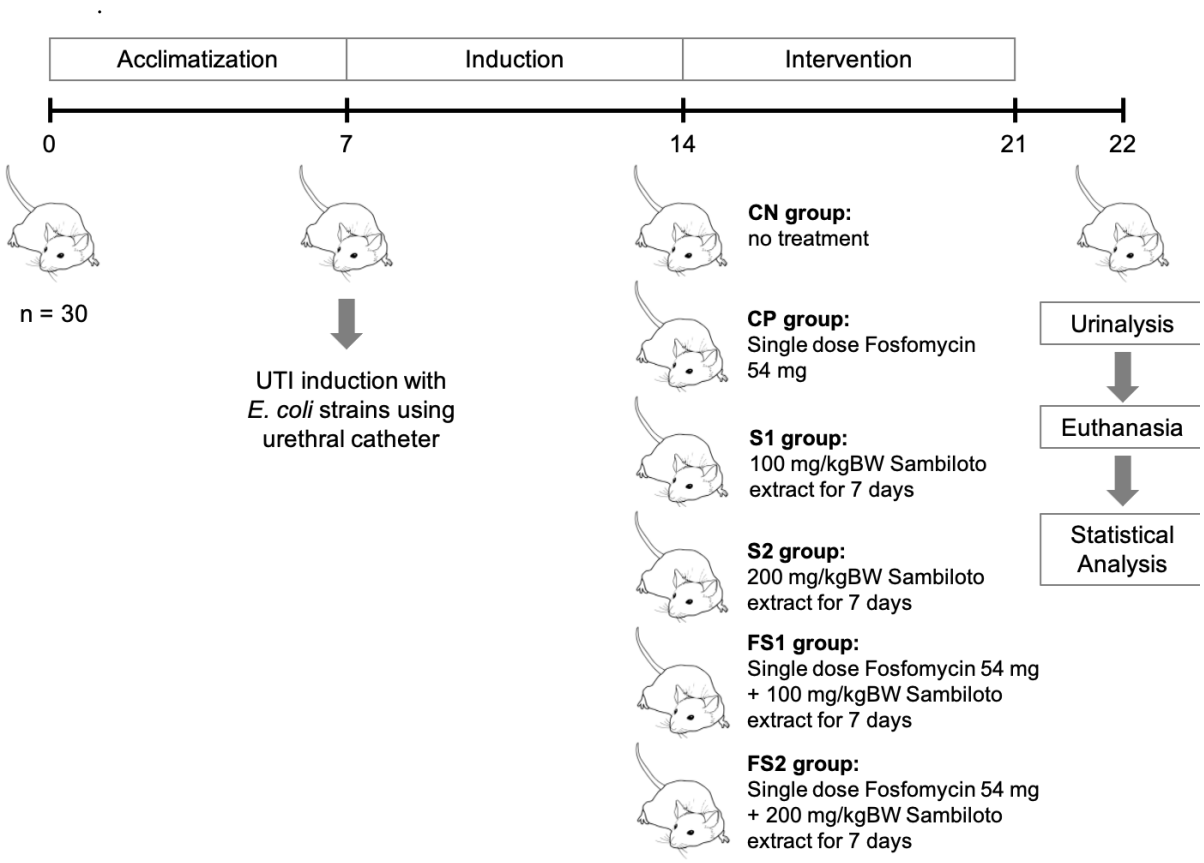

Figure 1. Study timeline

The Global Prevalence Study on Infections in Urology (GPIU) in 2003-2010 `reported that of 19,756 patients, 9.4% were diagnosed with HAUTI, and 70.4% were female.[6,7] According to the data obtained by the Ministry of Health of the Republic of Indonesia in 2018, the prevalence of UTI stands at 90-100 cases per 100,000 population annually, equating to roughly 180,000 cases per year.[8]

Various indicators can be employed to identify inflammation in the urinary tract, including counts of polymorphonuclear (PMN) leukocyte type in urethral swabs, prostate secretions, ejaculate fluid, urine sediment, and levels of PMN elastase.[9] Additionally, markers such as leukocyte esterase, C-reactive protein, and different interleukins can also be utilized for UTI detection.[10] Urinary biomarkers such as leukocyte esterase is useful for identifying UTIs, although it has low specificity. Interestingly, procalcitonin has become increasingly popular over the past decade for enhancing the diagnosis of bacterial infections.[11]

Treatment for UTI involves administering antibiotics. Fosfomycin is one of the most widely used antibiotic. However, the widespread and often imprudent use of numerous antibiotics has resulted in the development of antibiotic resistance. Consequently, microorganisms have evolved diverse resistance mechanisms due to the absence of new antibiotics for enhanced therapy.[12,13] Multiple studies have noted a rising trend of resistance to fosfomycin in E. coli, the most prevalent pathogen responsible for UTIs. Resistance to fosfomycin increased from 14.3% in 2013 to 20% in 2021.[14] Additionally, another study found fosfomycin resistance in 38.5% of E. coli isolates.[15]

An approach to tackle this resistance is through combination therapy, which involves administering two or more drugs simultaneously. These combinations often include multiple active ingredients, which can be natural compounds, a mix of natural substances, or a combination of natural and conventional antibiotics. The goal of combination therapy is to prevent the emergence of resistant bacterial strains. It also allows for lower doses of drugs to be used, reducing toxicity, while potentially enhancing treatment efficacy.[16] Currently, alternative UTI treatments other than chemical drugs are increasingly being used.[17] Traditional medicines that have antibacterial effects are increasingly common in developing countries.[18] Based on World Health Organization (WHO) data in 2004, herbal medicines often used as a traditional treatment, accounting approximately 65% of the population in developed countries and 80% in developing countries.[19]

One of the medicinal plants used in traditional medicine is Sambiloto (*Andrographis paniculata*) which is known to have beneficial compositions and properties. Based on the category of traditional medicines, Sambiloto is classified as a herbal medicine.[8] The Sambiloto plant is potential in regulating inflammatory responses and possesses antibacterial properties that can mitigate the side effects associated with chemical treatments for various inflammatory conditions, including lung infections and sepsis.[20–25] Previous research showed the ability of the Sambiloto plant to prevent the growth of biofilm isolates of *E. coli* in vitro.[9] Sambiloto also showed antibacterial properties in the

Table 1. Subjects Characteristics (Bacterial Count)

Group	Bacterial Count (CFU/ml)	
	Mean ± SD	p§
CN	858.60 ± 135.86*	<0,001•
CP	26.00 ± 10.27*	
S1	156.60 ± 56.06*	
S2	78.40 ± 21.48	
FS1	19.40 ± 7.64*	
FS2	4.80 ± 3.70*	

*Normal distribution (p > 0.05); §Kruskal-Wallis; •Significant (p < 0.05)

Table 2. Subjects Characteristics (Leukocyte Esterase)

Group	Leukocyte Esterase (cells/uL)	
	Median (min-max)	p§
CN	125 (125 -500)	<0.001•
CP	15 (0 - 15)	
S1	70 (70-125)	
S2	70 (15-70)	
FS1	0 (0 -15)	
FS2	0 (0 -15)	

*Normal distribution (p > 0.05); §Kruskal-Wallis; •Significant (p < 0.05)

Table 3. Subject Characteristics (Procalcitonin)

Group	Procalcitonin (ng/ml)	
	Mean ± SD	p⸸
CN	783.77 ± 56.40*	<0.001•
CP	28.40 ± 7.28*	
S1	239.29 ± 82.00*	
S2	110.74 ± 13.66*	
FS1	25.29 ± 6.24*	
FS2	4.66 ± 1.35*	

*Normal distribution (p > 0.05); ⸸One Way Anova; •Significant (p < 0.05)

urine of UTI patients tested in vitro.[10] Therefore, this research aims to assess the efficacy of Sambiloto leaf extract in conjunction with first-line antibiotics against infectious microorganisms to offer an alternative therapy that enhances the effectiveness of antibiotics. In addition, very little data is available on Sambiloto leaf extract in combination with standard antibiotic drugs as infection prevention therapy, especially in UTI.

METHODS

Design and Subject

The study adopted a post-test only control group design. Thirty healthy Rattus norvegicus rats, aged between 12 and 16 months, weighing 150-200 g, were sourced from the Laboratory of Experimental Animals at the Faculty of Medicine, Universitas Sebelas Maret. This study was conducted at September 2023. Treatment consisted of administering the fosfomycin, sambiloto leaf extract, as well as Sambiloto leaf extract-fosfomycin combination with variable measurement parameters, namely the germ number, leukocyte esterase levels, and urine procalcitonin levels.

Preparation of *A. paniculata* leaf extract

Sambiloto (*A. paniculata*) leaf extract was acquired from Sambiloto Herbal Supplement produced by Jamu Iboe brand. Each capsule of the supplement contains 500 mg of Sambiloto leaf extract. The capsule was opened to obtain the powdered form of Sambiloto leaf extract, which was then dissolved in a 1% Na-CMC (sodium carboxymethylcellulose) solution. The extract was orally administered according to the respective dose of each treatment group.

Experimental Design

The rats were fed with a standard feed and water ad libitum seven days prior to treatment. Thirty Rattus norvegicus rats were induced to develop urinary tract infection by administering 50 µl of *E. coli* bacterial inoculum for 7 days. The sample was then divided into six treatment groups. The negative control group (CN) consisted of rats that had not been given any treatment. The positive control group (CP) received a single dose of 54 mg of the antibiotic fosfomycin (Monuril®, Zambon, Jakarta, Indonesia). The Sambiloto 100 group (S1) was given 100 mg/kgBW of Sambiloto leaf extract for 7 days. The Sambiloto 200 group (S2) was given 200 mg/kgBW of Sambiloto leaf extract for 7 days. The combination group 100 (FS1) was given a combination of the fosfomycin 54 mg single dose and Sambiloto leaf extract at dose of 100 mg/kgBW for 7 days. The combination group 200 (FS2) was given a combination of the fosfomycin 54 mg single dose and Sambiloto leaf extract at dose of 200 mg/kgBW for 7 days. All treatment was given orally and the combination of Sambiloto leaf extract-fosfomycin was given simultaneously. Then, on the 22nd day after the UTI appeared, urinalysis was performed to measure the growth of the bacterial count, leukocyte esterase values, and procalcitonin levels in each group. The study timeline is shown at Figure 1.

Table 4. Comparative analysis of bacterial count, leukocyte esterase, and procalcitonin in all treatment groups

Group		p-value		
		Bacterial Count§	Leukocyte Esterase§	Procalcitonin⸸
CN	CP	0.009*	0.006*	<0.001*
	S1	0.009*	0.042*	<0.001*
	S2	0.009*	0.005*	<0.001*
	FS1	0.009*	0.006*	<0.001*
	FS2	0.009*	0.005*	<0.001*
CP	S1	0.009*	0.007*	0.025*
	S2	0.009*	0.014*	<0.001*
	FS1	0.248	0.549	0.973
	FS2	0.009*	0.221	0.010*
S1	S2	0.012*	0.093	0.126
	FS1	0.009*	0.007*	0.024*
	FS2	0.009*	0.006*	0.018*
S2	FS1	0.009*	0.011*	<0.001*
	FS2	0.009*	0.007*	<0.001*
FS1	FS2	0.009*	0.513	0.009*

*Significant ($p < 0.05$); §Mann-Whitney; ⸸Post Hoc Games-Howell

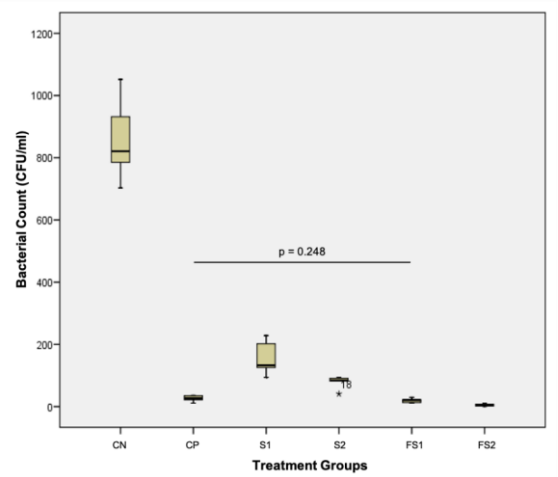

Figure 2. Comparison of bacterial count in all treatment groups analyzed by Mann-Whitney analysis. The differences in each group are statistically significant ($p < 0.05$) other than group CP vs FS1 ($p = 0.248$).

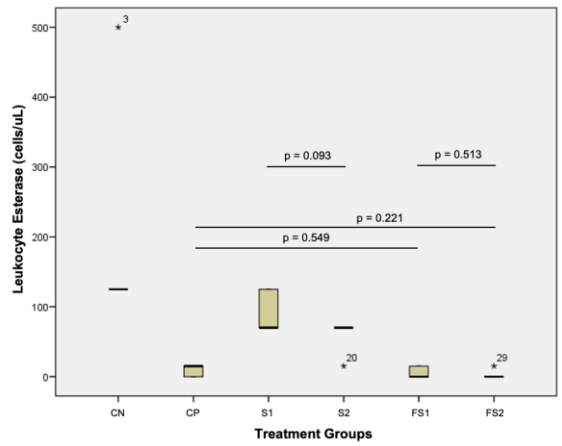

Figure 3. Comparison of leukocyte esterase levels in all treatment groups analyzed by Mann-Whitney analysis. The differences in each group are statistically significant ($p < 0.05$) other than group CP vs FS1 ($p = 0.549$); CP vs FS2 ($p = 0.221$); S1 vs S2 ($p = 0.093$); FS1 vs FS2 ($p = 0.513$).

Induction of Urinary Tract Infection

E. coli strains were retrieved from a storage facility maintained at -80°C in the Microbiology Laboratory, Faculty of Medicine, Universitas Diponegoro. The bacteria were cultured in Brain-Heart Infusion Broth (BHI) media and incubated for 24 hours at 37°C. From the BHI media, cultures were transferred to MacConkey agar plate media. A 0.5 Standard McFarland suspension was prepared, equivalent to 1.5 x 10^8 CFU/ml. This inoculum at a dose of 50 µl was then administered into the urinary tracts of experimental animals using urethral catheter.[26]

Determination of Bacterial Concentration

To assess the bacterial count, the procedure involved a microscopic examination. A technician will enumerate the number of bacteria per field of view (expressed in CFU/ml) with respective steps. The urine sample was diluted to a concentration of 1:1000, and then 0.1 ml of each dilution was pipetted and inoculated on the surface of the plate count agar (PCA) media. Using a sterile spreader, the sample was evenly distributed across the entire surface of the PCA media until it appeared dry. The plates were then inverted and incubated at 37˚C for 24 hours. After incubation, bacterial colonies were counted using a colony counter (Quebec Manual Darkfield Colony Counter, Reichert, USA).

Measurement of Leukocyte Esterase

When assessing leukocyte esterase levels, a dipstick (HEALGEN URS-10 T Reagent Strips for Urinalysis, Zhejiang Orient Gene Biotech Co., Ltd, China) was dipped into the urine sample obtained from the rats. Any color change on the dipstick was observed and the color of the sample's dipstick was compared to the standard color provided by the dipstick manufacturer.

Measurement of Procalcitonin Levels

Urine samples are collected from the experimental animals and then subjected to centrifugation with a speed of 2,000 rpm for 5 minutes. Following centrifugation, the supernatant is discarded, then the urine was furtherly analyzed using a procalcitonin immunoassay (Rat Procalcitonin Enzyme-Linked Immunosorbent Assay

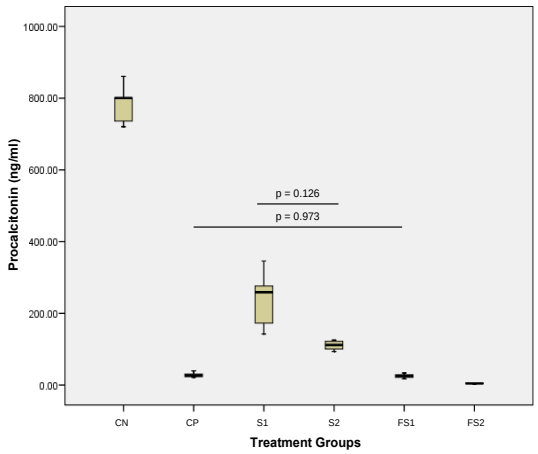

Figure 4. Comparison of procalcitonin levels in all treatment groups analyzed by Post Hoc Games-Howell analysis. The differences in each group are statistically significant ($p < 0.05$) other than group CP vs FS1 ($p = 0.973$); S1 vs S2 ($p = 0.126$).

Kit, Elabscience Biotechnology Co., Ltd., Wuhan, China; Detection range = 97-109%; Sensitivity = 9.38 pg/mL)

Statistical Analysis

The data were analyzed by using SPSS version 26.0. The data normality test was carried out using the Shapiro-Wilk test. Data that were not normally distributed was analyzed by using non-parametric statistics. Data with a ratio scale and not normally distributed were subjected to the Kruskal-Wallis test, followed by the post-hoc Mann-Whitney U test. The p-value was considered significant if $p < 0.05$ with a 95% confidence interval.

RESULTS

The subjects of this study included the germ number, leukocyte esterase, and urine procalcitonin levels. Based on the results of univariate analysis, the characteristics of the research subjects can be identified as shown in tables 1, 2 and 3.

The germ number data was normally distributed with $p > 0.05$ in CN, CP, S1, FS1, and FS2 groups. found to be not normally distributed, whereas the procalcitonin data were normally distributed. Therefore, Kruskal-Wallis test was employed to compare the germ number and leukocyte esterase data, while the One-Way ANOVA test was used for comparing the procalcitonin data. Based on these two tests, all data showed significant results with $p < 0.001$. The Mann-Whitney test was then carried out on the data on the germ number and leukocyte esterase and the Post Hoc Games-Howell test on the procalcitonin data to determine the differences in numbers between treatment groups. The results of the Mann-Whitney and Post Hoc analysis are shown in table 4 and figure 2, 3, and 4.

Among all treatment groups, the administration of Sambiloto leaf extract alone at a dose of 100 mg/kgBW showed the highest number of bacteria (156.60 ± 56.06 CFU/ml), as well as the highest level of leukocyte esterase (92.00 ± 30.13 cells/uL) and procalcitonin (239.29 ± 82.00 ng/ml). In contrast, the combination of Sambiloto leaf extract and conventional antibiotics, specifically fosfomycin in this study, resulted in reduced germ number and lower levels of leukocyte esterase and procalcitonin as compared to the negative control group. A combination of Sambiloto leaf extract and fosfomycin at 100 mg/kgBW reduced the germ number to 19.40 ± 7.64 CFU/ml. This combination also lower the leukocyte esterase and procalcitonin levels to 6 ± 8.22 cells/uL and 25.29 ± 6.24 ng/ml, respectively. A higher dose of fosfomycin at 200 mg/kgBW in combination with Sambiloto leaf extract exhibited an enhanced effect in lowering the germ number, leukocyte esterase, and procalcitonin levels to 4.80 ± 3.70 CFU/ml, 3.00 ± 6.71 cells/uL, and 4.66 ± 1.35 ng/ml, respectively, representing the lowest levels of the measured parameters in this study, even when compared to the positive control group.

The comparative analysis using Mann-Whitney test, as shown in Figure 2 and 3, demonstrated a significant difference ($p < 0.05$) of germ number in all treatment groups except for the positive control group and FS1 group ($p = 0.248$), and the differences of leukocyte esterase levels in each group are also statistically significant ($p < 0.05$) except for group CP vs FS1 ($p = 0.549$), CP vs FS2 ($p = 0.221$), S1 vs S2 ($p = 0.093$), and FS1 vs FS2 ($p = 0.513$). The Post Hoc analysis of procalcitonin levels, as shown in Figure 4, revealed a significant difference ($p < 0.05$) among all treatment groups other than CP vs FS1 ($p = 0.973$) and S1 vs S2 ($p = 0.126$).

DISCUSSION

The results show that 100 mg/BW of Sambiloto leaf extract, as an antimicrobial agent, proves to reduce the germ number to 156.60 ± 56.06 CFU/ml. In addition, administering 200 mg/BW reduces the germ number to 78.40 ± 21.48 CFU/ml. The findings of this study indicate that a higher dosage of Sambiloto extract displayed superior efficacy in reducing the germ number in the UTI rat model. Therefore, it can be concluded that increasing the dose of Sambiloto leaf extract is directly proportional to decreasing the number of germs. This aligns with research by Mishra et al., who find that sambiloto has the main compound in the form of andrographolide. Andrographolide can fight disease due to its strong antibacterial properties and its ability to activate B lymphocyte cells to produce antibodies.[27]

The results of the study also revealed a further reduction in germ number, even lower than the positive control group which received fosfomycin alone, following the administration of Sambiloto leaf extract administration at 100 mg/kgBW and 200 mg/kgBW with fosfomycin combination. Fosfomycin is a broad spectrum antibiotic which is effective against gram-positive and gram-negative bacteria, especially gram-negative bacteria that are the most common causes of urinary tract infections, including *Escherichia coli*, *Klebsiella* sp., *Enterobacter* sp. and *Proteus* sp.[28] Previous studies showed a synergistic effect between the combination of andrographolide with conventional antibiotics, including fosfomycin, was observed against *Pseudomonas aeruginosa* strains. A significant increase in the anti-infection property and anti-biofilm activity was observed when the antibiotics were combined with andrographolide.[29] Some benefits associated with

combining antimicrobial agents like antibiotics with andrographolide include heightened antibacterial activity, mitigation of side effects, reduction in the duration of long-term antimicrobial therapy, and prevention of the emergence of resistant microorganisms.[30] In normal rats, as observed in humans, the urine is sterile, with no bacteria found in microscopic examinations.[31] However, in UTI model rats, consistent with the study findings, administering a combination of fosfomycin and Sambiloto leaf extract at dose of 200 mg/kgBW resulted in a greater reduction in bacterial count compared to the combination of fosfomycin and Sambiloto leaf extract at dose of 100 mg/kgBW.

The reduction in the germ number when administering a combination of fosfomycin and Sambiloto leaf extract is also directly proportional to the decrease in leukocyte esterase and procalcitonin, which are used as markers for detecting UTI. It shows a decrease in leukocyte esterase with the greatest results after being given a combination of Sambiloto leaf extract 200 mg/BW and fosfomycin. This is caused by the anti-inflammatory effect of andrographolide, a compound found in Sambiloto leaf extract. Andrographolide can inhibit neutrophil adhesion/transmigration by suppressing MAC-1 upregulation. Studies have shown that andrographolide possesses anti-inflammatory properties by inhibiting the initial phase of neutrophil infiltration. This inhibition effectively reduces the release of esterase enzymes caused by leukocyte membrane lysis. As a result, the release of esterase enzymes due to inflammation-induced leukocyte lysis can be diminished.[32,33]

The largest reduction in procalcitonin levels is also obtained after being given a combination of the Sambiloto leaf extract 200 mg/BW and fosfomycin. Thus, it can be concluded that increasing the dose of Sambiloto leaf extract combined with fosfomycin can reduce the germ number, leukocyte esterase, and calcitonin levels higher than using Sambiloto leaf extract or fosfomycin alone. Based on research conducted by Li et al., andrographolide can significantly inhibit the expression of TNF-α, IL-6, and IL-1β due to LPS stimulation.[34] In the context of inflammation, heightened procalcitonin levels correlate with the presence of bacterial endotoxins and inflammatory cytokines.[35] Therefore, indirectly, andrographolide is able to reduce procalcitonin levels because it inhibits the expression of proinflammatory cytokines and bacterial endotoxin, both of which are procalcitonin activators.[34]

This research still presents several limitations. It is necessary to identify the synergistic interaction mechanism observed from the combination of fosfomycin and Sambiloto extract as a basis for conducting direct evaluations in humans with UTI. Such evaluations would provide valuable insights into the potential of this combination therapy as a novel alternative for UTI treatment in the future. Further research is recommended to investigate the interactions between other antimicrobials and Sambiloto leaf extract, determining whether these interactions are synergistic or antagonistic.

CONCLUSION

A combination of Sambiloto leaf extract and fosfomycin reduced the germ number, levels of leukocyte esterase, and procalcitonin in Rattus norvegicus UTI models. The greatest decrease in the number of germs, leukocyte esterase levels, and procalcitonin levels occurred when the combination of Fosfomycin and Sambiloto leaf extract was administered at a dose of 200 mg/kg BW. The combination of the antibiotic and Sambiloto leaf extract improved the antimicrobial effectiveness compared to using either the conventional antibiotic or the Sambiloto extract alone.

REFERENCES

1. Chu CM, Lowder JL. Diagnosis and treatment of urinary tract infections across age groups. Am J Obstet Gynecol. 2018 Jul;219(1):40–51.
2. Mody L, Juthani-Mehta M. Urinary tract infections in older women: a clinical review. JAMA. 2014 Feb;311(8):844–54.
3. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol. 2015 May;13(5):269–84.
4. Magill SS, Edwards JR, Bamberg W, Beldavs ZG, Dumyati G, Kainer MA, et al. Multistate point-prevalence survey of health care-associated infections. N Engl J Med. 2014 Mar;370(13):1198–208.
5. Tandogdu Z, Wagenlehner FME. Global epidemiology of urinary tract infections. Curr Opin Infect Dis. 2016 Feb;29(1):73–9.
6. Cek M, Tandoğdu Z, Wagenlehner F, Tenke P, Naber K, Bjerklund-Johansen TE. Healthcare-associated urinary tract infections in hospitalized urological patients--a global perspective: results from the GPIU studies 2003-2010. World J Urol. 2014 Dec;32(6):1587–94.
7. Choe H-S, Lee S-J, Cho Y-H, Çek M, Tandoğdu Z, Wagenlehner F, et al. Aspects of urinary tract infections and antimicrobial resistance in hospitalized urology patients in Asia: 10-Year results of the Global Prevalence Study of Infections in Urology (GPIU). J Infect Chemother Off J Japan Soc Chemother. 2018 Apr;24(4):278–83.
8. Nemin A, Widyaningsih Y, Bahrun U, Kurniawan L. Karakteristik Pasien Infeksi Saluran Kemih di Rumah Sakit Umum Lasinrang Kabupaten Pinrang Provinsi Sulawesi Selatan Tahun 2018-2019. Makassar: Universitas Hasanuddin; 2020.
9. Zdrodowska-Stefanow B, Ostaszewska-Puchalska I, Badyda J, Galewska Z. The evaluation of markers of prostatic inflammation and function of the prostate gland in patients with chronic prostatitis. Arch Immunol Ther Exp (Warsz). 2008;56(4):277–82.
10. Masajtis-Zagajewska A, Nowicki M. New markers of urinary tract infection. Clin Chim Acta. 2017 Aug;471:286–91.

11. Levine A, Tran M, Naut E. 640: THE DIAGNOSTIC VALUE OF PROCALCITONIN IN URINARY TRACT INFECTIONS. Crit Care Med [Internet]. 2018 Jan [cited 2024 Jul 10];46(1):306–306. Available from: https://journals.lww.com/ccmjournal/fulltext/2018/01001/640__the_diagnostic_value_of_procalcitonin_in.604.aspx
12. Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. Nat Med. 2004;10(12):S122–9.
13. Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev. 2010 Sep;74(3):417–33.
14. Ríos E, del Carmen López Diaz M, Culebras E, Rodríguez-Avial I, Rodríguez-Avial C. Resistance to fosfomycin is increasing and is significantly associated with extended-spectrum β-lactamase-production in urinary isolates of Escherichia coli. Med Microbiol Immunol [Internet]. 2022 Dec 1 [cited 2024 Jun 16];211(5–6):269–72. Available from: https://link.springer.com/article/10.1007/s00430-022-00749-2
15. Abdelraheem WM, Mahdi WKM, Abuelela IS, Hassuna NA. High incidence of fosfomycin-resistant uropathogenic E. coli among children. BMC Infect Dis [Internet]. 2023 Dec 1 [cited 2024 Jun 16];23(1):1–9. Available from: https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-023-08449-9
16. Mittal AK, Bhardwaj R, Mishra P, Rajput SK. Antimicrobials Misuse/Overuse: Adverse Effect, Mechanism, Challenges and Strategies to Combat Resistance. Open Biotechnol J. 2020;14(1):107–12.
17. Sobel GI (j, Editor S. Alternative Approaches to Conventional Treatment of Acute Uncomplicated Urinary Tract Infection in Women.
18. Anand U, Jacobo-Herrera N, Altemimi A, Lakhssassi N. A Comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics: Potential Avenues of Biocompatible Drug Discovery. Metabolites [Internet]. 2019 Nov 1 [cited 2024 Jun 13];9(11). Available from: /pmc/articles/PMC6918160/
19. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014 Jan;4:177.
20. Lin HC, Li CC, Yang YC, Chiu TH, Liu KL, Lii CK, et al. Andrographis paniculata diterpenoids and ethanolic extract inhibit TNFα-induced ICAM-1 expression in EA.hy926 cells. Phytomedicine. 2019;52:157–67.
21. Lu CY, Yang YC, Li CC, Liu KL, Lii CK, Chen HW. Andrographolide inhibits TNFα-induced ICAM-1 expression via suppression of NADPH oxidase activation and induction of HO-1 and GCLM expression through the PI3K/Akt/Nrf2 and PI3K/Akt/AP-1 pathways in human endothelial cells. Biochem Pharmacol. 2014;91(1):40–50.
22. Bao Z, Guan S, Cheng C, Wu S, Wong SH. A Novel Antiinflammatory Role for Andrographolide in Asthma via Inhibition of the Nuclear Factor-k B Pathway. Am J Respir Crit Care Med. 2009;179(16):657–65.
23. Lim JC-W, Goh F-Y, Sagineedu S-R, Yong AC-H, Sidik SM, Lajis NH, et al. A semisynthetic diterpenoid lactone inhibits NF-κB signalling to ameliorate inflammation and airway hyperresponsiveness in a mouse asthma model. Toxicol Appl Pharmacol. 2016 Jul;302:10–22.
24. Jiang CG, Li J Bin, Liu FR, Wu T, Yu M, Xu HM. Andrographolide inhibits the adhesion of gastric cancer cells to endothelial cells by blocking E-selectin expression. Anticancer Res. 2007;27(4 B):2439–47.
25. Ou H, Xiao X, Jiang Y, Peng Y, Yang M, Gao M. Expression of microRNA-23b in patients with sepsis and its effect on leukocytes and the expression of E-selectin and ICAM-1. Exp Ther Med. 2018;16:4707–11.
26. Zychlinsky Scharff A, Albert ML, Ingersoll MA. Urinary tract infection in a small animal model: Transurethral catheterization of male and female mice. J Vis Exp. 2017;2017(130):6–11.
27. Mishra US, Mishra A, Kumari R, Murthy PN, Naik BS. Antibacterial Activity of Ethanol Extract of Andrographis paniculata. Indian J Pharm Sci. 2009 Jul;71(4):436–8.
28. Raz R. Fosfomycin: an old--new antibiotic. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis. 2012 Jan;18(1):4–7.
29. Zeng X, Liu X, Bian J, Pei G, Dai H, Polyak SW, et al. Synergistic effect of 14-alpha-lipoyl andrographolide and various antibiotics on the formation of biofilms and production of exopolysaccharide and pyocyanin by Pseudomonas aeruginosa. Antimicrob Agents Chemother. 2011 Jun;55(6):3015–7.
30. Ativui S, Danquah CA, Ofori M, Gibbons S, Bhakta S, Doe P. Antibacterial and Antifungal Activities of Andrographolide in Combination with Antimicrobial Drugs. Res J Pharmacogn. 2022;9(4):21–7.
31. Sauer MB, Dulac H, Clark S, Moffitt KM, Price J, Dambach D, et al. Clinical Pathology Laboratory Values of Rats Housed in Wire-Bottom Cages Compared with Those of Rats Housed in Solid-Bottom Cages. J Am Assoc Lab Anim Sci. 2006;45(1):30–5.
32. Brunzel N. Fundamentals of Urine and Body Fluid Analysis. Philadelphia: WB Saunders Company; 2004. 119–263 p.
33. Shen Y-C, Chen C-F, Chiou W-F. Andrographolide prevents oxygen radical production by human neutrophils: possible mechanism(s) involved in its anti-inflammatory effect. Br J Pharmacol. 2002 Jan;135(2):399–406.
34. Li Y, He S, Tang J, Ding N, Chu X, Cheng L, et al. Andrographolide Inhibits Inflammatory Cytokines Secretion in LPS-Stimulated RAW264.7 Cells through Suppression of NF-κB/MAPK Signaling Pathway. Evid Based Complement Alternat Med. 2017;2017:8248142.

35. Faizah AK. Procalcitonin as Biomarker of Antibiotic Effectivity in Community-Acquired Pneumonia. Folia Medica Indones. 2015;51(1):96–100.

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Original Research Article

Revealing the Potency of Camelia sinensis and Serenoa repens as Purinoreceptor Inhibitor for Benign Prostatic Hyperplasia Treatment Through in Silico Study

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Abstract

Background: Benign prostatic hyperplasia (BPH) is the most common prostate disease in elderly men that leads to a significant deterioration in patients' quality of life (QoL). Pharmacological therapy of 5-alpha reductase inhibitor and alpha adrenoreceptors blocker often causes several side effects that decrease the QoL, so it is necessary to develop a new treatment for BPH. Purinoreceptor is a novel receptor that can inhibit electrically evoked nerve-mediated contractions in the prostate. Tea leaves (*Camellia sinensis*) and Saw palmetto (*Serenoa repens*) are herbs that have potential as alternative therapies for BPH

Objective: to reveal the potency of *Camelia sinensis* and *Serenoa repens* as purinoreceptor inhibitor for benign prostatic hyperplasia treatment through in silico study

Methods: This study using in silico method. Structures of active compounds were extracted from PubChem and protein from Protein Data Bank (PBD). The active compounds *Camellia sinensis* and *Serenoa repens* to the target protein *purinoreceptors, 5-alpha-reductase,* and *alpha adrenoreceptors* was evaluated *in silico* using a docking server with Finasteride dan Tamsolusin as a control. Molecular docking method using docking server application.

Results: Epigallocatechin gallate only compound that has potency in blocking purinoceptors and 5-alpha-reductase with free energy binding -6.81 kcal/mol and -6.79 kcal/mol. Capric acid, Caprylic acid, Lauric acid, Linoleic acid, and Myristic acid have the potential to bind to alpha adrenoreceptor ligands with free energy binding-4.37 kcal/mol, -4.04 kcal/mol, -3.75 kcal/mol, -3.08 kcal/mol, and -3.24 kcal/mol.

Conclusion: In silico study showed that *Camellia sinensis* have potential and effects as alternative therapies in benign prostatic hyperplasia on the target protein purinoreceptors, 5-alpha-reductase, and alpha adrenoreceptors. But, *Serenoa repens* have potential only through alpha adrenoreceptors. This study offers a potential alternative for BPH treatment using natural components. This is significant given the need for safer treatment options with fewer side effects compared to conventional therapies.

Keywords: *Camellia sinensis; Serenoa repens; Benign prostatic hyperplasia; In silico; Purinoreceptor*
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INTRODUCTION

Benign prostatic hyperplasia (BPH) is a non-malignancy growth of prostate tissue. BPH are common cause lower urinary tract symptom (LUTS) in elderly man. BPH is increases at the age of 90 years old with prevalence 8%-60%.[1]

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BPH can causes obstruction by increases compression due to increases prostate volume and smooth muscle[2]. Manifestation of hyperplasia prostate are urgency, nocturia, hesitancy, streaming, straining, and prolong micturition[3]. Common complications of BPH are urinary tract infection, hydronephrosis, nephrolithiasis.[2]

Early treatment of patient with BPH is modification of life style or pharmacology therapy. Pharmacology therapy that can use in with BPH is 5-alpha- reductase inhibitor such as Finasteride and alpha blocker such as Tamsolusin[4]. Mechanism action of 5-alpha-inhibit growth effect androgen in testosterone with reduction of testosterone conversion to dihydrotestosterone. Alpha-blocker works by relaxing smooth muscle of prostate and bladder neck with inhibit sympathetic nerve[3]. This two class therapy of BPH have any adverse effect such as impotence, decreased libido, and ejaculation dysfunction and hypotension.[3] So another treatment with minimal adverse effect is needed in BPH.

In early decade, another research showed that there was a P2X-purinoceptor in smooth muscle of prostate[5]. Purinoreceptor are ATP-gated and acetylcholine canal that location in musculus detrusor[6]. Blockade in this receptor can inhibit parasympathetic innervation that can make relaxation of detrusor[6]. This receptor is not found in blood vessel so it does not cause vasodilatation of blood vessels[6]. So, we need to evaluate the potency of P2X-purinoreceptor as target protein on BPH treatment. Indonesia is a country with more than 6000 herb that can use as traditional medication[7]. Tea (*Camelia sinensis*) is one of the Indonesian herb that has potential as BPH treatment[8] and prostate cancer[9]. Tea contains compounds epigallocatechin gallate, gallocatechin gallate, gallocatechin, catechin, epicatechin, gallate epicatechin dan epigallocatechin[10]. In addition saw palmetto (*Serenoa* repens) has potential as BPH treatment.[11] This herb contains *Lauric acid* (30,2%), *Myristic acid* (12,0%), *Oleic acid* (28,5%), *Palmitic acid* (9,5%), *Linoleic acid* (4,6%) dan *Capric acid* (2,5%).[10] However mechanism action is still unknown, further research is needed [11]. Based on explanation above, further research is needed to know the potential of Tea and Saw palmetto active compounds as alternative treatment on BPH through in silico study. In silico studies are essential for researching the potential of tea and saw palmetto compounds as BPH treatments due to their cost-effectiveness, time efficiency, and ability to screen large compound libraries quickly. Additionally, they facilitate personalized medicine by modelling compound effects on different genetic profiles, making them a crucial preliminary step before costly and time-consuming in vitro and in vivo studies.

MATERIALS AND METHODS

This study use in-silico method by analyzing the interaction of *Camellia sinensis* and *Serenoa repens* which contain capric acid, caprylic acid, catechin, epicatechin, epigallocatechin gallate, epigallocatechin, finasteride (K), gallate epicatechin, gallocatechin gallate, gallocatechin, lauric acid, myristic acid, oleic acid, linoleic acid and palmitic acid to three target proteins which are 5-alpha-reductase, purinoreceptor, dan alpha adrenoreceptor.

Material and tools

The structure of the active compounds *Camellia sinensis* and *Serenoa repens* which consist of Capric acid (ID:2969), Caprylic acid (ID:379), Catechin (ID:9064), Epicatechin (ID:72276), Epigallocatechin gallate (ID:65064), Epigallocatechin (ID:72277), Gallate epicatechin (107906), Gallocatechin gallate (ID:199472), Gallocatechin (ID:65084), Lauric acid (ID:3893), Myristic acid (ID:11005), Oleic acid (ID:445639), Linoleic acid (ID: 5280450) dan Palmitic acid (ID:985) are gained form Pubchem.com. Ligands for control using Finasteride (ID:57363) and tamsulosin (ID:121829) gained from www.pubchem.com. This research using target protein purinoreceptor (ID:5SVQ), and alpha adrenoreceptor (ID: P35368) gained from Protein Data Bank. Target protein of 5-alpha-reductase gained from NCBI GenBank with (ID AAC26863) and converted from FASTA into PDB format using Swiss-Model website (https://swissmodel.expasy.org/). Using hardware with specification RAM 4096 MB, *Intel ® core ™* I7, CPU @2.60 GHz, system operation with *Microsoft Windows 10 Pro 64-BIT*, internet connection and *software* based on *web autodock 4.0* at *docking* server (http://www.dockingserver.com).

In Silico test of *Camelia sinensis* and *Serenoa repens* Active Compound to 5-alpha-reductase, purinoreceptor, and alpha adrenoreceptor.

The ligand compounds were downloaded in Pubchem than continue to molecular docking test by using a docking server. The docking server accessed at (http://www.dockingserver.com).

Data Analysis Technique

In this study, Lipinski's Rule of Five was employed to assess the drug-likeness of plant compounds intended for medicinal use. Ligand pharmacological testing based on Lipinski's 5 rules (RO5) is carried out to analyze the potential of a chemical compound based on pharmacological and biological activity as an oral drug for humans. Lipinski's Rule of Five accessed from http://targetnet.scbdd.com/calcnet/calc_rule_text/ . Additionally, ADMET Rule Five data were analyzed to evaluate the compounds' absorption, distribution, metabolism, excretion, and toxicity properties. These methodologies were utilized to determine the suitability of plant compounds as potential medicines and to assess their potential toxicity profiles, which were accessed from https://biosig.lab.uq.edu.au/pkcsm/prediction. In silico test were observed with parameters of free binding energy, inhibition constant, surface interaction and amino acid residues between ligand and target protein.

RESULTS

The result of Lipski Rule of Five showed on table 1. The table evaluates various active compounds according to Lipinski's Rule of Five, which predicts the drug-likeness of a molecule based on its pharmacokinetic properties. The parameters include Topological Polar Surface Area (TPSA), Molecular Weight (MW), number of Hydrogen Bond Donors, and number of Hydrogen Bond Acceptors (HBA).

Table 1. Lipinski's Rule of Five

Active Compound	TPSA	MW	Molecular Weight	Hydrogen Bond Donor	HBA	Lipinski Rule of Five (%)
Capric acid	37.3	51.9558	172.2646	1.0	2.0	100
Caprylic acid	37.3	42.3418	144.21144	1.0	2.0	100
Catechin	110.38	74.3338	290.26806	5.0	8.0	75
Epicatechin	110.38	74.3338	290.26806	5.0	8.0	75
Epigallocatechin gallate	97.37	112.0645	458.37172	8.0	13.0	50
Epigallocatechin	130.61	76.3568	306.26746	6.0	9.0	75
Gallate epicatechin	177.14	110.0415	442.37232	7.0	12.0	50
Gallocatechin gallate	197.37	112.0645	458.37172	8.0	13.0	50
Gallocatechin	130.61	76.3568	306.26746	6.0	9.0	75
Lauric acid	37.3	61.5698	200.31776	1.0	2.0	100
Myristic acid	37.3	71.1838	228.37092	1.0	2.0	100
Oleic acid	37.3	89.9378	282.46136	1.0	2.0	75
Linoleic acid	37.3	89.4638	280.44548	1.0	2.0	75
Palmitic acid	37.3	80.7978	256.42408	1.0	2.0	75

TPSA, Topological Polar Surface Area; MW, Molecular Weight; HBA, Hydrogen Bond Acceptor.

Table 2. Pharmacokinetic Characteristics

Active Compound	Absorbtion	Distribution	Metabolism				Excretion	Toxicity
	Intestinal Absorbtion (% Absorbed)	BBB Permiability (log BB)	CYP2D6 (S/I)	CYP3A4 (S/I)	CYP1A2 (I)	CYP2C9 (I)	Total Clearance (log ml/min/kg)	Hepatotoxicity
Capric acid	94.06	0.142	N/N	N/N	N	N	1.552	N
Caprylic acid	94.75	0.225	N/N	N/N	N	N	1.48	N
Catechin	68.82	-1.054	N/N	N/N	N	N	0.183	N
Epicatechin	68.82	68.820	N/N	N/N	N	N	0.183	N
Epigallocatechin gallate	47.39	-2.184	N/N	N/N	N	N	0.292	N
Epigallocatechin	54.12	-1.377	N/N	N/N	N	N	0.328	N
Gallate epicatechin	62.09	-1.847	N/N	N/N	N	N	-0.169	N
Gallocatechin gallate	47.39	47.39	N/N	N/N	N	N	0.292	N
Gallocatechin	54.12	-1.377	N/N	N/N	N	N	0.328	N
Lauric acid	93.37	0.057	N/N	N/N	N	N	1.623	N
Myristic acid	92.69	-0.027	N/N	N/N	N	N	1.693	N
Oleic acid	91.82	-0.168	N/N	Y/N	Y	N	1.884	N
Linoleic acid	92.32	-0.142	N/N	Y/N	Y	N	1.936	Y
Palmitic acid	92.00	-0.111	N/N	Y/N	N	N	1.763	N

S, substrate; I, inhibitor; Y, Yes; N, No

The percentage compliance with Lipinski's Rule of Five is also listed. Compounds like Capric acid, Caprylic acid, and Lauric acid show 100% compliance, indicating high potential as drugs due to favourable properties such as lower molecular weight, appropriate hydrogen bonding capacity, and suitable lipophilicity. Conversely, compounds like Gallocatechin gallate and Gallic acid have only 50% compliance, suggesting potential issues with bioavailability and absorption due to their higher molecular weight and excessive hydrogen bonding characteristics.

The result of pharmacokinetics characteristics showed on table 2. The table presents the pharmacokinetic characteristics of various active compounds, focusing on absorption, distribution, metabolism, excretion, and toxicity. Intestinal absorption percentages range from 47.39% to 94.75%. Blood-Brain Barrier (BBB) permeability values (log BB) vary from -2.184 to 0.225. Metabolic interactions are noted with several CYP enzymes, where most compounds do not act as substrates or inhibitors (N/N). Total clearance rates, measured in log ml/min/kg, span from -0.169 to 1.936. Only Gallic acid showed hepatotoxicity. Based on these factors, compounds such as Capric acid and Lauric acid appear to be the safest, while Gallic acid is the least safe due to its hepatotoxicity.

The result of molecular docking showed on table 3. The result of molecular docking between 5-alpha-reductase ligand and epigallocatechin gallate compound has a lower free energy than the control tamsulosin while other compounds have a greater free binding energy than the control. *Purinoreceptor* ligand showed that epigallocatechin gallate, gallate epicatechin, and gallocatechin gallate, has lower free binding energy than control finasteride.

Table 3. Result of Molecular Docking

Ligand	Active Compound	Free Energy Binding	Inhibition constant	Surface Interaction	Molecule Interaction	
					Hydrogen Bond	Hydrophobic bond
5-alpha-reductase	Capric acid	-2.50 kcal/mol	14.60 mM	405.45	-	LEU88 MET90 PHE91 HIS94
	Caprylic acid	-2.44 kcal/mol	16.17 mM	354.927	-	LEU87 LEU88 MET90 PHE91 HIS94
	Catechin	-4.22 kcal/mol	808.60 uM	439.409	SER63 TYR95	PRO62
	Epicatechin	-4.84 kcal/mol	282.64 uM	447.609	-	TRP56
	Epigallocatechin gallate	-6.79 kcal/mol	10.48 uM	710.29	-	LEU16 LEU66 PRO67
	Epigallocatechin	-4.86 kcal/mol	276.01 uM	450.051	SER63 PRO62	PRO62
	Gallate epicatechin	-6.54 kcal/mol	16.13 uM	581.701	SER63	GLN20 GLU60 TYR95
	Gallocatechin gallate	-6.17 kcal/mol	30.03 uM	600.881	SER63	SER63 PHE91
	Gallocatechin	-4.25 kcal/mol	761.31 uM	491.999	-	LEU66 PRO67
	Lauric acid	-2.77 kcal/mol	9.35 mM	449.793	-	LEU87 MET90 PHE91 HIS94 TYR95
	Myristic acid	-2.83 kcal/mol	8.42 mM	515.864	-	PRO62 LEU66 PRO67 PHE91 TYR95
	Oleic acid	-3.09 kcal/mol	5.42 mM	603.891	-	PRO67 LEU87 LEU88 PHE91 HIS94 TYR95
	Linoleic acid	-2.98 kcal/mol	6.59 mM	614.199	-	PHE91 LEU66 LEU16 PRO67 LEU88
	Palmitic acid	-2.01 kcal/mol	33.40	603.467	-	PHE91 LEU16 LEU66 PRO67 LEU88
	Finasteride (C)	-6.56 kcal/mol	15.49 uM	527.241	SER63	PRO62 LEU66 PRO67 LEU88 PHE91

Table 3. Cont.

Ligand	Active Compound	Free Energy Binding	Inhibition constant	Surface Interaction	Molecule Interaction	
					Hydrogen Bond	Hydrophobic bond
Purinoreceptor	Capric acid	-3.03 kcal/mol	6.00 mM	451.101	-	PHE171
	Caprylic acid	-2.93 kcal/mol	7.08 mM	398.097	-	PHE171
	Catechin	-4.48 kcal/mol	516.25 uM	444.259	-	MET166
	Epicatechin	-4.81 kcal/mol	295.77 uM	456.051	-	MET166
	Epicatechin	-4.86 kcal/mol	275.42 uM	453.106	-	MET166
	Epigallocatechin gallate	-6.81 kcal/mol	10.23 uM	483.764	-	MET166
	Epigallocatechin	-4.85 kcal/mol	276.55 uM	446.898	-	MET166
	Gallate epicatechin	-6.21 kcal/mol	28.07 uM	453.048	-	MET166
	Gallocatechin gallate	-5.56 kcal/mol	83.68 uM	560.567	-	
	Gallocatechin	-4.29 kcal/mol	720.41 uM	461.379	-	
	Lauric acid	-3.00 kcal/mol	6.34 mM	497.382	-	LEU69 PHE171
	Linoleic acid	-3.68 kcal/mol	2.00 mM	518.236	ARG204	PHE171
	Myristic acid	-3.05 kcal/mol	5.77 mM	465.91	-	PHE171
	Oleic acid	-3.25 kcal/mol	4.12 mM	592.546	ARG204	LEU69 VAL74 PHE171
	Palmitic acid	-3.32 kcal/mol	3.66 mM	568.769	-	VAL74 PHE171
	Tamsolusin (C)	-7.19 kcal/mol	5.39 uM	559.081	MET166 GLU169 ASN170	MET166 PHE229
	Finasteride (C)	-5.45 kcal/mol	101.23 uM	566.818	VAL309	LEU308 LEU265 ILE56
alpha adrenoceptor	Capric acid	-4.37 kcal/mol	621.98 uM	385.899	-	LEU75 CYS110 ILE114 LEU117 VAL277 PHE281 TRP285
	Caprylic acid	-4.04 kcal/mol	1.10 mM	345.563	-	LEU68 ILE114 LEU117 VAL277 PHE281 TRP285
	Lauric acid	-3.75 kcal/mol	1.78 mM	423.903	SER319	LEU68 LEU75 ILE114 LEU117 VAL277 PHE281 TRP285

Table 3. Cont.

Ligand	Active Compound	Free Energy Binding	Inhibition constant	Surface Interaction	Molecule Interaction	
					Hydrogen Bond	Hydrophobic bond
alpha adrenoceptor	Linoleic acid	-3.08 kcal/mol	5.51 mM	622.075	-	ILE276 CYS280 LEU283 CYS284 ILE321 ILE324 ILE325 CYS328
	Myristic acid	-3.24 kcal/mol	4.25 mM	525.535	-	ILE276 CYS280 ILE321 ILE324 ILE325 CYS328
	Catechin	+24.13 kcal/mol		498.394	SER113 TRY326 ASN322	LEU75 LEU117 LEU68 VAL277
	Epicatechin	+20.08 kcal/mol		495.015	TRY326 CYS110 ASN322	PHE281 LEU117 ILE114 VAL277 LEU68
	Epigallocatechin gallate	+122.24 kcal/mol		598.386	TRP285 CYS110 TYR326 SER113	SER319 ILE65 ILE120 ALA71 LEU274
	Epigallocatechin	+19.98 kcal/mol		494.835	CYS110 TYR326 ASN322	PHE281 LEU117 ILE114 VAL277 LEU68
	Gallate epicatechin	+110.87 kcal/mol		594.1	SER319 TRP285 CYS110 SER113 TYR326	LEU68 LEU274 PHE281 VAL277 ILE114 LEU75 ILE120 LEU117
	Gallocatechin gallate	+140.55 kcal/mol		621.448	TYR326 SER113 ASN322	LEU274 ILE120 VAL277 LEU117 LEU68
	Gallocatechin	+40.63 kcal/mol		595.415	ASN322 CYS110 SER113 TYR326	VAL277 LEU75 LEU117 LEU68
	Oleic acid	+10.36 kcal/mol		604.343	-	LEU117 ALA112 TRP151 VAL277 ALA71 LEU147 ILE120

Table 3. Cont.

Ligand	Active Compound	Free Energy Binding	Inhibition constant	Surface Interaction	Molecule Interaction	
					Hydrogen Bond	Hydrophob bond
alpha adrenoceptor	Palmitic acid	+0.65 kcal/mol		537.351	CYS110	ILE200 CYS118 LEU68 LEU75 PRO196 ILE114 PHE281 LEU117
	Tamsolusin (C)	+1.74 kcal/mol		668.874	CYS280 LYS272 ILE276 SER329	ILE276 CYS280 LEU283 ILE321 ILE325 CYS328

In the adrenoceptor ligand shown that capri acid, caprylic acid, lauric acid, linoleic acid, and myristic acid has lower free binding energy than tamsulosin as a control. The five active compounds are predicted can bind spontaneously and better than finasteride as control. The active compounds that have the same hydrogen bonds as the finasteride control on the 5-alpha-reductase ligand are catechin, epigallocatechin, gallate epicatechin, and gallocatechin gallate compounds. Purinoreceptor ligands do not have the same hydrogen bound like tamsulosin and finasteride as control. Adenoreceptor do not have hydrogen bound like tamsulosin. The result of free binding energy will used to assess the spontaneity and stability of the bound.

Inhibition constant shown that only epigallocatechin gallate compound has a lower inhibition constant than finasteride at 5-alpha-reductase ligand. Epigallocatechin gallate compound predicted have a lower inhibition constant value than the control wen its binds to the ligand. The result of alpha adrenoreceptor ligand and purinoreceptor shown that all compound has high inhibition constant value compared with tamsulosin. Data of value inhibition constant will be used to assess the magnitude of the binding inhibition that affected in the protein-ligand bound shows in figure 1-3. The surface interaction of the 5 alpha reductase ligands showed that gallocatechin gallate, gallate epicatechin, and epigallocatechin gallate had higher value than the finasteride as control.

All the compound of alpha adrenoreceptor ligand has lower interaction compared with tamsulosin. The value of the surface interaction will be used to assess the probability of protein-ligand interaction as indicated by the size and molecule area.

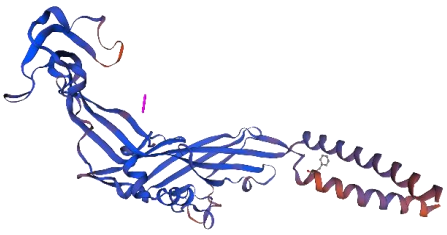

Figure 1. Crystal Structure of purinoreceptor

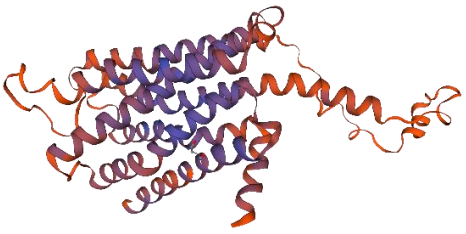

Figure 2. Crystal Structure of adrenoreceptor

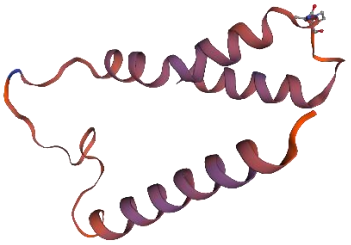

Figure 3. Crystal Structure of 5-alpha-reductase

DISCUSSION

The typical development and operation of the prostate rely on the conversion of testosterone into dihydrotestosterone (DHT) through the action of 5-alpha reductase (5-AR) enzymes, specifically types 1 and 2. There's a hypothesis suggesting that an excess of DHT could play a role in the development of both benign prostatic hyperplasia (BPH) and prostate cancer.[4]The result of docking process with 5-alpha-reductase ligand with herbs active compound showed that lowest free energy at epigallocatechin gallate. In another compound showed the value of free energy is negative but is higher than control. The amount of free energy (ΔG) are

describe the spontaneity and stability of the binding of active compound with target protein. It's suggested that epigallocatechin gallate can binding with 5-alpha-reductase ligand spontaneously and stable more than finasteride. In other compounds can also bind spontaneously, but it less reactive compared with epigallocatechin gallate compound. Herbs' active compound was predicted have ability to bind with protein target and interact spontaneously and reactively if it has a lower or equal free energy than control.[12] In every spontaneous process, increasing protein-ligand happen when there is transformation of free energy binding (ΔG) that have negative value.[13]

Inhibition constant epigallocatechin compound had lover value than control finasteride at 5-alpha-reductase ligand. Epigallocatechin gallate compound predicted has a lower inhibitory value than control when its bind to the ligand.[15] The lowest constant inhibition of free binding energy shows that ligand and target protein was bind strongly. This is due to increasing the tortional from this complex energy makes a stable complex compound and energy.[14] Decreasing value of inhibition constant indicates the smaller inhibition that occurs in increasing the ligand bound.[15]

Surface interaction value at 5-alpha-reductase ligand showed gallocatechin gallate, gallate epicatechin, and epigallocatechin gallate, oleic acid, linoleic acid, and palmitic acid has a higher value than finasteride. If the value of interaction surface higher it's shown more stable binding and give more higher biology activity.[16] Surface interaction also affected by the size of the ligand and give a higher chance for ligand and target protein to binding[16]. In this research predicted that gallocatechin gallate, gallate epicatechin, and epigallocatechin gallate compounds can bind ligand more stable and produce higher biological activity than control.

Based to the data was obtained in this research, it can conclude that the epigallocatechin gallate compound has the highest potential compared to controls and other compound to binding with 5-alpha-reductase ligands. Finasteride can inhibit 5-alpha-reductase enzyme that catalysis conversion of testosterone to the androgen dihydrotestosterone.[18] It is assume that epigallocatechin gallate has a better potential than finasteride in binding to 5-alpha-reductase ligand. The lowest free energy value, lowest inhibition constant, and high surface interaction value compared with finasteride control supported potential of epigallocatechin gallate. While other compounds have weak potential to bind with 5-alpha-reductase ligands.

Hydrogen bonds between ligand molecules and amino acids in the receptor binding pocket can significantly influence the binding energy and specificity of the interaction. For instance, studies on glycine receptor ligands have shown that hydrogen bond formation between the ligand and receptor amino acids (like lysine and aspartic acid) can estimate the binding energy, which correlates with the ligand's inhibitory activity.[17] The efficacy of ligands in inhibiting receptors can also be influenced by hydrogen bonding. Studies on the histamine H2 receptor have shown how hydrogen bond strength, affected by ligand deuteration, can alter ligand-receptor interactions, affecting agonist and antagonist binding and providing insights into receptor function and ligand efficacy.[17]

Active compound again purinoreseptor ligand

In this study used purinoreceptor ligands with tamsulosin and finasteride as control. P2X-Purinoreceptor is responsible for prostate contraction with P2X1-purinoreceptor subtype in humans[18]. Blockade of P2X1-purinoreceptors is known to inhibit electrically nerve-mediated contraction.[20] P2X1 purinoreceptor combined with α1A adrenoreceptor antagonist, may provide move effective relaxation of prostate smooth muscle.[21] Functional study in human prostate has shown that adrenoreceptor antagonists can suppress contractile response at all electric field.[22]

The result of docking with purinoreceptor ligands showed that epigallocatechin gallate compound, gallate epicatechin and gallocatechin gallate had a lower free energy value than finasteride. It is assumed that bounds formed in all compound are occur spontaneously and stable because all compounds have negative value of free energy. However, the best binding compound with the ligand is epigallocatechin gallate compound. In all compound that connected to purinoreceptor ligands, there is no compound had a lower free energy value than tamsulosin. from the data suspected that epigallocatechin gallate, gallate epicatechin and gallocatechin gallate compounds could bind strongly to purinoreceptor ligands. This is due to the low value of free binding energy is able to binding the target protein strongly and can increase potential biological activity.[23]

All inhibition constant showed that all compounds had a higher inhibitory constant value than tamsulosin. In this research predicted that the inhibition at formation protein-ligand interaction is greater than control-ligands. Because of constants inhibition show a greater barrier between ligand and protein target. The lower value inhibition constants indicate the smaller inhibition that occurs in the protein-ligand bound.[16]

The surface interaction showed that gallocatechin gallate, oleic acid and palmitic acid compounds had higher values than tamsulosin. The result indicated that gallocatechin gallate, oleic acid, and palmitic acid compound have potential to bind ligands stably. Increasing surface interaction will increase the docking stability.[24] In this research predicted that the value of surface interaction is depend on the size of molecule and the surface area of the ligand molecule, this causes a higher chance of bounding between the ligand and the compound. The binding of ligands with large hydrophobic areas to enzyme active sites often results in increased stability due to the exclusion of water molecules from the binding interface.[25]

Active compound against alpha adrenoreceptor ligands

This study uses tamsulosin as control. Tamsulosin is a selective antagonist adrenoreceptor α1 with a greater selective for prostate tissue (1A-adrenoceptor dominant) than for vascular tissue (1B dominant). Mechanism's action of tamsulosin is blocking 1A adrenoceptors in the prostate gland. Inhibit smooth muscle contraction and promotes dynamic micturition as well as increase the urinary flow rate (Qmax).[26] Blockade of adrenoceptor

α1A and α1D in the bladder result in inhibit of detrusor muscle contraction, reduced detrusor muscle instability and reduce storage symptoms.[27] Study in human prostate have shown that the contractile response to electrical field stimulation is almost completely suppressed by adrenoceptor antagonists.[19]

Data in this research shown that drug as a control had a positive free energy. Meanwhile, the ligand bounds with capric acid, caprylic acid, lauric acid, linoleic acid and myristic acid compounds have negative free energy. In every spontaneous process bounding of protein-ligand happen if there is change of free energy gibbs (ΔG) at negative system when system reach equilibrium state or constant temperature.[28] Because of the degree of protein-ligand association determined by the negative value of free energy (ΔG), determined complex stability of certain protein-ligand, or as alternative, affinity increasing ligand to certain acceptor. Therefore, the researchers suspected that the binding occur in the control and ligand was not spontaneous and less stable.

Inhibition constant showed that all compounds had a higher inhibition constant value than the tamsulosin. It is assumed that inhibition in the formation of protein-ligand interaction is greater than control-ligand. Because of the value of the inhibition constants indicate the magnitude of the barrier between the ligand and the target protein. Lower value of inhibition constant indicates the smaller inhibition that occurs in the protein-ligand bound.[15]

The value of surface interaction shown all compound had a lower value than tamsulosin. its suggested that there is potential of the compound to bound ligand less stable than control. This is due to the value of surface interaction showed that the bounding is more stable and there is a higher biology activity [16]. Surface interaction are also influenced by the size of the ligand molecule, the large of surface are, the higher chance for bounding between the ligand and the target protein.[18]

Based on all this data, we can conclude that capric acid, caprylic acid, lauric acid, linoleic acid, and myristic acid have the potential to bind to alpha adrenoreceptor ligand. All of those compounds meet the criteria of Lipinski's Rule of Five. Lipinski's Rule of Five provides valuable guidelines for assessing the oral bioavailability of compounds but does not directly address drug toxicity. However, there is a relationship between the physicochemical properties defined by these rules and potential toxicity. Several drugs that violate Lipinski's rules are still effective, particularly those designed for specific targets or used in non-oral delivery methods, which may bypass some toxicity concerns.[29]

When the compound binds to alpha adrenoreceptor ligand, it's predicted that 1A Adrenoceptor blockade prostate gland and will inhibit smooth muscle contraction. Meanwhile, blockade of 1A and 1D adrenoreceptors in the bladder will inhibit detrusor muscle contraction.[19] Lipinski's Rule of Five has significantly shaped the field of medicinal chemistry by providing a simple and effective filter for assessing drug-likeness.[30] However, its limitations necessitate the development of more comprehensive models and guidelines to enhance drug discovery and accommodate a broader range of therapeutic agents.[30]

CONCLUSION

Camellia sinensis' active compounds, such as epigallocatechin gallate, were predicted to potentially affect benign prostatic hyperplasia by targeting the protein 5-alpha-reductase. Additionally, compounds like gallocatechin gallate, oleic acid, and palmitic acid were predicted to have similar effects on the protein purinoreceptors. *Serenoa repens*' active compounds, including capri acid, caprylic acid, lauric acid, linoleic acid, and myristic acid, were also suggested to potentially impact benign prostatic hyperplasia through alpha adrenoreceptors. This study presents a potential alternative for BPH treatment using natural components, which is significant due to the demand for safer treatment options with fewer side effects compared to conventional therapies. By identifying the potential of *Camellia sinensis* and *Serenoa repens* as purinoreceptor inhibitors, the study opens avenues for new insights into the mechanisms of action in BPH treatment.

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REFERENCES

1. Lim KB. Epidemiology of clinical benign prostatic hyperplasia. Asian J Urol. 2017 Jul;4(3):148-151. doi: 10.1016/j.ajur.2017.06.004. Epub 2017 Jun 9. PMID: 29264223; PMCID: PMC5717991.
2. Patel ND, Parsons JK. Epidemiology and etiology of benign prostatic hyperplasia and bladder outlet obstruction. Indian J Urol. 2014 Apr;30(2):170-6. doi: 10.4103/0970-1591.126900. PMID: 24744516; PMCID: PMC3989819
3. Lokeshwar SD, Harper BT, Webb E, Jordan A, Dykes TA, Neal DE Jr, Terris MK, Klaassen Z. Epidemiology and treatment modalities for the management of benign prostatic hyperplasia. Transl Androl Urol. 2019 Oct;8(5):529-539. doi: 10.21037/tau.2019.10.01. PMID: 31807429; PMCID: PMC6842780.
4. Lerner LB, McVary, KT, Barry MJ et al: Management of lower urinary tract symptoms attributed to benign prostatic hyperplasia: AUA Guideline part I, initial work-up and medical management. J Urol 2021; 206: 806.
5. Ventura S, Oliver Vl, White CW, Xie JH, Haynes JM, Exintaris B. Novel drug targets for the pharmacotherapy of benign prostatic hyperplasia (BPH). Br J Pharmacol. 2011 Jul;163(5):891-907. doi: 10.1111/j.1476-5381.2011.01332.x. PMID: 21410684; PMCID: PMC3130937.
6. Ford AP, Gever JR, Nunn PA, Zhong Y, Cefalu JS, Dillon MP, Cockayne DA. Purinoceptors as therapeutic targets for lower urinary tract dysfunction. Br J Pharmacol. 2006 Feb;147 Suppl 2(Suppl 2):S132-43. doi: 10.1038/sj.bjp.0706637. PMID: 16465177; PMCID: PMC1751490.
7. Jadid N, Kurniawan E, Himayani CES, Andriyani, Prasetyowati I, Purwani KI, Muslihatin W, Hidayati D, Tjahjaningrum ITD. An ethnobotanical study of medicinal plants used by the Tengger tribe in Ngadisari village, Indonesia. PLoS One. 2020 Jul 13;15(7):e0235886.

doi: 10.1371/journal.pone.0235886.
PMID: 32658902; PMCID: PMC7357767.

8. Katz A, Efros M, Kaminetsky J, Herrlinger K, Chirouzes D, Ceddia M. A green and black tea extract benefits urological health in men with lower urinary tract symptoms. Ther Adv Urol. 2014 Jun;6(3):89-96. doi: 10.1177/1756287214526924. PMID: 24883106; PMCID: PMC4003843
9. Putra MMR, Seputra KP. The Potential of *Cammelia sinensis* (Tea Leaves) Active Compound as Alternative Therapy on castrate-resistant prostate cancer (CRPC) with Androgen Receptor Inhibition: In Silico Study. BHSJ [Internet]. 2022 Apr. 28 [cited 2022 Jun. 27];5(1):25-8. Available from: https://www.e-journal.unair.ac.id/BHSJ/article/view/32147
10. Koch W, Zagórska J, Marzec Z, Kukula-Koch W. Applications of Tea (*Camellia sinensis*) and its Active Constituents in Cosmetics. Molecules. 2019 Nov 24;24(23):4277.
doi: 10.3390/molecules24234277.
PMID: 31771249; PMCID: PMC6930595.
11. Chua T, Eise NT, Simpson JS, Ventura S. Pharmacological characterization and chemical fractionation of a liposterolic extract of saw palmetto (*Serenoa repens*): effects on rat prostate contractility. J Ethnopharmacol. 2014 Mar 14;152(2):283-91. doi: 10.1016/j.jep.2013.12.030. Epub 2014 Jan 21. PMID: 24463033.
12. Damayanti, D. S., Utomo, D. H., and Kusuma, C, 2017. Revealing the potency of *Annona muricata* leaves extract as FOXO1 inhibitor for diabetes mellitus treatment through computational study. In Silico Pharmacology, 5(1)
13. Gilson MK, Zhou HX. Calculation of protein-ligand binding affinities. Annu Rev Biophys Biomol Struct. 2007;36:21-42.
14. Tambunan US, Alamudi S. 2010. Designing cyclic peptide inhibitor of dengue virus NS3-NS2B protease by using molecular docking approach. Bioinformation. 5(6):250-254.
15. Mardianingrum R, Herlina T, Supratman U. Isolasi dan molecular docking senyawa 6,7-dihidro17-hidroksierisotrin dari daun dadap belendung (*Erythrina poppigiana*) terhadap aktivitas sitotoksik antikanker payudara MCF-7. Chimica et Natura Acta. 2015;3(3):90-9.3
16. Bikadi Z, Hazai E. Application of the PM6 semiempirical method to modeling proteins enhances docking accuracy of AutoDock. J Cheminform. 2009;1(15).
17. Karolak-Wojciechowska J, Mrozek A, Kieć-Kononowicz K, Handzlik J. Structure and activity studies of glycine receptor ligands. Part 8. Arylidene-imidazoline-4-one aminoacids. J Mol Struct. 2003;649:25-36.
18. Mubarika SAZ, Dewi AR, Damayanti DS. In silico study: Anthelmintic potential of garlic's active compound (*Allium sativum L.*) by inhibiting acetylcholinesterase, beta tubulin and activating voltage-dependent L type calcium channel. Faculty of Medicine, University of Islam Malang. 2019;83-93.
19. Ventura, S., Oliver, V. l., White, C. W., Xie, J. H., Haynes, J. M., and Exintaris, B, 2011. Novel drug targets for the pharmacotherapy of benign prostatic hyperplasia (BPH). British journal of pharmacology, 163(5), 891–907.
20. North RA, Jarvis MF. P2X receptors as drug targets. Mol Pharmacol. 2013;83(4):759-69. doi:10.1124/mol.112.083758.
21. Alsufyani HA, Docherty JR. Roles for α1-adrenoceptors during contractions by electrical field stimulation in mouse vas deferens. Korean J Physiol Pharmacol. 2021;25(6):525-32. doi:10.4196/kjpp.2021.25.6.525.
22. Batty M, Pugh R, Rathinam I, Simmonds J, Walker E, Forbes A, et al. The role of α1-adrenoceptor antagonists in the treatment of prostate and other cancers. Int J Mol Sci. 2016;17(8):1339. doi:10.3390/ijms17081339.
23. H. Purnomo, M. Tegar, A. E. Nugroho. 2013. Interaksi Senyawa Aktif dari *Aegle marmelos Correa* sebagai Anti Inflamasi dengan Reseptor COX1 dan COX-2. Traditional Medicine Journal. Vol.18:80-87
24. Verma A, Kumar A, Debnath M. Molecular docking and simulation studies to give insight of surfactin amyloid interaction for destabilizing Alzheimer's Aβ42 protofibrils. Med Chem Res. 2016;25:1616-22. doi: 10.1007/s00044-016-1594-y.
25. Ortega G, Kurnik M, Dauphin-Ducharme P, Li H, Arroyo-Currás N, Caceres A, Plaxco K. Surface attachment enhances the thermodynamic stability of Protein L. Angew Chem Int Ed Engl. 2019;58(6):1714-8. doi: 10.1002/anie.201812021.
26. Leibacher, Nathan Walter, Marcelo Henrique Araujo da Silva. The adverse Effect of Tamsulosin in Men With Benign Prostatic Hyperplasia, A Review of the Literatur. American Journal of life science. 2019 ; 7 (4). 68-74
27. Dunn CJ, Matheson A, Faulds DM. Tamsulosin: a review of its pharmacology and therapeutic efficacy in the management of lower urinary tract symptoms. Drugs Aging. 2002;19(2).
28. Du X, Li Y, Xia YL, Ai SM, Liang J, Sang P, et al. Insights into protein-ligand interactions: mechanisms, models, and methods. Int J Mol Sci. 2016;17(2):144.
29. Karami TK, Hailu S, Feng S, Graham R, Gukasyan HJ. Eyes on Lipinski's Rule of Five: A New "Rule of Thumb" for Physicochemical Design Space of Ophthalmic Drugs. J Ocul Pharmacol Ther. 2022;38(1):43-55. doi: 10.1089/jop.2021.0069.
30. Poongavanam V, Doak B, Kihlberg J. Opportunities and guidelines for discovery of orally absorbed drugs in beyond rule of 5 space. Curr Opin Chem Biol. 2018;44:23-29.
doi:10.1016/j.cbpa.2018.05.010.

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Original Research Article

The Effect of Administration of Sapodilla Leaf Extract Cream (*Manilkara Zapota (L.) P. Royen*) On the Expression of PDGF And Il-10

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Abstract

Background: *Sunburn* is an acute inflammatory skin condition caused by exposure to UV rays. Excessive exposure increases the production of ROS which, if accumulated, can lyse *growth factors*, one of which is PDGF and also form IL-10 immune suppression. This condition can be influenced by providing antioxidants and anti-inflammatories such as those contained in sapodilla leaf extract (*Manilkara zapota (L.) P. Royen*) which has many benefits such as anti-inflammatory, anti-pyretic, anti-tumor, antioxidant, anti-microbial, anti- diabetic, anti-lipid and anti-aging. In previous research, the polyphenol content in Sapodilla leaves was a potential source of inhibiting ROS. However, until now the role of Sapodilla leaf extract on UVB burns has not been studied.

Objective: The aim of this research is to determine the effect of administering Sapodilla leaf extract cream on PDFG and IL-10 in Wistar rats that experienced burns due to exposure to UVB.

Method: Experimental research with a *posttest only control group design* approach. This research used 24 Wistar rats exposed to UVB rays which were divided into 4 groups (normal control, control with cream, 25% Sapodilla leaf extract cream, and 50% Sapodilla leaf extract cream). The ELISA (Enzyme-Linked Immunosorbent Assay) method was used to analyze PDGF and IL-10 levels in skin tissue.

Results: The highest ratio of PDGF levels was found in (K3) 2.915 ± 0.368. The results of the *one-way Anova* analysis had a *p value* of 0.024 ($p<0.05$) which stated that there were significant differences between treatment groups. In IL-10 levels there was an increase in K3 255.9 ± 35,563. In IL-10, the results of *one-way Anova* analysis had a *p value* of 0.240 ($p>0.05$), which stated that there were no significant differences between treatment groups.

Conclusion: Administration of sapodilla leaf extract cream at a dose of 25% had a significant effect on increasing PDGF levels and a slight increase in IL-10 in mice that experienced burns due to exposure to UVB.

Keywords: *Manilkara zapota (L.) P. Royen*, PDFG, IL-10, ELISA Method
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INTRODUCTION

Indonesia is a country with a tropical climate with quite a lot of exposure to sunlight. Ultraviolet light consists of 3 zones according to its wavelength, namely UVA (UVA 315-400 nm), UVB (280-315 nm), and UVC (100-280 nm).[1] Exposure to UV light can cause acute skin inflammation.[2] Excessive exposure will cause skin damage, including immunosuppression.[3] When cells and tissues are exposed to UVB rays, molecular dissociation (radiolysis) occurs and free radicals are produced in the form of *Reactive Oxygen Species* (ROS). The accumulation of ROS by UVB exposure can cause inflammation which can lyse several *growth factors* including *Platelet Derived Growth Factor* (PDGF) and also form interleukin-10 (IL-10) anti inflammation.

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Indonesia is rich in natural resources, researchers have turned to using it as an alternative medicine, one of which is sapodilla leaf extract which has been proven to contain active polyphenol substances and has anti-inflammatory properties.[4] In previous research, ethanol sapodilla leaf extract reduced edema in mice given carrageenan, activity significantly anti-inflammatory.[5] However, research on sapodilla leaf extract which is linked to burns due to UVB exposure is still scarce, so further research is needed.

Based on previous research in the US in 2015, of 31,162 US citizens, 34% of respondents had experience of experiencing *sunburn*. The highest incidence in Fitzpatrick types I-III is at a young age of around 18-29 years.[6] Sunburn due to exposure to UV rays and DNA damage will increase the risk of melanoma and non-melanoma skin cancer.[7] Most of the Indonesian population does a lot of outside activities, around 57.3 % focuses on UV light exposure.[8]

An imbalance in ROS production initiates inflammation and activates proinflammatory cytokines from epidermal keratinocytes such as PDGF.[9] In injured areas PDGF is an important chemokine and mitogen for fibroblasts, keratinocytes and vascular endothelium, and also stimulates macrophages to produce and secrete growth factors such as TGF-β.[10] PDGF can influence the function of dendritic cells and induce regulatory T cells via C-type lectin like receptor member 2 (CLEC-2) expression. Furthermore, regulatory B cells interact with pro-inflammatory mediators, platelet-activation factor and produce Interleukin-10 as an immunosuppressive reaction to UVB exposure.[11] *Sunburn* conditions by UVB induced skin inflammation. Its potential to recover after eliminate the cause and get appropriate therapy. Polyphenols are found in many types of plants. Polyphenols are used as antioxidants, anti-inflammatory and anti-tumor. There is a lot of research on the benefits of polyphenols in reducing damage caused by UVB exposure.[12]

The *Manilkara zapota (L.) P. Royen* plant has many benefits such as anti-inflammatory, anti-pyretic, anti-tumor, antioxidant, anti-microbial, anti-diabetic, anti-lipid and anti-aging.[13] In previous research, the polyphenol content in *Manilkara zapota leaves (L.) P. Royen* is a potential source in inhibiting ROS.[4] However, until now the role of Sapodilla leaf extract in burns caused by UVB has not been studied, so this research was conducted to determine the effect of administering Sapodilla leaf extract cream at concentrations of 25% and 50% against PDGF and IL-10 in Wistar rats that experienced UVB burns.

MATERIALS AND METHODS

This research is an experimental study using *a post test only control group design* which was carried out at *the Integrated Biomedical Laboratory* IBL, Faculty of Medicine, Universitas Islam Sultan Agung from December to January 2024. Ethical clearence of research was given by the bioethics commission of medical/health research, Faculty of Medicine Sultan Agung Islamic University. The research subjects were male Wistar rats aged 2-3 months with body weight 190-210 grams which was declared healthy and suitable for use for research by veterinarians from the Animal House Integrated Biomedical Laboratory-IBL, Faculty of Medicine, Universitas Sultan Agung, Semarang. Wistar rats underwent adjustment for 7 days. Mice were placed in separate cages at a fixed temperature and given a normal diet and access to water.

Sampling uses *probability sampling* techniques, namely by taking samples from a population that has the same opportunity to be selected as a sample. The system used is very simple random sampling (*simple random sampling*). All 28 Wistar rats that met the criteria for the study were divided into 4 treatment groups randomly. There is one control group and another as a treatment group.

The sample used was 1 kg sapodilla leaf (*Manilkara zapota (L.) P. Royen*), taken from Tuban city. The samples were first cleaned of adhering dirt, then dried in an oven at 40oC. The results are checked for water content using a *moisture balance*. If the water content results are below 10% then the drying results are considered good. Simplicia is then dry sorted to remove any dirt remaining during the drying process, cut into small pieces and weighed. Then blend it into powder. Then sifted with a 20 mesh sieve. 450 grams of simplicia leaf powder was extracted using the maceration method with 1500 ml of 96% ethanol solvent. The simplicia leaf powder is put into a separate dark colored bottle. Then the simplicia is soaked using ethanol solvent for 3 days and occasionally stirred 3 times a day, after 3 days it is filtered and the dregs are macerated again for 2 days with 1500 ml of 96% ethanol. repetition is done twice. The collected filtrate is then thickened using a *rotary evaporator* at a temperature of 40ºC until a thick extract is obtained, 110 grams [14,15]

Making a 20-gram cream preparation is done by mixing 15 grams of cream base with 25% sapodilla leaf extract (5 grams) and 10 grams of cream base with 50% sapodilla leaf extract (10 grams). Stirring is done until homogeneous. Sapodilla leaf extract cream was used every day at 0.5 grams per rat, so the dose of sapodilla leaf extract used was 0.125 grams for a 25% dose and 0.25 grams for a 50% dose. Samples taken randomly came from 28 Wistar rats, which were divided into 4 groups. The mice were housed in 4 cages consisting of normal controls, controls with cream, 25% Sapodilla leaf extract, and 50% Sapodilla leaf extract. Each cage contains 7 mice.

The rats were then exposed to UVB 160 mj/cm2/day for 3 days and continued to apply sapodilla leaf extract cream. After treatment for 6 days, tissue was taken. Previously, all Wistar rats were euthanized using anesthesia. Make a tissue incision on the part of the skin exposed to UV B, using scissors and tweezers. Tissue samples were cut and weighed 1 gram, then the tissue was added with PBS (PH 7.4). Then sonicate the sample for 15 seconds or until the tissue melts. Take 1ml/1000uL and put it in a 1.5 ml tube to become supernatant. Next, a protein test is carried out by mixing 500 µL of the sample plus 500 µL of 10% NaOH and 500 µL of 0.1% $CuSO_4$ and then observing the color change to bluish purple if it is positive for protein. Next, the samples were frozen at -20ºC.[16]

Table 1. Results of measurements of sapodilla leaf extract flavonoids

ppm concentration	Absorbents	Initial total flavonoid content (mg/ml)	Average Total Flavanoid Content (mg/ml)
1000	0,894	55,9	
1000	0,950	59,4	56,8
1000	0,882	55,1	

Meanwhile, the average total phenol content in sapodilla leaf extract was 170.1 mg/ml ± 12.4.

Table 2. Results of Sapodilla Leaf Extract Phenol Measurements

ppm Concentration	Absorbents	Initial total flavonoid content (mg/ml)	Average Total Phenol Content (mg/ml)
500	0,989	184,4	
500	0,855	159,6	170,1
500	0,891	166,2	

The skin tissue samples that were obtained were then analyzed for PDGF and IL-10 levels using the ELISA method. PDGF and IL-10 ELISA analysis was carried out using a kit from BioEnzy . The working principle of the ELISA examination in this practicum is an antigen-antibody reaction using a quantitative technique based on the number of specific antigen-antibody bonds determined by the absorbance value from a spectrophotometer.

The collected data processed, edited and tabulated for descriptive tests, followed by data normality using the *Shapiro Wilk* test and data homogeneity testing using the *Levene* test. Data analysis was conducted using One Way Anova SPSS version 26 followed by the Post Hoc LSD and Duncan tests to determine the differences between each group.

RESULTS

This study used 24 samples, no one was excluded during the research. This study consisted of 4 groups consisting of normal control, positive control and 2 treatment groups. Normal control (K1) consists of 6 samples without intervention and treatment. The positive control (K2) consisted of 6 samples exposed to UVB light and applied with a cream base. The first treatment group (K3) consisted of 6 samples smeared with 25% sapodilla leaf extract cream and the second treatment group (K4) consisted of 6 samples smeared with 50% sapodilla leaf extract cream.

Assessment of Total Flavonoid and Phenol Content

Sapodilla leaf extract in this study was obtained by maceration using ethanol solvent and producing a sapodilla leaf extract bath. Results of the assessment of total flavonoids and phenol in sapodilla leaf extract using the spectrophotometric method. In 1 gr of sapodilla leaf extract there is an average total flavonoid content of 56.8 mg/ml ± 2.1.

Effect of UVB Illumination on the Microscopic Image of Sunburn Cells

The group of mice that were given UVB irradiation with an energy intensity of 160 mJ/cm2/day for 3 days and those without irradiation were then subjected to a *sunburn* cell histology validation test. The examination was carried out on day 4 with microscopic observation, the results obtained were as shown in Figure 1.

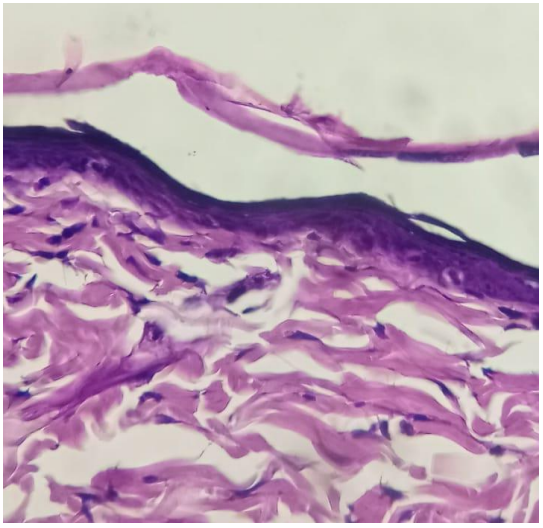

Figure 1.A Histology of healthy skin (Normal Control)

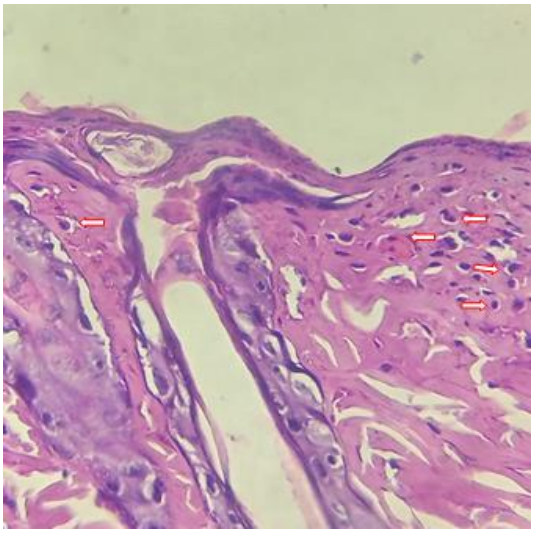

Figure 1.B Histology of exposed skin to UVB (Negative Control)

Table 3. Research Data the Effect of Giving Sapodilla Leaf Extract Cream on PDGF and IL-10 Levels

Variabel	Group				pvalue
	K1 n=6 Mean± SD	K2 n=6 Mean± SD	K3 n=6 Mean± SD	K4 n=6 Mean± SD	
Kadar PDGF	2.440±0.447	2.496±0.209	2.915±0.368	2.341±0.099	
Saphiro wilk	0.912	0.470	0.462	0.097	
Levene test					0.010
One way Anova					0.024
Kadar IL-10	214.142±38.707	220.713±31.147	255.9±35.563	217.615±47.027	
Saphiro wilk	0.817	0.947	0.819	0.936	
Levene test					0.659
One way Anova					0.240

Figure 1.A is a histological picture of healthy skin. The outermost layer is visible, namely the epidermis with a thin layer of keratin lying on top. The lower part of the epidermis is the dermis layer which consists of connective tissue and dense elastic tissue. Figure 1.B shows the histology of mouse skin exposed to UVB light. The main histological changes in the epidermis include *dyskeratotic* and *vacuolated* keratinocytes (*sunburn* cells) marked by red arrows. Microscopically, the two images show differences in the presence of *sunburn* cells.

In this study, the results of PDGF and IL-10 levels were obtained in a mouse model with *sunburn* burns due to UVB exposure treated with sapodilla leaf extract. In the normal control group (K1) the ratio of PDGF levels was 2,440 ± 0.447, in the positive control group (K2) 2,496 ± 0.209, in treatment group 1 (K3) 2,915 ± 0.368, in treatment group 2 (K4) 2,341 ± 0.099. Based on statistical tests, the level data for each group is normally distributed but not homogeneous with a significance value of the *Shapiro Wilk* test > 0.05 for each group and the *Levene test* of 0.010. The results of the *one way Anova* analysis had a *p value* of 0.024 (p<0.05) which stated that there were significant differences between treatment groups. Differences between groups were continued with the *Games-Howell post hoc* test.

In this study, IL-10 levels were also analyzed, in K1 it was 214,142 ± 38,707, for K2 it was 220,713 ± 31,147, there was an increase in K3 255.9 ± 35,563, in K4 it was 217,615 ± 47,027. Based on statistical tests, the IL-10 levels for each group were normally distributed and homogeneous with a significance value of the *Shapiro Wilk* test > 0.05 for each group and the *Levene test* of 0.659. The results of the *one way Anova* analysis had a p value of 0.240 (p>0.05) which stated that there were no significant differences between treatment groups.

Effect of Giving Sapodilla Leaf Extract Cream on PDGF Levels of Wistar Rats with UVB Burns

In the descriptive data, the PDGF levels of each group were tested for normality using Shapiro Wilk, obtaining a significance value for all groups of p>0.05. These results show that the data is normally distributed. In the *Levene test* assessment, a value of 0.010 (p<0.05) was obtained, which shows that the data is not homogeneous. Followed by the *one way Anova* test to assess significant differences between groups, a p value of 0.024 (p<0.05) was obtained, which stated that there were significant differences between groups. The results of further tests using *Games-Howell* are presented in graphical form in figure 2 and table 4. In this study, the results showed that the average PDGF levels between K3 (25% sapodilla leaf extract cream) and K4 (50% sapodilla leaf extract cream) had is significantly different because it has a significant value smaller than 0.05, namely 0.042. Based on the data above, it can be seen that administering a 25% dose of sapodilla leaf extract cream increases the expression of PDGF levels compared to a 50% dose of sapodilla leaf extract cream.

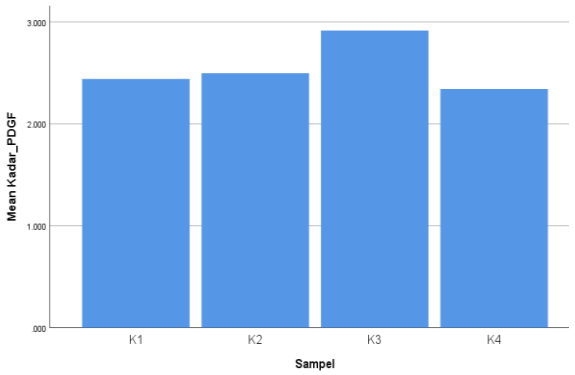

Figure 2. Graph The Effect of Giving Sapodilla Leaf Extract Cream on PDGF levels in all groups

Table 4. Games-Howell test for PDGF levels between research groups

Group	Comparison Group	Significance
K1	K2	0.992
	K3	0.250
	K4	0.950
K2	K1	0.992
	K3	0.152
	K4	0.417
K3	K1	0.250
	K2	0.152
	K4	0.042*
K4	K1	0.950
	K2	0.417
	K3	0.042*

*The mean difference is significant at the p < 0,05 level

Effect of Giving Sapodilla Leaf Extract Cream on IL-10 Levels of Wistar Rats with UVB Burns

In the descriptive data, the IL-10 levels of each group were tested for normality using Shapiro Wilk, obtaining a significance value for all groups of p>0.05. These results show that the data is normally distributed. In the *Levene test* assessment, a value of 0.659 (p>0.05) was obtained, which shows homogeneous data. Followed by *the one way Anova* test to assess significant differences between groups, a *p value* of 0.240 (p>0.05) was obtained, which stated that there were no significant differences between groups. In graph 3, there is a slight

increase in IL-10 levels in the K3 group who received 25% sapodilla leaf extract cream treatment. In the results of further tests using LSD in table 5, it was found that there was no real difference in IL-10 levels in the research samples, which was indicated by the larger sig value. of 0.05 across sample test comparisons.

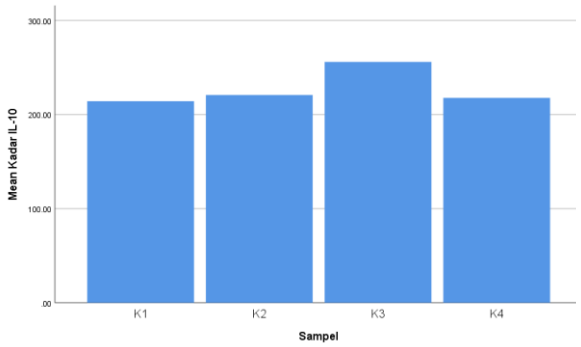

Figure 3. Graph The Effect of Giving Sapodilla Leaf Extract Cream on IL-10 levels in all groups

Table 5. Post Hoc LSD Test for IL-10 Levels Between Research Groups

Group	Comparison Group	Significance
K1	K2	0.771
	K3	0.075
	K4	0.878
K2	K1	0.771
	K3	0.130
	K4	0.891
K3	K1	0.075
	K2	0.130
	K4	0.101
K4	K1	0.878
	K2	0.891
	K3	0.101

*The mean difference is significant at the p <0,05 level

DISCUSSION

UVB exposure can cause *sunburn* which is characterized by redness, pain and inflammation of the skin. On the histological picture, *sunburn cells* were found. This is because UVB penetrates the outermost layer of the skin, the epidermis, there is an increase in *reactive oxygen species* (ROS) which activates the NF-kB pathway and leads to the release of inflammatory mediators and activation of immune cells.[17] The initial response to UVB exposure is the release of pro-cytokines inflammation, such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), which contribute to the redness, pain, and swelling associated with UVB exposure.[18] However 20, UVB exposure can also induce anti-inflammatory responses in the skin, such as the release of interleukin-10 (IL-10) and PDGF, which are anti-inflammatory and promote tissue repair.[19]

Flavonoid and phenolic compounds have benefits as antioxidants, they can induce the release of Lipooxygenase and cyclooxygenase enzymes which can suppress inflammatory reactions. The antioxidant content in the ability to prevent DNA damage and influence cellular signaling pathways is one of the properties of flavonoids. Other benefits include scavenging free radicals, as a UV absorber, and as a cytoprotective, anti-inflammatory, and anti-apoptotic factor. Previous research has shown that plants high in polyphenols are effective photoprotectants against UV carcinogenesis.[20] The results of this study found that the average total flavonoids per gram of extract was 56.8 mg/ml ± 2.1. Meanwhile, the average total phenol per gram of extract was 170.1 mg/ml ± 12.4. This is impressive as suppressing the NF-kB pathway is expected to reduce the inflammatory response.

This study determined the effect of sapodilla leaf extract cream on IL-10 and PDGF levels in Wistar rats that experienced *sunburn*. The sample of male Wistar rats was chosen as a model for analyzing the anti-inflammatory benefits of sapodilla leaf extract cream, because Wistar rats are mammals with a skin structure similar to humans.

This study analyzed the levels of IL-10 and PDGF, which are anti-inflammatory factors and *growth factors*. Molecular levels of PDGF and IL-10 in this study were checked using ELISA. The results showed that there was an increase in PDGF levels in the 25% sapodilla leaf extract treatment group compared to the control and 50% extract. The decrease in PDGF levels at a dose of 50% is thought to be because inflammation has been controlled. This is because PDGF is produced by macrophages, injured endothelial cells so that the inflammatory signal decreases, causing PDGF production to decrease. The structure of flavonoids also acts as a binder for hydroxyl free radicals, so that ROS produced due to UVB rays can be suppressed.[21] The PDGF receptor is activated by binding to the PDGF ligand, which is secreted by platelets, macrophages and other cells. PDGF signaling is also involved in physiological and pathological processes, including wound healing, tissue repair in *sunburn*.[22] In Konuku et al's research, high doses of sapodilla leaf ethyl acetate extract were more effective in suppressing inflammatory effects in a mouse model with leg swelling.[23] In Swarnakumari's research, The higher the dose of sapodilla leaf ethanol extract, the faster the repair of wounds on the cornea.[24]

In areas exposed to UV light, Langerhans cells will be lost because these cells migrate into the *draining lymph nodes* (DLN). These Langerhans cells lose their ability to present antigen because they cannot produce IL-12, but they can activate T-natural killer cells which trigger T-regulatory cells to produce IL-10 which is also an anti-inflammatory cytokine.[25] In this study, The results of the IL-10 study showed a slight increase in the 25% sapodilla leaf extract group compared to the control and 50% sapodilla leaf extract, but there was no significant difference between the groups. It is suspected that the inflammatory process has been controlled, the treated mice have entered the next phase, namely the proliferation and remodeling phase.

Overall, the results of this study show that sapodilla leaf extract cream has antioxidant activity and a dose of 25% induces growth factors and acts as an anti-inflammatory. This shows that sapodilla leaf extract has the potential to be developed as a *sunburn* therapy.

CONCLUSION

Based on the research results, it can be concluded that administering a 25% dose of sapodilla leaf extract cream had a significant effect on increasing PDGF levels in the skin of model mice that experienced burns due to exposure to UVB, while a 50% dose did not show a significant effect. The administration of 25% and 50% sapodilla leaf extract cream did not have a significant effect on increasing IL-10 levels in the skin of model mice that experienced burns due to exposure to UVB.

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REFERENCES

1. Lee LY, Liu SX. Pathogenesis of photoaging in human dermal fibroblasts. Int J Dermatology Venereol. 2020 ;3(1):37–42. doi:10.1097/JD9.0000000000000068
2. Mahobiya P. Protection against ultraviolet b radiation-induced oxidative damage by antioxidants on liver of female wistar rat. Inter J of Sci Study. 2020;12(7):95-8. doi:10.13140/RG.2.2.27371.98081
3. González Maglio DH, Paz ML, Leoni J. Sunlight effects on immune system: Is there something else in addition to uv-induced immunosuppression? Biomed Res Int. 2016;2016(1):1-10. doi:10.1155/2016/1934518
4. Mohd Tamsir N, Mohd Esa N, Omar SNC, Shafie NH. Manilkara zapota (L.) P. Royen: potential source of natural antioxidants. Malaysian J Med Heal Sci. 2020;16(8):196–204.
5. Ganguly A, Mahmud Z Al, Uddin MMN, Rahman SMA. In-vivo anti-inflammatory and anti-pyretic activities of manilkara zapota leaves in albino wistar rats. Asian Pacific J Trop Dis. 2013;3(4):301–7. doi:10.1016/S2222-1808(13)60073-0
6. Holman DM, Ding H, Guy GP, Watson M, Hartman AM, Perna FM. Prevalence of sun protection use and sunburn and association of demographic and behaviorial characteristics with sunburn among US adults. JAMA Dermatology. 2018;154(5):561–8. doi:10.1001/jamadermatol.2018.0028
7. Gaddameedhi S, Selby CP, Kemp MG, Ye R, Sancar A. The circadian clock controls sunburn apoptosis and erythema in mouse skin. J Invest Dermatol. 2015;135(4):1119–27. doi.org/10.1038/jid.2014.508
8. Jordaniel Setiabudi, Made Wardhana, I Gusti Ayu Agung Elis Indira NMDP. Profil pra kanker dan kanker kulit RSUP Sanglah periode 2015 - 2018. J Med Udayana. 2021;10(3):83–9. doi:10.24843.MU.2021.V10.i3.P13
9. Ansary TM, Hossain MR, Kamiya K, Komine M, Ohtsuki M. Inflammatory molecules associated with ultraviolet radiation-mediated skin aging. Int J Mol Sci. 2021;22(3974):1-14. doi.org/10.3390/ijms22083974.
10. Yang X, Zhan P, Wang X, Zhang Q, Zhang Y, Fan H, et al. Polydopamine-assisted PDGF-BB immobilization on PLGA fibrous substrate enhances wound healing via regulating anti-inflammatory and cytokine secretion. PLoS One. 2020;15(9):1–18. doi: 10.1371/journal.pone.0239366.
11. Agrawal S, Ganguly S, Hajian P, Cao JN, Agrawal A. PDGF upregulates CLEC-2 to induce T regulatory cells. Oncotarget. 2015;6(30):28621–32. doi: 10.18632/oncotarget.5765
12. Saric S, Sivamani RK. Polyphenols and sunburn. Int J Mol Sci. 2016;17(9):1–22. doi: 10.3390/ijms17091521.
13. Chunhakant S, Chaicharoenpong C. Antityrosinase, antioxidant, and cytotoxic activities of phytochemical constituents from Manilkara zapota L. bark. Molecules. 2019;24(15):1–18. doi: 10.3390/molecules24152798.
14. Octaviani M, Syafrina S. Uji aktivitas antibakteri ekstrak etanol daun dan kulit batang sawo (Manilkara zapota (L.) Van Royen). J Ilmu Kefarmasian Indones. 2018;16(2):131-136. doi: 10.35814/jifi.v16i2.520
15. Puspitasari AD, Yuita NE, Sumantri S. Krim antioksidan ektrak etanol daun kopi arabika(Coffea Arabica). J Ilm Teknosains. 2017;3(2). 82-8. doi: 10.26877/jitek.v3i2.1884
16. CMHC Committee. Prosedur Pemeriksaan ELISA [internet]. Publikasi Jurnal Ilmiah News. 2022. Available from: https://cattleyapublicationservices.com/?p=383#google_vignette
17. Nobile V, Zanoletti V, Manzoni V, Romagnoli S, Cestone E. Soothing effect of a cosmetic product on skin discomforts induced by a chemical irritant (Capsaicin) and UV-radiation, and after mosquito bites and sunburn in a real-world setting. Cosmetics. 2022;9(6):1-14. doi: 10.3390/cosmetics9060130
18. Nakamura M, Haarmann-Stemmann T, Krutmann J. COX inhibition enhances inflammatory immune cell infiltration in UV-irradiated human skin: Implications for the treatment of sunburn. Exp Dermatol. 2015;24(10):734–5. doi: 10.1111/exd.12783
19. Gallo RL, Bernard JJ. Innate immune sensors stimulate inflammatory and immunosuppressive responses to UVB radiation. J Invest Dermatol. 2014;134(6):1508–11. doi: 10.1038/jid.2014.32
20. Anbualakan K, Tajul Urus NQ, Makpol S, Jamil A, Mohd Ramli ES, Md Pauzi SH, et al. A Scoping review on the effects of carotenoids and flavonoids on skin damage due to ultraviolet radiation. Nutrients. 2023;15(1):1–17. doi: 10.3390/nu15010092
21. Putri R, Putra A, Chodidjah. Clitorea ternatea flower extract induces platelet derived growth factor (PDGF) and GPx gene overexpression in ultraviolet (UV)B irradiation- induced collagen loss. Med Glas. 2023;20(1):15-21. doi: 10.17392/1530-22

22. Lopes DM, Mcmahon SB. Ultraviolet radiation on the skin: A painful experience? CNS Neurosci Ther. 2016;22(2):118–26. doi: 10.1111/cns.12444.
23. Konuku K, Karri KC, Gopalakrishnan VK, Hagos Z, Kebede H, Naidu TK, et al. Anti-inflammatory activity of Manilkara zapota leaf extract. Int J Curr Pharm Res. 2017;9(4):130-134. doi:10.22159/ijcpr.2017v9i4.20977.
24. S. S. Phytochemical screening and in vitro corneal wound healing activities of the leaves of Manilkara zapota in newer herbal drug development. World J Pharm Res. 2017;6(9):639–47. doi: 10.20959/wjpr20179-9224
25. Putranti IO, Sistina Y, Tengah J, Jenderal U, et al. Tinjauan pustaka fotobiologi ultraviolet pada jaringan kulit. 2021;13(2):33-55. doi: 10.20884/1.mandala.2023.16.1.8379.

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Original Research Article

Antibiotic-Resistant Phenotype and Genotype of S. suis serotype 2 (SS2) Isolated from Humans in Bali, Indonesia

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Abstract

Background: In recent times, meningitis, an infection primarily attributed to the zoonotic bacteria *Streptococcus suis*, has emerged as a significant public health concern in Bali, Indonesia. Their resistance to a multitude of antibiotics has emerged as a contemporary threat, as opposed to their virulence. There is a current lack of reported information regarding the genetic or phenotypic susceptibility pattern of *S. suis* to antibiotics in Bali.

Objective: The objective of this research endeavor was to ascertain the antibiotic susceptibility pattern of *S. suis* isolates in Bali, either through phenotypic or genetic means.

Methods: Glycerol stock isolates of *S. suis* from various specimen sources, including CSF, blood, and pleural fluid from April 2016 until April 2022 which had been assessed for species identification and antimicrobial susceptibility test (AST) using the VITEK 2 Compact (Biomeriuex®) were subjected to determine the serotype and antibiotic resistance genetically.

Results: Successful isolation of sixty-six *S. suis* isolates occurred primarily from cerebrospinal fluid. The results demonstrated that all isolates exhibited phenotypic resistance to tetracycline, with one isolate (MKPNH0071) demonstrating co-resistance to tetracycline and erythromycin. It is additionally corroborated genetically through the amplification of the *tetM* gene in every isolate, including those that exhibited concurrent resistance to erythromycin and tetracycline. The *intTn* gene, a member of the conjugate transposon Tn916 family which plays a role as horizontal media gene transfer on plasmids for carrying the resistance genes *ermB* and *tetM*, was amplified in an isolate that exhibited tetracycline co-resistance with erythromycin (MKPNH0071).

Conclusion: This research represents the initial investigation into the antibiotic resistance phenotype and genotype of *S. suis* serotype 2 (SS2) isolated from human subjects in Bali, Indonesia. Our findings suggest that the isolate of SS2 (MKPNH0071), which demonstrated tetracycline co-resistance with erythromycin, might be facilitated by the horizontal acquisition of the genetic element Tn916.

Keywords: *Streptococcus suis; antibiotic co-resistance; Bali*
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INTRODUCTION

Pigs harbor the zoonotic pathogen *Streptococcus suis*. The upper respiratory tract of piglets, including the pharynx and tonsils, becomes colonized by this bacterium. Despite the fact that this bacterial colonization frequently results in the absence of symptoms (asymptomatic carrier status), the risk of invasive diseases such as sepsis, meningitis, endocarditis, pneumonia, and arthritis must be considered.[1,2]

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Human infections are also caused by emerging *S. suis* in Southeast Asia (Hong Kong, Thailand, Vietnam, and China).[1,2,3] This zoonotic pathogen is the most common cause of bacterial meningitis in adults in Vietnam and Hongkong.[3] Asia is documented as the region with the highest incidence of infections caused by this pathogen, at 0.8 cases per 100,000 individuals.[2,3,4] There have been reports of this infection also emerging in Western European countries, albeit with an incidence rate that is ten times lower.[4] In 2007, Wertheim documented 409 human cases of *S. suis* infection, the majority of which originated in Southeast Asia.[5] In 2008, instances of *S. suis* infection were identified in pig joint fluid samples collected in the Timika region of Papua, Indonesia.[6] Susilawathi *et al*. recently reported that 44 of the 71 cases of bacterial meningitis collected at Sanglah Central General Hospital between 2014 and 2017 were confirmed to be meningitis caused by *S. suis*.[7]

Among the 29 serotypes of *S. suis*, serotype 2 (SS2) is the most prevalent in both pigs and humans, causing infections.[8,9,10] Serotype 2 continues to be the most prevalent in Europe, North America, South America, Asia, and Australia. Infection cases caused by *S. suis* exhibit a diverse distribution of serotypes across all geographical regions. North America (Canada) exhibits the highest prevalence of serotypes 1, 1/2, and 2. This is in contrast to South America, where serotypes 1/2, 2, and 3 predominate. It has been reported that serotypes 2, 4, 7, and 9 are more prevalent in Europe.[4,11] Susilawathi *et al*. reported that serotypes 2 and 1/2 are the most frequently found in Indonesia.[7]

Given the lack of a viable vaccine against *S. suis*, there has been a growing reliance on antibiotics to manage and control infections caused by SS2. Among these, macrolides, β-lactams, tetracyclines, and sulfonamides are the most commonly prescribed.[12,13] With the escalating utilization of antibiotics to treat infections induced by SS2, a novel peril emerges: antibiotic resistance to this pathogen on a global scale. The most frequently reported antibiotic resistance among *S. suis* isolates was to macrolides (>70%) and tetracyclines (>90%).[14] A research investigation was conducted at Prof. I.G.N.G. Ngoerah Hospital regarding antibiotic susceptibility of *S. suis* in Bali. The findings revealed that throughout the period from 2016 to 2021, all 55 clinical isolates of *S. suis* exhibited resistance to tetracycline.[15] Human infections with *S. suis* that are co-resistant to tetracycline and macrolide/lincosamide have been documented in multiple studies.[4,16,17] The co-resistance mechanism between tetracycline and macrolide/lincosamide is purportedly facilitated through the action of transposons Tn916, which belong to the transposon conjugate family and serve as a conduit for horizontal gene transfer on the SS2 plasmid.[16]

As the prevalence of antibiotic resistance in *S. suis* infections rises globally, preventative measures and suitable treatment are required to contain instances of this pathogen-caused antibiotic resistance. Consequently, this research was conducted to further the sample study initiated by Dwijayanti *et al*.[15] Additionally, the genetic analysis will be explored more deeply including the finding of co-resistance to tetracycline and macrolide/lincosamide isolate in this study.

MATERIALS AND METHODS

Ethics approval

This study was approved by The Research Ethics Committee of the Faculty of Medicine, Universitas Udayana (Denpasar, Bali, Indonesia) No:1387/UN14.2.2.VII.14/LT/2023 dated May 25, 2023

Isolate information data

The isolate data, including species identification and antibiotic susceptibility, were obtained using the VITEK 2 Compact (bioMérieux®) with the VITEK® 2 GP card and VITEK® 2 AST-ST03 card. These results were adjusted according to the 2021 Clinical Laboratory Standards Institute (CLSI) guidelines.[18] The antibiotics Benzylpenicillin, Ampicillin, Cefotaxime, Ceftriaxone, Levofloxacin, Erythromycin, Clindamycin, Linezolid, Vancomycin, and Tetracycline were evaluated for susceptibility. Subsequently, the antibiotic susceptibility patterns of the *S. suis* serotype 2 clinical isolates in Bali were ascertained utilizing Microsoft Excel 2019.

Bacterial isolate

From April 2016 to April 2022, we gathered 66 bacterial isolates from various specimen sources e.g., cerebrospinal fluid, blood, and pleural fluid (Supplementary data). The inclusion criteria for this study were as follows: all *S. suis* isolates identified as *S. suis* by the VITEK® 2 GP card, with antimicrobial susceptibility testing (AST) performed using the VITEK® 2 AST-ST03 card; a complete microbiology request form; and a medical record.

Bacterial culture conditions

A collection of 66 isolates of *S. suis* was maintained at a temperature of -80 °C in tryptic soy broth (TSB) media containing 50% glycerol. For further study, 66 glycerol stock isolates of *S. suis* were cultivated on 5% defibrinated sheep blood agar plate (DSBAP) and incubated in 5% CO_2 at 37 °C for 18 to 24 hours. All 66 isolates were grown as colonies on DSBAP, reconfirmed as *S. suis* using VITEK 2 Compact (bioMérieux®), and then subjected to further investigation.

Bacterial DNA isolation

S. suis chromosomal DNA was isolated utilizing a Roche High Pure PCR Isolation Kit Template (Roche Life Science, Indianapolis, U.S.A.). However, for antibiotic resistance genes that predominantly carried by plasmid DNA were extracted using the QIAprep® Spin Miniprep Kit (Qiagen, Hilden, Germany). *S. suis* colonies were suspended in 200 µl of phosphate-buffered saline (PBS) with a pH of 7.3. Isolation of DNA from the bacterial suspensions was carried out in accordance with the guidelines provided by the manufacturer.

Polymerase Chain Reaction (PCR) condition

Primers designated for identification, genotyping, and antibiotic resistance genes were utilized in this investigation. The PCR was performed with Go Taq® Green Master Mix (Promega, Madison, USA) with primer concentrations of 0.3 M were utilized. Then, the amplicons were electrophoresed for 35 min on a 1.5% agarose gel in TBE buffer at 100 volts. The DNA was

Table 1. Target gene and PCR primers used in this study

Target genes	Primer sequence 5' – 3'	Amplicon size (bp)	Temperature	References
***S. suis* genes target**				
recN	CTACAAACAGCTCTCTTCTAGTC ACAACAGCCAATTCATGGCGTGATT	336	60^{0}C	Ishida *et al.*
Major *S. suis* serotype capsular genes				
cps2J	GTTGAGTCCTTATACACCTGTT CAGAAAATTCATATTGTCCACC	459	60^{0}C	Nutravong *et al.*
Macrolide Resistance Genes				
ermB	GAAAAGGTACTCAACCAAATA AGTAACGGTACTTAAATTGTTTAC	639	48^{0}C	Gygax *et al.*
Lincosamide Resistance Genes				
lnuB	CCTACCTATTGTTTGTGGAA ATAACGTTACTCTCCTATTC	944	47^{0}C	Gygax *et al.*
Tetracycline Resistance Genes				
tetM	GTTAAATAGTGTTCTTGGAG CTAAGATATGGCTCTAACAA	657	45^{0}C	Agerso *et al.*
Tn916-Like Transposon Family				
intTn	GGTCTTCGTATTTCAGAGTTTGG GTTGCATGTGCGTAATAGTTCAG	473	53^{0}C	Agerso *et al.*

visualized using GelRedTM Nucleic Acid Gel Stain (Biotium, Hayward, CA 94545) and then documented using Gel Doc (Bio-Rad). The PCR conditions corresponding to each primer pair are detailed in Table 1.

DNA sequencing

The positive control in this study was not included; however, the sample that successfully amplified the *tetM*, *ermB*, and *lnuB* genes target, respectively was then subjected to perform DNA sequencing commercially in 1stBase (Selangor, Malaysia). The DNA sequencing results from each target gene were analyzed using SnapGene version 7.2.1. Then, the nucleotide sequence was aligned with sequence database using the BLAST® tool, which is available at the National Centre for Biotechnology Information (NCBI) platforms (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

RESULTS

The majority of specimen sources come from cerebrospinal fluid (CSF)

Based on the register data, 66 samples that satisfied the inclusion criteria were identified as *S. su*is. Based on the analysis of the 66 samples data, it was determined that cerebrospinal fluid (CSF) provided the greatest number of specimens, 51 (77.27%), followed by blood with 14 (21.21%), and one specimen source derived from pleural fluid (1.51%).

Almost every isolate of *S. suis* was identified as SS2.

Based on the results of bacterial identification tests conducted using VITEK 2 Compact Automatic System (BioMérieux®) and the VITEK® 2 GP card, *S. suis* was identified in 66 of the samples analyzed. All samples underwent a second confirmation through the detection of *recN*, a gene that is conserved in *S. suis*. The findings indicated that the gene *recN* was amplified in every sample (data not shown). The entire sample was subsequently subjected to an additional serotype test, and the results determined that it belonged to *S. suis* serotype 2 (SS2) (data not shown).

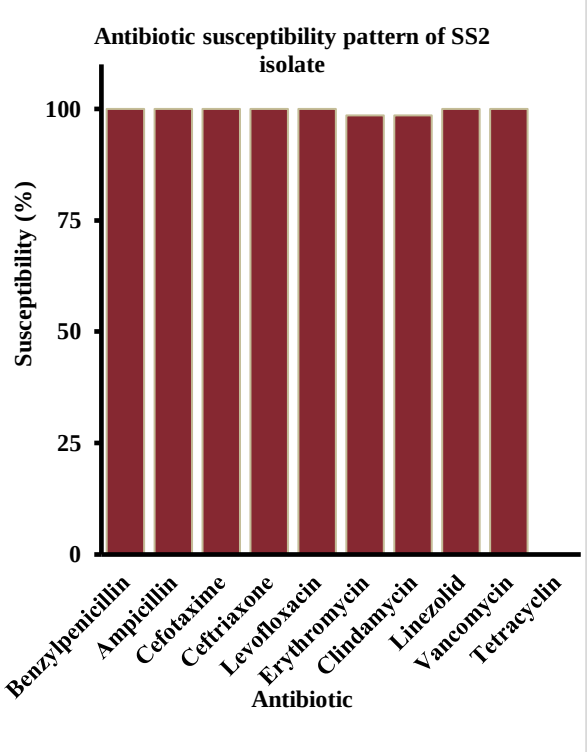

Figure 1. Antibiotic susceptibility pattern of SS2

Antibiotic susceptibility data was obtained from VITEK 2 compact. This result showed that all isolates (100%) were susceptible toward Benzylpenicillin (BZP), Ampicillin (AMP), Cefotaxime (CTX), Ceftriaxone (CRO), Levofloxacin (LVX), Linezolid (LNZ), Vancomycin (VAN). However, all isolates (100%) resistance toward Tetracycline (TCY). Isolate MKPNH0071 which exhibited resistant to Tetracycline (TCY), also show resistance toward Erythromycin (ERY), and Clindamycin (CLI).

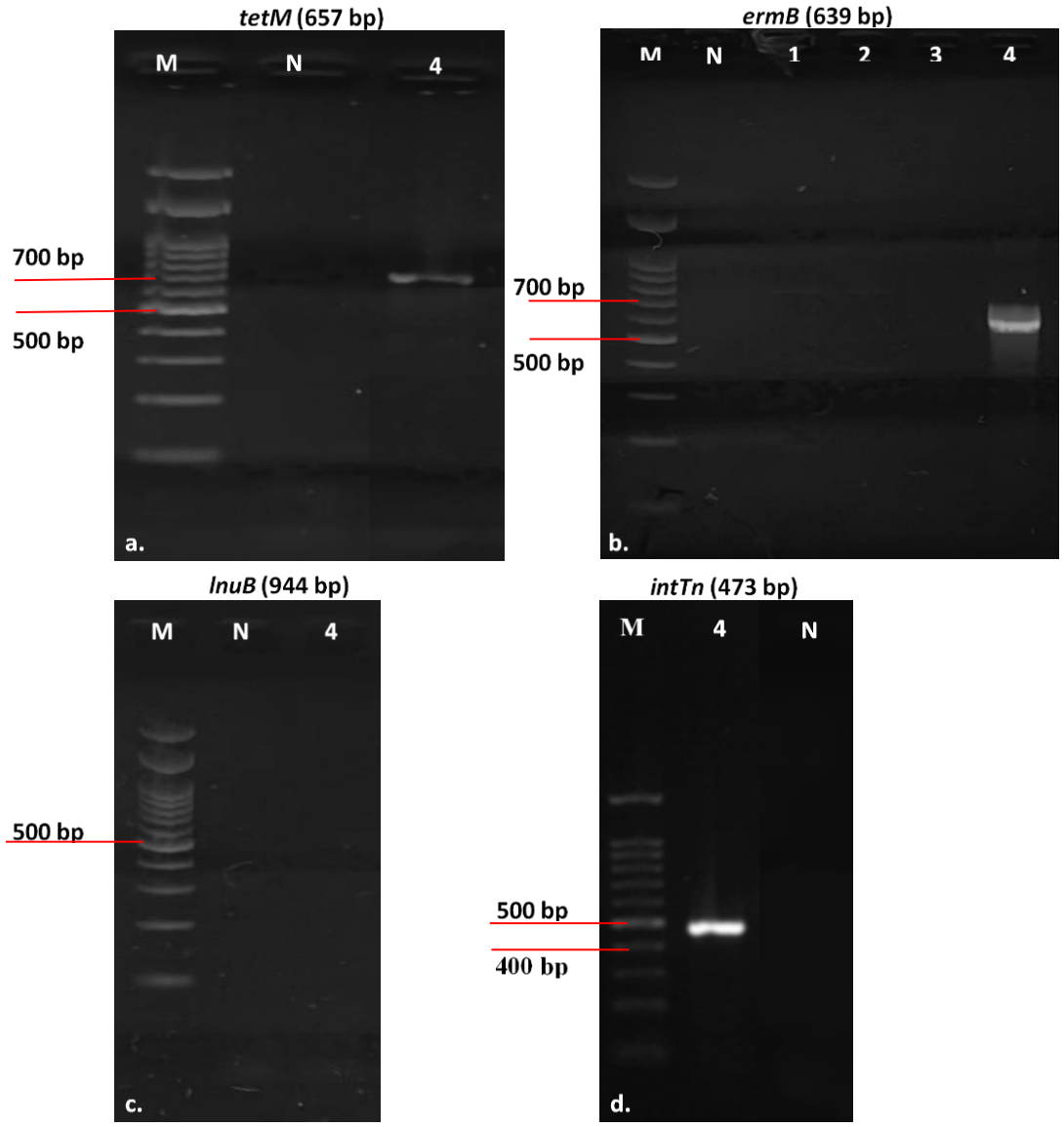

Figure 2. Agarose electrophoresis result of *tetM*, *ermB*, *lnuB*, and *intTn* gene at figure **a**, **b**, **c**, and **d,** respectively. **M**, indicated as a marker, 1 kb DNA ladder; **N**, indicated as negative control; **1**, **2**, **3**, and **4,** indicated as isolate ID number MKPNH0068, MKPNH0069, MKPNH0070, and MKPNH0071, respectively. **a.** The isolate MKPNH0071 successfully amplified the *tetM* gene with the expected PCR product size 657 bp, **b.** Among four isolates, only isolate MKPNH0071 successfully amplified the *ermB* gene with expected PCR product size 639 bp, **c.** The isolate MKPNH0071 did not amplify the *lnuB* gene with the expected PCR product size 944 bp, **d.** Isolate MKPNH0071 also successfully amplified the *intTn* gene with an expected PCR product size 473 bp.

Antibiotic susceptibility pattern of SS2

Based on the data obtained from the 2021 Clinical Laboratory Standard Institute (CLSI) antibiotic susceptibility testing results using the VITEK 2 Compact Automatic System (BioMérieux®) with the VITEK® 2 AST-ST03 card, it was observed that all samples (100%) showed that they were still susceptible to the following antibiotics: Benzylpenicillin/BZP; Ampicillin/AMP; Cefotaxime/CTX; Ceftriaxone/CRO; Levofloxacin/LVX; Linezolid/LNZ; Vancomycin/VAN. Conversely, all sample (100%) exhibited resistant toward Tetracycline/TCY. It is noteworthy that a sample MKPNH0071, which exhibited resistant to Tetracycline/TCY, also demonstrated resistant to Erythromycin/ERY and Clindamycin/CLI (Figure 1).

The *intTn* gene was detected in the co-resistance tetracycline and erythromycin mechanism

When a test is carried out to detect the antibiotic resistance gene, namely tetracycline (*tetM*), erythromycin (*ermB*), and clindamycin (*lnuB*), the results obtained were that all samples (100%) amplified the tetracycline resistance gene (*tetM*) (Figure 2a) and only one sample (MKPNH0071) amplified the erythromycin resistance gene (*ermB*) (Figure 2b). None of the samples amplify the clindamycin resistance gene (*lnuB*) (Figure 2c). In line with the discovery of the resistance gene to tetracycline (*tetM*) and erythromycin (*ermB*) on one sample (MKPNH0071), followed by gene detection *intTn* on that sample. The results showed that this sample also amplified the gene *intTn* (Figure 2d).

All target genes (*tetM*, *ermB*, and *intTn*) in sample MKPNH0071 were subjected to sequencing. The nucleotide sequences from the sequencing result were

aligned with references sequence using the BLAST® tool at NCBI. The aligning result showed that *tetM*, *ermB*, and *intTn* gene have 100% identity similarity with *S. suis* GX1 *tet(M)* gene for tetracycline resistance (GN_0482244.1), 100% identity similarity with *Streptococcus pyogenes* pDB101 *erm(B)* gene for 23S rRNA (NG_242280.1), and 99% identity similarity with *Streptococcus agalactiae* strain PHEGBS0082 transposon Tn916 (OP715838.1), respectively.

DISCUSSION

All isolates exhibited resistance to tetracycline, as indicated by the antibiotic susceptibility data and validated by the amplified tetracycline resistance gene (*tetM*) (100%). Both the *tetM* and *tetO* genes, which encode ribosomal protection protein, were prevalent in the tetracycline-resistant *S. suis* bacterium. Additionally, other studies have documented a higher carrier rate of the *tetM* gene in comparison to the *tetO* gene.[23,24] Subsequently, our research aligns with that of Uruen *et al*., who compiled publication data regarding the prevalence of antimicrobial resistance (AMR) across various antibiotic classes in *S. suis* isolates originating from Europe, Asia, and America. Notably, their study documented the highest rate of tetracycline resistance.[14] The escalating prevalence of tetracycline resistance can be attributed to the widespread use of this antibiotic to treat infectious diseases in food production animals, especially in intensive pig farming.[14] Free access to antibiotics without a doctor's prescription in Indonesia has led to unrestricted usage; consequently, become an AMR threat in Indonesia.[25,26]

The phenotypic findings derived from the VITEK 2 data indicated that a single sample exhibited resistance to tetracycline and erythromycin/clindamycin. This was corroborated by the amplification of the tetracycline resistance gene (*tetM*) and the erythromycin resistance gene (*ermB*), but not the clindamycin resistance gene (*lnuB*). The observed co-resistance of *S. suis* serotype 2 to tetracyclines and macrolides/lincosamides, is still infrequent. Similar findings were also reported by other researchers.[27,28,29] *Streptococcus* sp. from clinical isolates that are resistant to macrolide frequently possess resistance genes encoded by ribosomal methylase (*erm*) and efflux (*mef*) genes.[21] Our research aligns with the findings of Ye *et al*., who identified the *ermB* gene in every erythromycin-resistant isolate, thus validating its prevalence in *S. suis* type 2 in China.[23]

Furthermore, one sample of co-resistance to erythromycin and tetracycline also exhibits amplification of the *intTn* gene. The literature indicates that the *intTn* gene, which is a member of the conjugate transposon Tn916 family, was horizontally media gene transfer transferred for tetracycline resistance gene (*tetM*) and erythromycin resistance gene (*ermB*) onto plasmids *S. suis* serotype 2. This plasmid contains the tetracycline resistance gene (*tetM*) and the erythromycin resistance gene (*ermB*), which frequently results in co-resistance to both antibiotics.[4,16] Furthermore, the existence of components associated with Tn916 and *tetM* in *S. suis* serotype 2 may significantly contribute to the pathogenic nature of this bacterial pathogen.[16] In contrast to the *tetM* and *ermB* genes, the *lnuB* gene was exclusively found in the TnGBS2 family and not the Tn916 family.[4] The peculiar aspect is that instances of co-resistance to tetracycline and erythromycin antibiotics are predominantly documented in pigs.[16] It is possible that the transmission of the antibiotic resistance gene to humans occurred via contaminated meat harboring *S. suis*.

In contrast, while none of the isolates exhibited amplification of the clindamycin resistance gene (*lnuB*), one isolate demonstrated phenotypic resistance to clindamycin. This phenomenon can be delineated through the examination of two clindamycin-specific resistance mechanisms: the first pertains to an ABC transporter that is encoded by the *lsaE* gene, and the second concerns the target's modification by a nucleotidyl-transferase, which is either encoded by the *lnuB* or *lnuC* genes. In order to validate the resistance of our isolate to clindamycin, it is necessary to examine the remaining two clindamycin-resistant genes, namely *lsaE* and *lnuC*.[4] Clindamycin resistance was uncommon in this study, which is a relatively low number in comparison to the resistance rates observed with tetracycline and erythromycin.

CONCLUSIONS

This is the first investigation into the antibiotic resistance genotype and phenotype of *S. suis* serotype 2 (SS2) obtained from human sources in Bali, Indonesia. According to the findings, the isolate of SS2 (MKPNH0071) which demonstrated the occurrence tetracycline co-resistance with erythromycin might be facilitated by the horizontal acquisition of the genetic element Tn916. Further characterization of the mobile resistome of *S. suis* is imperative due to its potential to function as a reservoir of resistance genes for other species inhabiting the same habitats. This pathogen poses a threat to the health of both animals and humans and may also facilitate the transmission of antimicrobial resistance (AMR) genes between these species. In the coming years, this will be one of the global challenges that must be addressed in order to preserve essential antimicrobial activity.

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REFERENCES

1. Segura M. *Streptococcus suis*: An emerging human threat. J Infect Dis. 2009;199(1):4–6. https://academic.oup.com/jid/article-lookup/doi/10.1086/594371
2. Feng Y, Zhang H, Wu Z, Wang S, Cao M, Hu D, et al. *Streptococcus suis* infection: An emerging/reemerging challenge of bacterial infectious diseases? Virulence. 2014;5(4):477–97. http://www.tandfonline.com/doi/abs/10.4161/viru.28595

3. Huong VTL, Ha N, Huy NT, Horby P, Nghia HDT, Thiem VD, et al. Epidemiology, clinical manifestations, and outcomes of *Streptococcus suis* infection in humans. Emerg Infect Dis. 2014;20(7):1105–14. http://wwwnc.cdc.gov/eid/article/20/7/13-1594_article.htm
4. Dechêne-Tempier M, Marois-Créhan C, Libante V, Jouy E, Leblond-Bourget N, Payot S. Update on the Mechanisms of Antibiotic Resistance and the Mobile Resistome in the Emerging Zoonotic Pathogen *Streptococcus suis*. Microorganisms. 2021 Aug 18;9(8):1765. https://www.mdpi.com/2076-2607/9/8/1765
5. Wertheim H.F.L., Nghia H.D.T., Taylor W, Schultsz C. *Streptococcus suis*: An Emerging Human Pathogen. Clin Infect Dis. 2009 Mar;48(5):617–25. https://academic.oup.com/cid/article-lookup/doi/10.1086/596763
6. Nugroho W, Cargill CF, Putra IM, Kirkwood RN, Trott DJ, Salasia SIO, et al. Investigations of selected pathogens among village pigs in Central Papua, Indonesia. Trop Anim Health Prod. 2016 Jan 17;48(1):29–36. http://link.springer.com/10.1007/s11250-015-0913-5
7. Susilawathi NM, Tarini NMA, Fatmawati NND, Mayura PIB, Suryapraba AAA, Subrata M, et al. *Streptococcus suis* –Associated Meningitis, Bali, Indonesia, 2014–2017. Emerg Infect Dis. 2019 Dec;25(12):2235–42. http://wwwnc.cdc.gov/eid/article/25/12/18-1709_article.htm
8. Lunha K, Chumpol W, Samngamnim S, Jiemsup S, Assavacheep P, Yongkiettrakul S. Antimicrobial Susceptibility of *Streptococcus suis* Isolated from Diseased Pigs in Thailand, 2018–2020. Antibiotics. 2022;11(3):1–12. https://www.mdpi.com/2079-6382/11/3/410
9. Wilson T, Jeffers J, Rappgabrielson V, Martin S, Klein L, Lowery D, et al. A novel signature-tagged mutagenesis system for *Streptococcus suis* serotype 2. Vet Microbiol. 2007 May 16;122(1–2):135–45. https://linkinghub.elsevier.com/retrieve/pii/S0378113507000077
10. Princivalli MS, Palmieri C, Magi G, Vignaroli C, Manzin A, Camporese A, et al. Genetic diversity of *Streptococcus suis* clinical isolates from pigs and humans in Italy (2003-2007). Euro Surveill. 2009;14(33):1–7. http://dx.doi.org/10.2807/ese.14.33.19310-en
11. Estrada AA, Gottschalk M, Rossow S, Rendahl A, Gebhart C, Marthaler DG. Serotype and genotype (multilocus sequence type) of *Streptococcus suis* isolates from the United States serve as predictors of pathotype. J Clin Microbiol. 2019;57(9). https://journals.asm.org/doi/10.1128/JCM.00377-19
12. Hu P, Yang M, Zhang A, Wu J, Chen B, Hua Y, et al. Comparative Genomics Study of Multi-Drug-Resistance Mechanisms in the Antibiotic-Resistant *Streptococcus suis* R61 Strain. Read TD, editor. PLoS One. 2011 Sep 26;6(9):e24988. https://dx.plos.org/10.1371/journal.pone.0024988
13. Pérez-Trallero E, Montes M, Orden B, Tamayo E, García-Arenzana JM, Marimón JM. Phenotypic and genotypic characterization of *Streptococcus pyogenes* isolates displaying the M.L.S.B. phenotype of macrolide resistance in Spain, 1999 to 2005. Antimicrob Agents Chemother. 2007;51(4):1228–33. https://journals.asm.org/doi/10.1128/AAC.01054-06
14. Uruén C, García C, Fraile L, Tommassen J, Arenas J. How *Streptococcus suis* escapes antibiotic treatments. Vol. 53, Veterinary research. BioMed Central; 2022. 91 p. https://doi.org/10.1186/s13567-022-01111-3
15. Dwijayanti N, Puspita Y, Susilawathi N, Tarini N. Antibiotic Susceptibility Pattern of *Streptococcus suis* Isolate from Clinical Specimens at Sanglah General Hospital, Denpasar, Bali. Int J Med Rev Case Reports. 2023;7(0):1. https://www.mdpub.net/?mno=122982
16. Zhang C, Zhang Z, Song L, Fan X, Wen F, Xu S, et al. Antimicrobial Resistance Profile and Genotypic Characteristics of *Streptococcus suis* Capsular Type 2 Isolated from Clinical Carrier Sows and Diseased Pigs in China. Biomed Res Int. 2015;2015:1–9. http://www.hindawi.com/journals/bmri/2015/284303/
17. Nedbalcova K, Kucharovicova I, Zouharova M, Matiaskova K, Kralova N, Brychta M, et al. Resistance of *Streptococcus suis* Isolates from the Czech Republic during 2018–2022. Antibiotics. 2022;11(9):1–15. https://www.mdpi.com/2079-6382/11/9/1214
18. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 31st ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2021. https://clsi.org/about/press-releases/clsi-publishes-m100-performance-standards-for-antimicrobial-susceptibility-testing-31st-edition/
19. Ishida S, Tien LHT, Osawa R, Tohya M, Nomoto R, Kawamura Y, et al. Development of an appropriate PCR system for the reclassification of *Streptococcus suis*. J Microbiol Methods. 2014;107:66–70. http://dx.doi.org/10.1016/j.mimet.2014.09.003
20. Nutravong T, Angkititrakul S, Jiwakanon N, Wongchanthong W, Dejsirilerts S, Nawa Y. Identification of major *Streptococcus suis* serotypes 2, 7, 8 and 9 isolated from pigs and humans in upper northeastern Thailand. Southeast Asian J Trop Med Public Health. 2014;45(5):1173–81. https://pubmed.ncbi.nlm.nih.gov/25417521/
21. Gygax SE, Schuyler JA, Kimmel LE, Trama JP, Mordechai E, Adelson ME. Erythromycin and clindamycin resistance in group B streptococcal clinical isolates. Antimicrob Agents Chemother. 2006;50(5):1875–

7. https://journals.asm.org/doi/10.1128/AAC.50.5.1875-1877.2006
22. Agersø Y. The identification of a tetracycline resistance gene tet(M), on a Tn916-like transposon, in the *Bacillus cereus* group. FEMS Microbiol Lett. 2002 Sep 10;214(2):251–6. http://doi.wiley.com/10.1016/S0378-1097(02)00883-2
23. Ye C, Bai X, Zhang J, Jing H, Zheng H, Du H, et al. Spread of *Streptococcus suis* Sequence Type 7, China. Emerg Infect Dis. 2008 May;14(5):787–91. http://wwwnc.cdc.gov/eid/article/14/5/07-0437_article.htm
24. Tian Y, Aarestrup FM, Lu CP. Characterization of *Streptococcus suis* serotype 7 isolates from diseased pigs in Denmark. Vet Microbiol. 2004 Oct;103(1–2):55–62. https://linkinghub.elsevier.com/retrieve/pii/S0378113504002652
25. Siahaan, Selma, Herman, Max J., Fitri, Nyoman. Antimicrobial Resistance Situation in Indonesia: A Challenge of Multisector and Global Coordination. *J of Trop Med*. 2022; 2783300, 10 pages. https://doi.org/10.1155/2022/2783300
26. Kurniawan K, Posangi J, Rampengan N. Association between public knowledge regarding antibiotics and self-medication with antibiotics in Teling Atas Community Health Center, East Indonesia. Med J Indones. 2017 May;26(1):62-9. https://mji.ui.ac.id/journal/index.php/mji/article/view/1589
27. Vela AI, Moreno MA, Cebolla JA, González S, Latre M V., Domínguez L, et al. Antimicrobial susceptibility of clinical strains of *Streptococcus suis* isolated from pigs in Spain. Vet Microbiol. 2005 Jan;105(2):143–7. https://linkinghub.elsevier.com/retrieve/pii/S0378113504003815
28. Chu YW, Cheung TKM, Chu MY, Tsang VYM, Fung JTL, Kam KM, et al. Resistance to tetracycline, erythromycin and clindamycin in *Streptococcus suis* serotype 2 in Hong Kong. Int J Antimicrob Agents. 2009 Aug;34(2):181–2. https://linkinghub.elsevier.com/retrieve/pii/S0924857909000454
29. Marie J. Antimicrobial susceptibility of *Streptococcus suis* isolated from swine in France and from humans in different countries between 1996 and 2000. J Antimicrob Chemother. 2002 Aug 1;50(2):201–9. https://academic.oup.com/jac/article-lookup/doi/10.1093/jac/dkf099

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Original Research Article

Sorghum Tempeh on Cholesterol Levels and Histopathology of Aorta in High-Fat Diet-Induced Rat Model

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Abstract

Background: Hypercholesterolemia is strongly linked to increased mortality rates and reduced productivity. It is also a major risk factor for the development of cardiovascular disease. Sorghum is recognized for its bioactive components and anti-cholesterol characteristics. Sorghum tempeh involves fermentation, resulting in increased fiber content and improved protein digestibility as compared to white sorghum.

Objective: This study examined the effects of sorghum tempeh on cholesterol levels and the histopathology of the aorta in rats fed a high-fat diet.

Methods: A total of 24 male 8-weeks-old Sprague Dawley rats were randomly divided into four groups: a standard diet group (SD), a high-fat diet control group (FD), a high-fat diet group supplemented with sorghum tempeh dose of 0.75 g/200g of BW (T1), and a high-fat diet group supplemented with sorghum tempeh dose of 1.50 g/200g of BW (T2). Cholesterol levels were measured using the total cholesterol ELISA method. After four weeks of intervention, the histopathology of aorta tissue was analyzed using Hematoxylin-Eosin staining.

Results: Serum total cholesterol levels in the T1 and T2 groups had significant differences ($p<0.05$) against the SD and FD groups. The decreased mean changes of the before-after intervention were observed in the T1 group (-93.195±5.920) and the T2 group (-121.143±4.276), while the mean effect size was observed ($\eta^2 = 0.996$). Histopathological analysis of aortic tissues from rats in the high-fat diet with sorghum tempeh group revealed improved condition of aortic tissues and reduction of foam cells.

Conclusion: Administering sorghum tempeh in the diet can significantly impact changes in body weight and cholesterol levels, with a potential effect of over 90%. The recommended dose of sorghum tempeh is 0.75 g per 200 g of body weight, which may lead to a decrease in body weight and a reduction in cholesterol levels.

Keywords: *sorghum tempeh; cholesterol; high-fat diet; aorta histopathology*
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INTRODUCTION

Based on data from the American Heart Association (AHA), between 2015 and 2018, 38.1% of people had hypercholesterolemia, 27.8% had low-density lipoprotein cholesterol levels ≥130 mg/dL, and 17.2% had low levels of high-density lipoprotein cholesterol, which were below 40 mg/dL. In 2020, cardiovascular disease caused 19 million deaths worldwide.[1] Increased amounts of very-low-density lipoprotein (VLDL) released into the plasma due to heightened liver-mediated cholesterol synthesis are responsible for elevated LDL and total plasma cholesterol levels.

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Elevated plasma cholesterol concentrations are typically attributed to higher levels of dietary cholesterol. Increased concentrations of both total and LDL cholesterol in the bloodstream are linked to hypercholesterolemia, a significant risk factor for the development of cardiovascular diseases.[2,3]

Sorghum is a type of cereal that contains a significant amount of starch and a bioactive compound called 3-deoxyanthocyanin (3-DXA). The concentration of 3-DXA in sorghum is 3-4 times higher compared to other types of grains. The substance also includes phenolic substances, flavonoids, β-glucans, and dietary fiber, which function as antioxidants, anti-inflammatory agents, and cholesterol-lowering agents.[4–6] Shen et al. conducted a study on the impact of sorghum on mice fed a high-fat diet. The group that consumed a diet containing sorghum experienced a 27.27% rise in HDL (high-density lipoprotein) and a 24.47% decrease in serum cholesterol levels.[7]

Sorghum tempeh is a sorghum-based product that undergoes fermentation by Rhizopus sp. This fermentation process produces a protease enzyme with proteolytic activity, which in turn enhances the levels of phenolic acids. As a result, the bioavailability of micronutrients is increased and protein digestibility is improved.[8] The tempeh fermentation process leads to enhanced nutritional quality modifications. Several studies indicate that sorghum may reduce the possibility of developing coronary heart disease by regulating lipid profiles and enhancing insulin sensitivity.[9,10] This study focuses on sorghum tempeh as a functional food product that specifically aims to decrease the risk of hyperlipidemia. The objective of this study was to examine the effects of sorghum tempeh on cholesterol levels and the histology of the aorta in rats that were fed a high-fat diet.

MATERIALS AND METHODS

Sample preparation

The white sorghum seeds were immersed in water at a ratio of 1:3 (w/v) for 24 hours. Following 10 minutes of boiling, the consistency of the sorghum seeds had an adjustment, becoming more tender. The sorghum seeds were inoculated with a 0.1% (by weight) concentration of tempeh yeast. The sorghum was wrapped in permeable plastic and allowed to remain at a room temperature of 29±1oC for 72 hours. Tempeh sorghum was drained at 90°C for 5 minutes to inhibit microbial activity. The intervention was made from 60% sorghum tempeh consisting of 18.7 g of sorghum tempeh, 10 g of skimmed milk, 1.5 g of canola oil, and 1 g of maltodextrin, dissolved in 100 cc of water.

Proximate analysis

An analysis was conducted at the Chem-mix Pratama Laboratory in Yogyakarta to compare the composition of sorghum tempeh and white sorghum. A proximate study was performed on modified sorghum to measure the levels of protein, fat, carbohydrates, antioxidant activity, and dietary fiber. The total carbohydrate content was calculated by subtracting the sum of other components from the total. The Kjeldahl method was applied to do protein analysis proximal. The immediate outcomes were acquired as a percentage (%) value. The examination of fat was conducted using the Soxhlet technique. The AOAC's enzymatic-gravimetric method (1995) was used to measure the total, soluble, and insoluble dietary fiber. Each analysis was conducted twice. Each data point was calculated based on a dry basis. The assessment of antioxidant activity was conducted using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) technique and protein digestibility using the in-vitro method.

Animals and treatment

This study used a true experimental design with a randomized pre-post-test-controlled group design for the total cholesterol levels and post-test-only control group design for the histopathology of aorta. A total of 24 male Sprague Dawley rats, 8 weeks old, were obtained from the Experimental Animal Laboratory of the Center for Food and Nutrition Studies, Universitas Gadjah Mada. Rats weigh 150-200 grams in healthy condition. The conditioning period for rats was eight weeks and the intervention period for rats was four weeks. Additionally, they were divided by simple random sampling into four groups: a standard diet group (SD), a high-fat diet group (FD), a high-fat diet group supplemented with 0.75 g/200g of BW (T1), and a high-fat diet group supplemented with 1.50 g/200g of BW (T2). The cage room individually had an ambient temperature of 28 – 32 ^{o}C with a lighting cycle of 12 hours dark and 12 hours light. Furthermore, the food was measured to 20 g/day using standard feed of Comfeed PARS and high-fat diet consisted of 60% standard feeds, 27.8% wheat flour, 2% pure cholesterol, 0.2% cholic acid, and 10% lard.

During the intervention period, the body weight was measured on a weekly basis. Following the experimental periods, samples of blood and aortic tissue were collected. Cholesterol levels were analyzed using the ELISA method with the total cholesterol kit. Rats had their retro-orbital plexus utilized to withdraw blood ±1% of their total body weight.

Ethical clearance

The study protocol (Ethical Clearance number 52/EC/H/FK-UNDIP/VI/2022) was approved by the Ethics Committee of the Faculty of Medicine, Universitas Diponegoro.

Histopathology of aorta

The aorta was separated and fixed in a 10% formalin buffer solution. The fixed aorta was prepared with paraffin blocks and stained with Hematoxylin-Eosin (HE). The phases involved in the preparation and HE staining process were fixation, dehydration, clearing, and paraffin infiltration. The histopathological slides were examined using a light microscope at a magnification of 100x. The observations identified structural damage to the aorta tissue and foam cells were assigned a score of 0, indicating normal histology. Score 1 indicates the widening of elastic fibers with a small number of foam cells. Score 2 indicates the fragmentation of elastic lamellae with a large number of foam cells. Score 3 indicates the proliferation of smooth muscle, infiltration of lipids in the middle layer, and fibrosis. Score 4 indicates the presence of an ulcerated or lipid-based plaque.[11–13]

Table 1. Comparison of Sorghum Tempeh and White Sorghum

Composition	White sorghum	Sorghum tempeh	*p-value*
Carbohydrate (%)	65.238±0.341	51.054±0.138	0.000*
Fat (%)	4.959±0.035	11.664±0.048	0.000*
Protein (%)	8.661±0.108	16.078±0.044	0.000*
Energy (kcal/100g)	333.107±1.458	370.772±0.087	0.001*
Antioxidant activity (%)	70.079±0.079	79.581±0.079	0.000*
Total dietary fiber (%)	16.328±0.126	19.807±0.177	0.002*
Insoluble fiber (%)	15.206±0.132	18.585±0.137	0.002*
Soluble fiber (%)	1.117±0.006	1.222±0.040	0.067
Protein digestibility (%)	50.915±1.331	63.580±0.200	0.006*

*One-way ANOVA significant difference ($p<0.05$)

Table 2. Food intake throughout the intervention period

Groups	Energy (kcal)	Protein (g)	Fat (g)	Carbohydrate (g)	Fiber (g)
SD	74.922±6.378	3.472±0.297	0.366±0.028[b]	14.255±1.213	1.098±0.919[b]
FD	74.632±7.201	3.460±0.335	0.363±0.034[b]	14.198±1.367	1.091±0.108[b]
T1	71.583±7.720	3.315±0.358	0.500±0.037[a,b]	13.292±1.470	1.323±0.110[a,b]
T2	77.326±4.742	3.578±0.217	0.682±0.025[a,b]	14.053±0.900	1.701±0.070[a,b]
p-value	0.528	0.537	0.000*	0.530	0.000*

*One-way ANOVA significant difference ($p<0.05$), [a]significant difference against control group (*Bonferroni* test), [b]significant difference against intervention group (*Bonferroni* test)

Table 3. Body weight changes of 4 weeks intervention (g)

Groups	W1	W4	*p-value*	Δ	*p-value*
SD	243.67±3.98	269.83±3.54	0.000*	26.17±2.40[a,b]	0.000**
FD	313.83±3.31	365.50±3.51	0.000*	51.67±1.21[a,b]	
T1	304.00±3.46	271.50±7.89	0.000*	-32.50±9.56[a,b]	$\eta^2 = 0.971$
T2	304.50±3.94	307.67±5.57	0.260	3.17±6.11[a,b]	

*Paired t-test, **One-way ANOVA, significant difference ($p<0.05$), [a]significant difference against control group (*Tamhane* test), [b]significant difference against intervention group (*Tamhane* test)

Data analysis

Statistical analyses were performed using IBM SPSS Statistics 25 SPSS software. The mean±SD was used to express all the data. The Shapiro-Wilk test was employed to analyze the normality of research data. The data of proximate analysis, food intake and the mean changes of total cholesterol and body weight were examined using a one-way ANOVA and Tamhane post hoc as a follow-up test. Significance was set at $p < 0.05$. The effect size of the observed differences was evaluated using the eta squared (η^2), along with its 95% confidence interval. The threshold values were defined as 0.01 considered small, 0.06 medium, and 0.14 large effects.

RESULTS

Sorghum tempeh and white sorghum comparison analysis

The proximate analysis results, shown in Table 1, compare the percentage of carbohydrate, fat, protein, energy, and antioxidant activity between sorghum tempeh and white sorghum ($p < 0.005$). Sorghum tempeh also has greater levels of total dietary fiber and insoluble fiber ($p = 0.002$), measuring 19.8% and 18.58%, respectively, compared to white sorghum. However, the soluble fiber of sorghum tempeh and white sorghum were not significantly different ($p = 0.067$). The carbohydrate content of sorghum tempeh was found to be lower (51.05%) compared to white sorghum (65.23%). On the other hand, the fat, protein, and energy content of sorghum tempeh (11.66%; 16.07%; 370.77 cal/100 g) was higher compared to that of white sorghum (4.95%; 8.66%; 333.10 cal/100g). According to the DPPH method antioxidant test results, the antioxidant content of sorghum tempeh was determined to be 79.58%. The protein digestibility of sorghum tempeh ($p = 0.006$) was shown to be enhanced (63.58%) after fermentation, in comparison to white sorghum (50.91%).

Food intake and body weight changes of rats

Table 2 showed the food intake of standard feed and sorghum tempeh administration during the intervention period. The SD and FD groups were only fed a standard diet, whereas the T1 and T2 groups were fed a standard diet together with sorghum tempeh at varying doses. The results of the one-way ANOVA test indicated statistically significant differences in fat and fiber intake ($p < 0.005$). The T1 group consumed 0.5 grams of fat per day, while the T2 group consumed 0.68 grams of fat per day. The T2 group had a higher fiber intake of 1.7 g/day compared to the T1 group, which had an intake of 1.32 g/day. The energy intake of the T1 group was 71.58 kcal/day, while the T2 group had an intake of 77.32 kcal/day. However, there was no significant difference when compared to the control groups ($p = 0.528$). Carbohydrate and protein intake were not significantly different ($p = 0.530$ and $p = 0.537$, respectively).

Table 4. Total Cholesterol Levels Pre and Post Intervention (mg/dL)

Groups	Pre-intervention	Post-intervention	*p-value*	Δ	*p-value*
SD	88.685±1.365	89.986±2.089	0.008*	1.302±0.741[b]	0.000**
FD	217.885±4.225	220.365±3.847	0.001*	2.480±0.929[b]	
T1	214.356±2.757	121.161±4.111	0.000*	-93.195±5.920[a,b]	$\eta^2 = 0.996$
T2	218.980±2.652	97.836±2.504	0.000*	-121.143±4.276[a,b]	

*paired t-test, **One-way ANOVA *p* value significant difference (p<0.05), [a]Significantly different from control group (SD and TD) (*Tamhane* test), [b]Significantly different from intervention group (T1 and T2) (*Tamhane* test)

Table 5. Histopathology scoring

Groups	Histopathology Aorta Score					Median (Min-Max)	*p*
	0	1	2	3	4		
SD	6	0	0	0	0	0 (6-6)[a]	0,003*
FD	0	6	0	0	0	1 (6-6)[b]	
T1	2	4	0	0	0	0.67 (2-4)[c,d]	
T2	1	5	0	0	0	0.83 (1-5)[b]	

*Kruskal Wallis significant difference (p<0.05), [a]Significantly different from FD and T2 (Mann Whitney Post hoc test), [b]Significantly different from normal control (SD), [c]Not significantly different from control group (SD and FD), [d]Not significantly different from intervention (T1 and T2).

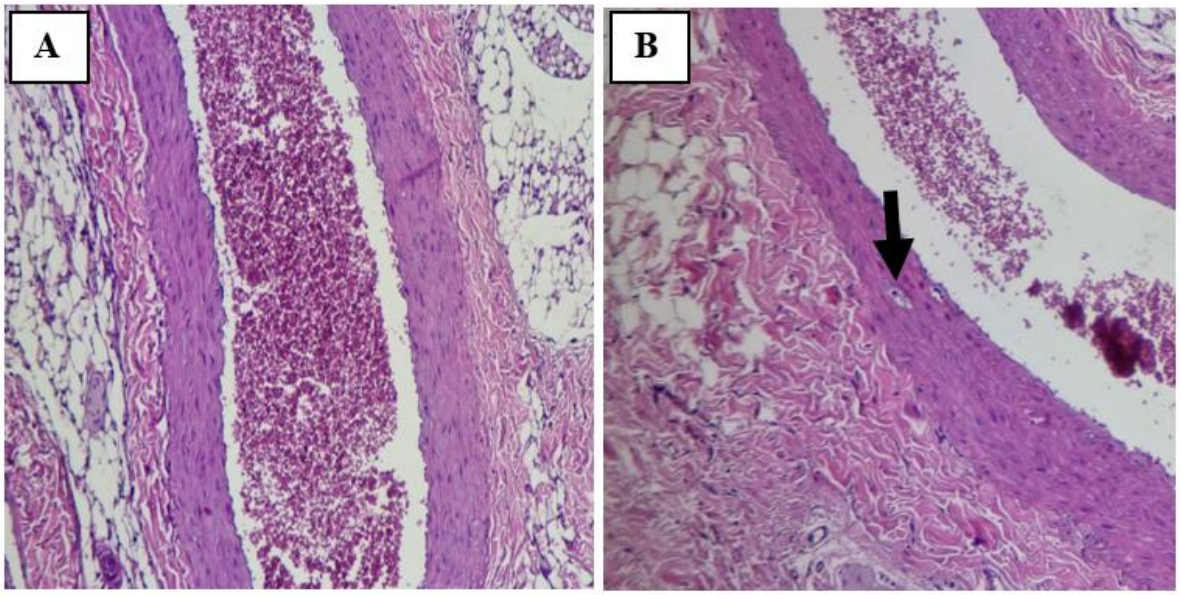

Figure 1. Histopathology of Aorta (Hematoxylin-eosin stain; original magnification: 100x). Microscopic view of a normal aorta cross section (score 0) (A), cross section of aorta containing foam cells (score 1) Histopathology showing the foam cell (arrow) within tunica intima (B).

According to Table 3, after four weeks of intervention, the SD, FD, and T1 groups showed significant differences between week 1 and week 4 of intervention time ($p < 0.005$). Meanwhile, the body weight of the T2 group was not significantly different over the intervention period ($p = 0.260$). The T1 group observed a drop in body weight with a mean change of -32.5 g, while the other group did not see any decrease in body weight.

The One-way ANOVA test showed a statistically significant difference in mean changes of body weight ($p < 0.005$). The effect size was calculated as eta squared ($\eta^2 = 0.971$).

Improvements in total cholesterol levels

The blood samples of rats were analyzed to determine their total cholesterol levels, as presented in Table 4. In the pre-intervention condition, the groups who were induced with a high-fat diet showed greater levels of total cholesterol in FD, T1, and T2 compared to the group that had a standard diet. The paired t-test showed statistically significant differences in the total cholesterol levels of the SD group ($p = 0.008$), FD, T1, and T2 groups before and after the intervention ($p < 0.005$). The One-way ANOVA test showed a statistically significant difference in mean changes of total cholesterol levels among the various groups ($p < 0.005$). The Tamhane test showed a statistically significant difference in the lowering of total cholesterol levels between the T1 and T2 groups compared to the SD and FD groups. The results indicated a reduction in total cholesterol levels in both the T1 and T2 groups following the intervention, with mean reductions of -93.19 mg/dL

and -121.14 mg/dL, respectively. The effect size was calculated as eta squared ($\eta^2 = 0.996$).

Effects of sorghum tempeh on Histopathology of Aorta

Figure 1 demonstrates that the SD group, which was fed a normal diet, displayed a normal microscopic image of the aorta in Sprague Dawley rats. These images showed that the aortic layer had three distinct layers: the tunica intima, tunica media, and tunica adventitia. The preparation displayed a picture of an artery exhibiting the presence of foamy macrophages infiltrating the intima. No pictures of intra/extracellular mucin or thrombus were present. There was no presence of plaque detected over the entire 8-weeks period of the high-fat diet. The histopathology of the aorta in rats given a high-fat diet shows histological abnormalities observed in the tunica intima, including the presence of foam cells. In Table 5, the FD and T2 groups showed statistically significant differences compared to the SD group. Histopathological examination of the aorta showed the normal tissue with no presence of foam cells (score = 0), the T2 group had only one sample of normal tissue (n = 6), while the T1 group contained two samples of normal tissues (n = 6).

DISCUSSION

This study determines that a high-fat diet can lead to hypercholesterolemia, which is defined by elevated levels of total cholesterol in the blood and the production of foam cells at early stages. The total cholesterol levels of hypercholesterolemic rats supplemented with two different doses of sorghum tempeh (0.75g and 1.50g per 200g of BW) were significantly lower compared to the control groups, as in prior research has found that sorghum tempeh effectively reduces LDL and MDA levels in rats after a high-fat diet.[9] Antioxidant activity of sorghum tempeh was slightly higher than white sorghum, which might be related to the fermentation, demonstrating the improvement of sorghum's nutrition characteristics.[14] The fermentation process generates a protease enzyme with proteolytic properties, leading to an elevation in phenolic acids. Consequently, this procedure enhances the bioavailability of micronutrients and improves protein digestibility; according to this study, the protein digestibility of sorghum tempeh was shown to be higher (63.58%) compared to sorghum that was not fermented (50.91%).[8] Study by Murtini et al. reported that sorghum tempeh fermented for 72 hours produced protein (10.27%), starch (45.56%), and fat (0.56%).[6] The protein of sorghum tempeh in this study was also found to be higher (16.07%) compared to the protein of sorghum (8.66%). The fermentation method applied to the production of tempeh additionally improves its bioavailability, but it also breaks down the glycosidic linkages to generate phenolics. The increase in phenolic compounds contributes to a higher level of antioxidant activity.[15] In addition, the antioxidant properties present in sorghum tempeh help reduce the buildup of cholesterol caused by a high-fat diet.

The high fiber content seen in sorghum tempeh is associated with a reduction in blood cholesterol levels. The composition of sorghum tempeh includes two types of fiber: soluble fibers (10.1–25.0%) and insoluble fibers (75.0–90.0%). This research additionally showed that the content of insoluble fiber (18.58%) is higher than soluble fiber (1.22%).[8] Short-chain fatty acids (SCFA) from intestine may lower blood cholesterol levels via decreasing hepatic cholesterol synthesis.[16] These findings propose a potential method to decrease the risk of hyperlipidemia and cardiovascular disease.[17]

The findings of this study demonstrate that the administration of sorghum tempeh for 4 weeks leads to a considerable reduction in total cholesterol levels. When administered at a higher dose of 1.50 g/200g of BW, it shows a significant decrease rate. The average total calorie intake from feed and enteral formula was 71.58 kcal/day in the T1 group and 77.32 kcal/day in the T2 group. Food intake of standard feed and sorghum tempeh administration showed different energy, fat, and fiber intake. The high-dose group received more energy and fat intake than the low-dose group, and the result showed the T1 group experienced a weight loss of 32.5 grams, but the T2 group exhibited a weight gain of 3.167 grams. The high-dose group had a higher fiber intake (1.70 g) compared to the low-dose group (1.32 g). Furthermore, the decreased mean changes before and after intervention were observed in the T1 group (-93.19 mg/dL) and the T2 group (-121.14 mg/dL). The effect size in this study was used to determine the practical significance of the study results and to understand the practical implications of the findings. The administration of sorghum tempeh had a 99.6% impact on changes in cholesterol levels and 97.1% on body weight changes, suggesting a large effect.

The histopathology results of this study demonstrated that the ingestion of a high-fat diet for 8-weeks leads to the development of hypercholesterolemia in rats, as previously observed, and promote the accumulation of foam cells in the tunica intima of the aorta.[13,18–20] Cholesterol buildup leads to a rise in lipoproteins in the inner lining of blood vessels, which are then oxidized by oxygen-free radicals generated by endothelial cells or macrophages. This accumulation occurs specifically in macrophages and other phagocytes, resulting in the formation of foam cells.[21]

After the sorghum tempeh was administered, only one or two out of the six samples exhibited improvement, characterized by the absence of foam cells. A possible explanation is that the samples had different levels of fat accumulation at the start of the intervention. As a result, some samples may have had more advanced conditions that were less susceptible to the intervention, other factors might contribute to this outcome. The rats included in the study may exhibit modest variations in their genetic compositions, which can influence their metabolic processes and responses to dietary intervention of sorghum tempeh.[22] The genetic variations may impact the rats' lipid metabolism, antioxidant defenses, and vulnerability to the formation of foam cells.

The gut microbiota also has a substantial impact on the breakdown of nutrients from food and the subsequent effects on health. Differences in the gut microbes of the rats may cause variations in the availability and efficacy of the active chemicals in sorghum tempeh, leading to varied outcomes.[23] In addition, the immune system, which contributes to the development of foam cells through macrophage activity, can differ across individual rats. Variations in immunological responses and

phenotypic alterations of macrophages during the accumulation of foam cells may result in differences in the cellular mechanisms involved in the formation or reduction of foam cells when exposed to the intervention.[24] In this study, the one rat showing an absence of foam cells could have had a combination of factors that made it more receptive to the positive effects of sorghum tempeh. In contrast, the other rats did not exhibit the same level of responsiveness. Further studies using larger sample sizes and the inclusion of other variables, such as the gut microbiota and macrophage activity, may be required to determine the exact cause.

The outcome of this study had a significant effect on overall cholesterol levels despite the presence of foam cells. Consequently, supplementation with sorghum tempeh may reduce the production of foam cells and enhance the histological appearance of the aorta. However, it was unable to achieve the total same condition as the healthy control group. The results showed that giving sorghum tempeh at a dose of 0.75 g/200g of BW was significant to improve the development of atherosclerosis, including reducing high cholesterol levels and preventing the formation of foam cells. The limitation of this study was that the researcher did not analyze any antioxidant markers to identify the phenolic compound of sorghum tempeh, the organ weight of rats, or histology of other organs, such as the liver. There was a lack of fatty acid analysis to clarify the factors contributing to the greater fat content in sorghum tempeh.

CONCLUSION

Sorghum tempeh is a type of functional food that offers enhanced nutritional benefits, including a higher content of total dietary fiber, predominantly insoluble fiber, increased antioxidant activity, and improved protein digestibility. Administering sorghum tempeh in the diet can significantly impact changes in body weight and cholesterol levels, with a potential effect of over 90%. The recommended dose of sorghum tempeh is 0.75 g per 200 g of body weight, which may lead to a decrease in body weight and a reduction in cholesterol levels. It is possible to restore the normal condition of the aorta histopathology by inhibiting the development of foam cells.

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REFERENCES

1. Tsao CW, Aday AW, Almarzooq ZI, et al. Heart Disease and Stroke Statistics—2022 Update: A Report From the American Heart Association. *Circulation*. 2022;145(8). doi:10.1161/CIR.0000000000001052
2. Wang HH, Garruti G, Liu M, Portincasa P, Wang DQH. Cholesterol and Lipoprotein Metabolism and Atherosclerosis: Recent Advances in Reverse Cholesterol Transport. *Annals of Hepatology*. 2017;16:S27-S42. doi:10.5604/01.3001.0010.5495
3. Xu J, Wang W, Zhao Y. Phenolic Compounds in Whole Grain Sorghum and Their Health Benefits. *Foods*. 2021;10(8):1921. doi:10.3390/foods10081921
4. Ham YM, Song HS, Kwon JE, et al. Effects of fermented Sorghum bicolor L. Moench extract on inflammation and thickness in a vascular cell and atherosclerotic mice model. *J Nat Med*. 2019;73(1):34-46. doi:10.1007/s11418-018-1231-9
5. Moraes ÉA, Marineli R da S, Lenquiste SA, et al. Sorghum flour fractions: Correlations among polysaccharides, phenolic compounds, antioxidant activity and glycemic index. *Food Chemistry*. 2015;180:116-123. doi:10.1016/j.foodchem.2015.02.023
6. Murtini EDS, Sutrisno A, Radite AG. Karakteristik Kandungan Kimia dan Daya Cerna Tempe Sorgum Coklat (Sorghum bicolor) [Characteristics of Chemical Content and Digestibility of Brown Sorghum Tempeh]. *J Teknol Industri Pangan*. 2011;22(2):150. https://journal.ipb.ac.id/index.php/jtip/article/view/4270
7. Shen Y, Song X, Chen Y, et al. Effects of sorghum, purple rice and rhubarb rice on lipids status and antioxidant capacity in mice fed a high-fat diet. *Journal of Functional Foods*. 2017;39:103-111. doi:10.1016/j.jff.2017.10.017
8. de Morais Cardoso L, Pinheiro SS, Martino HSD, Pinheiro-Sant'Ana HM. Sorghum (*Sorghum bicolor* L.): Nutrients, bioactive compounds, and potential impact on human health. *Critical Reviews in Food Science and Nutrition*. 2017;57(2):372-390. doi:10.1080/10408398.2014.887057
9. Khoirun Nisa A, Afifah DN, Djamiatun K, Syauqy A. The effect of Sorghum Tempeh (Sorghum bicolor L. Moench) on low-density lipoprotein (LDL) and malondialdehyde (MDA) levels in atherogenic diet-induced rats. *Potr S J F Sci*. 2021;15:662-671. doi:10.5219/1589
10. Setyowati D, Muna AN, Septiyani A, et al. Sorghum flour's effect on improving plasma lipid profile and atherogenic index in diabetic rats. *Aceh Nutri J*. 2023;8(2):186. doi:10.30867/action.v8i2.735
11. Forood A, Malekpour-Afshar R, Mahdavi A. Serum level of plasminogen activator inhibitor type-1 in addicted patients with coronary artery disease. *Addict Health.* 2014;6(3-4):119-126. PMID: 25984279 PMCID: PMC4354217
12. Harefa K, Sulastri D, Nasrul E, Ilyas S. Atherosclerotic Biomarkers (Interleukin-6 and CD40) and Tunica Intima Thickness in Obese Rats after the Administration of Plectranthus amboinicus (Lour.) Spreng Ethanol Extract. *Open Access Maced J Med Sci*. 2020;8(A):852-857. doi:10.3889/oamjms.2020.4349
13. Sarihati IGAD, Suastika K, Wita IW, Astawa INM, Adi AAAM. Atherosclerosis towards rat relating with high cholesterol feed. *irjeis*. 2017;3(2):150-156. https://sloap.org/journals/index.php/irjeis/article/view/542

14. Londoño-Hernandez, L, Bolívar G, Ramírez T. C. Effect of solid state fermentation with Rhizopus oryzae on biochemical and structural characteristics of sorghum (Sorghum bicolor (L.) Moench). *IJFFT*. 2018;8(1). doi:10.30954/2277-9396.01.2018.4
15. Salazar-López NJ, González-Aguilar GA, Rouzaud-Sández O, Loarca-Piña G, Gorinstein S, Robles-Sánchez M. Sorghum bran supplementation ameliorates dyslipidemia, glucose dysregulation, inflammation and stress oxidative induced by a high-fat diet in rats. *CyTA - Journal of Food*. 2020;18(1):20-30. doi:10.1080/19476337.2019.1702105
16. Soliman GA. Dietary Fiber, Atherosclerosis, and Cardiovascular Disease. *Nutrients*. 2019;11(5):1155. doi:10.3390/nu11051155
17. Mo Y nan, Cheng F, Yang Z, et al. Antioxidant Activity and the Potential Mechanism of the Fruit From Ailanthus altissima Swingle. *Front Vet Sci*. 2021;8:784898. doi:10.3389/fvets.2021.784898
18. Murwani S, Ali M, Muliartha K. Diet Aterogenik pada Tikus Putih (Rattus novergicus strain Wistar) Sebagai Model Hewan Aterosklerosis. *JKB*. 2013;22(1):6-9. doi:10.21776/ub.jkb.2006.022.01.2
19. Gui Y, Zheng H, Cao RY. Foam Cells in Atherosclerosis: Novel Insights Into Its Origins, Consequences, and Molecular Mechanisms. *Front Cardiovasc Med*. 2022;9:845942. doi:10.3389/fcvm.2022.845942
20. Volobueva A, Zhang D, Grechko AV, Orekhov AN. Foam cell formation and cholesterol trafficking and metabolism disturbances in atherosclerosis. *Cor Vasa*. 2019;61(1):48-55. doi:10.1016/j.crvasa.2018.06.006
21. Javadifar A, Rastgoo S, Banach M, Jamialahmadi T, Johnston TP, Sahebkar A. Foam Cells as Therapeutic Targets in Atherosclerosis with a Focus on the Regulatory Roles of Non-Coding RNAs. *IJMS*. 2021;22(5):2529. doi:10.3390/ijms22052529
22. Golforoush P, Yellon DM, Davidson SM. Mouse models of atherosclerosis and their suitability for the study of myocardial infarction. *Basic Res Cardiol*. 2020;115(6):73. doi:10.1007/s00395-020-00829-5
23. Shevchenko A, Shalaginova I, Katserov D, Matskova L, Shiryaeva N, Dyuzhikova N. Post-stress changes in the gut microbiome composition in rats with different levels of nervous system excitability. Wilson BA, ed. *PLoS ONE*. 2023;18(12):e0295709. doi:10.1371/journal.pone.0295709
24. Lee J, Choi JH. Deciphering Macrophage Phenotypes upon Lipid Uptake and Atherosclerosis. *Immune Netw*. 2020;20(3):e22. doi:10.4110/in.2020.20.e22