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PENGANTAR EDITOR

Salam sehat,

Alhamdulillah edisi ke dua volume ke tiga Jambura Medical and Health Science Journal

(JMHSJ) kembali dapat diterbitkan. Sebagaimana pada edisi sebelumnya, maka jurnal terbitan Fakultas Kedokteran (FK) UNG pada edisi kali ini menerbitkan 5 *original article* dari beragam institusi, yakni Universitas Muslim Indonesia Makassar, Universitas Tadulako Palu, dan Universitas Negeri Gorontalo. Hal ini menunjukkan JMHSJ juga diminati oleh para peneliti, akademisi, hingga praktisi dari berbagai penjuru Indonesia.

Topik yang diangkat pun bervariasi mulai dari analisis fitokimia kandungan antikanker dan antimikroba dari herbal daun Beta-beta, efisiensi penggunaan physioex dalam pembelajaran mahasiswa kesehatan, karakteristik penderita LUTS pada peserta PROLANIS, anemia dan status gizi pada remaja sekolah, serta morfometri pulau pancreas setelah diberikan metformin in vivo. Semoga kedepan akan lebih banyak tulisan dari berbagai disiplin ilmu kedokteran yang diangkat dalam JMHSJ dari berbagai penjuru instansi. Hal ini diharapkan dapat menjadi indikator baiknya kualitas pengelolaan jurnal yang sebentar lagi akan diakreditasi pada indeksasi nasional (SINTA). Selamat membaca.

Gorontalo, September 2024

Dr.dr. Muhammad Isman Jusuf,Sp.N

Chief Editor

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Phytochemical Screening of Ethyl Acetate Extract of Beta-beta Leaves (*Lunasia amara* Blanco) as Anticancer and Antimicrobial Based on Prediction of Activity Spectra for Substances (PASS) Online

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ABSTRACT

Introduction: Beta-beta leaves (*Lunasia amara* Blanco) have anticancer and antibacterial activity, but the secondary metabolites involved are still not clearly known. The aim of this research is to screen secondary metabolites from beta-beta leaves that have potential anticancer and antibacterial properties using the Passonline server and determine potential compound target proteins using the Superpred online server.

Method: Extraction of beta-beta leaves was carried out using the maceration method using ethyl acetate and identification of secondary metabolites using Liquid Chromatography High-Resolution Mass Spectrometry (LC HRMS). Prediction of the anticancer, antibacterial and toxicity potential of secondary metabolites of beta-beta leaves using Prediction of Activity Spectra for Substances (PASS) Online. Analysis of similarity to drugs using Lipinski rule of five and prediction of target proteins using the online superpred server.

Results: There are 10 secondary metabolites each which have anticancer and antibacterial bioactivity. The compounds methyl cinnamate and 3,5-pyridinedicarboxylic acid are similar to drugs based on Lipisnki rule of five analysis and are non-toxic based on online PASS analysis. There are 3 target proteins for methyl cinnamate which are involved in anticancer and antibacterial activity, namely NFκβ P105, ADAM10 and Catephsin D.

Conclusion: Ethyl acetate extract from beta-beta leaves contains secondary metabolites as anticancer and antibacterial based on Passonline server analysis. Methyl cinnamate is a potential secondary metabolite candidate as an anticancer and antibacterial with 3 target proteins, namely NFκβ P105, ADAM10 and Catephsin D.

Key words: Anticancer, antibacterial, beta-beta, *Lunasia amara,* passonline, prediction

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Introduction

Cancer and bacterial infections have now become major diseases that threaten physical and mental health worldwide. The number of patients that continues to increase every year is a particular concern because the mortality rate is also increasing. Chemotherapy treatment efforts for cancer patients are not always acceptable to patients or their families because of the side effects that cause a decrease in quality of life.[1,2] Treatment of infectious diseases with antibiotics is currently also starting to cause polemics because several species of bacteria are resistant to antibiotics. Therefore, it is necessary to develop new anticancer drugs that can selectively inhibit the growth of cancer cells, reduce chemoresistance, and cancer recurrence after treatment. Likewise with the development of antibiotics. One alternative effort that can be made is to use herbal plants in the surrounding environment. The use of medicinal plants as medicine is preferred for the treatment of diseases because it is efficient, easy to obtain, economical, and well tolerated by the body.[3] One of the Indonesian plants that has various bioactivities is Beta-beta (*Lunasia amara* Blanco).

Beta-beta is an endemic plant in Indonesia that has medicinal properties. Traditional uses of beta-beta include increasing stamina, reducing swelling, cleaning the eyes, treating snake bites, and as an antidiabetic.[4] Several research results have shown that Beta-beta leaf and bark powder have aphrodisiac effects. [5,6] Beta-beta crude extract is reported to have bioactivity as an anti-inflammatory,[7,8] antiparasitic,[9] antioxidant,[10,11,12] anticancer,[12,13] and antidiabetic.[14] Recent research has successfully proven that the ethanol extract of beta-beta leaves *Staphylococcus aureus, E. coli, Klebsiella pneumonia, Pseudomonas aeroginosa*, and *Salmonella typhimurium*.[15,16]

Although it is known to have antibacterial effects, the types of secondary metabolites from beta-beta leaves and the receptor proteins involved are still unclear because they have never been reported. Through this study, beta-beta leaves will be extracted using LCHR-MS, screening their potential secondary metabolites as anticancer and antibacterial along with the target proteins involved. Thus, the purpose of this study is to screen secondary metabolites from beta-beta leaves that have the potential as anticancer and antibacterial using the Passonline server and determine the target proteins of potential compounds using the Superpred online server.

Methods

Extraction and identification of secondary metabolites from leaves Beta-beta

A total of 250 grams of beta-beta leaf powder was extracted using the maceration

method using ethyl acetate solvent. The extract obtained was then identified for its secondary metabolite content using LCHRMS based on previous research.[14,17]

Prediction of anticancer and antibacterial activity of secondary metabolite from beta-beta leaves

Secondary metabolites contained in the ethyl acetate extract of Beta-beta leaves were predicted for their potential as antibacterial, anticancer, and toxicity using the passonline server (https://www.way2drug.com/passonline/predict.php). Canonical smiles of each secondary metabolite were obtained from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) then entered into the passonline server and then run to obtain bioactivity data from secondary metabolites.

Drug-likeness Prediction

Canonical smiles of each secondary metabolite were obtained from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) then entered into the http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp and then run to obtain bioactivity data from secondary metabolites.

Prediction of LD50 activity of secondary metabolite from leaves beta-beta

The canonical smile of each secondary metabolite was obtained from Pubchem and inputted into Protox II (https://tox-new.charite.de/protox_II/) to obtain data on the cytotoxic activity of each secondary metabolite.

Prediction of protein targets from potential secondary metabolites

To find out the target proteins from potential secondary metabolites, the Canonical smile of each compound is inputted into SuperPred (https://prediction.charite.de/).

Result

Metabolite secondary from beta-beta leaves

Based on the results of LCHR-MS analysis, it is known that there are 139 secondary metabolites contained in the ethyl acetate extract of beta-beta leaves (Figure 1). All of these secondary metabolites were then screened using the Passonline server to see their anticancer and antibacterial activity.

Result of screening secondary metabolites from ethyl acetate extract Beta-beta leaves as anticancer and antibacterial.

Based on the screening results using Passonline, it is known that there are 10 secondary metabolites that have the potential as anticancer and 10 secondary metabolites that have the

potential as antibacterial (Pa value > 0.7). The types of secondary metabolites that have the potential as anticancer and antibacterial are shown in Table 1.
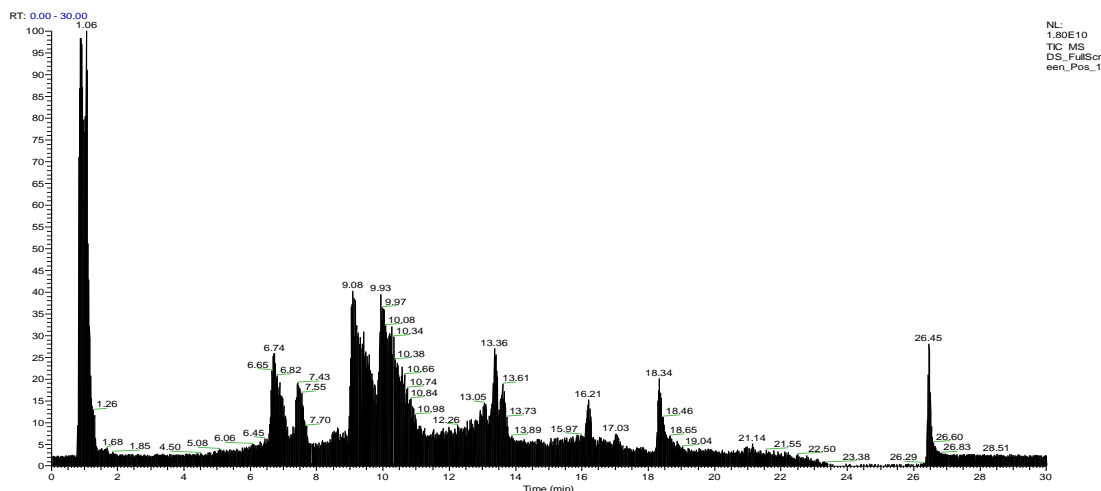
Figure 1. Chromatogram of secondary metabolites from Beta-beta leaves

Table 1. Anticancer and antibacterial activity prediction of secondary metabolites from Beta-beta leaves

No	Secondary metabolites	Structure	Mass weight	Anticancer activity(Pa value)	Antibacterial activity (Pa value)
1	Giberelic acid (diterpenoid)	$C_{19}H_{22}O_6$	363	0.95	
2	Dextrorphan	$C_{17}H_{23}NO$	257	0.92	
3	Tangeritin	$C_{20}H_{20}O_7$	372	0.82	0.89
4	Schaftoside	$C_{26}H_{28}O_{14}$	564	0.85	
5	Nobiletin	$C_{21}H_{22}O_8$	402	0.85	0.91
6	2,2,6,6-Tetramethyl-1-piperidinol (TEMPO)	$C_9H_{19}NO$	157	0.85	
7	Stanolone	$C_{19}H_{30}O_2$	290	0.81	0.88
8	-(-) Caryophyllene oxide	$C_{15}H_{24}O$	220	0.95	
9	Galaxolidone	$C_{18}H_{24}O_2$	272	0.81	
10	Corymboside	$C_{26}H_{28}O_{14}$	564	0.85	
11	Dibutyl phthlate	$C_{16}H_{22}O_4$	278		0.827
12	3,5-Pyridinedicarboxylic acid	$C_{14}H_{21}NO_4$	289		0.83
13	Trietylene glicol monobutyl eter	$C_{10}H_{22}O_4$	206		0.8
14	Metyl cinnamate	$C_{10}H_{10}O_2$	162		0.81
15	Scopoletin	$C_{10}H_8O_4$	192		0.82
16	Chlorogenic acid	$C_{16}H_{18}NO_2$	354		0.86
17	Benzyl butyl phthalate	$C_{19}H_{20}O_4$	312		0.81

Pa : *prediction activity*

Result of similarity with drugs (lipinski rule of five) , LD50 and toxicity

From the results of the similarity analysis with drugs (Table 2), it is known that schaftoside does not meet the Lipinski rule of five. Based on the results of the toxicity analysis using passonline, 2 compounds were obtained that were not toxic (Pa> 0.7), namely 3,5-pyridinedicarboxylic acid and methyl cinnamate. The results of analysis using protox 2 stated that the LD50 dose for methyl cinnamate was 1910 mm/kg and was categorized as level IV toxicity. While the LD50 dose for 3,5-pyridinedicarboxylic acid was 3720 mm/kg and was categorized as level V toxicity. The structures of the two compounds are shown in Figure 2.

Table 2. Result of screening secondary metabolites similarity with Lipinski rule of five, LD50 value and toxicity

No	Secondary Metabolites	Mass mol.	H accept	H donor	Log P	Molar refrac-Tibity	Predict LD50 (mm/kg)	Toxicity
1	Dibutyl phthlate	278	4	0	3,6	77	3474	Toxic
2	3,5-pyridinedicarboxylic acid	167	5	2	0,4	38	3720	Non toxic
3	Trietyleneglicol monobutyleter	206	4	1	0,8	54	3900	Toxic
4	Methyl cinnamate	162	2	0	1,8	47	1910	Non toxic
5	Tangeritin	371	7	0	3,3	98	5000	Toxic
6	Nobiletin	402	8	0	3,3	105	5000	Toxic
7	Androstanolone	290	2	1	3,9	82	3000	Toxic
8	Scopoletin	192	4	1	1,3	49	3800	Toxic
9	Chlorogenic acid	354	9	6	-0,6	82	5000	Toxic
10	Benzyl butyl phthlate	312	4	0	4	87	2330	Toxic
11	Giberellic acid	346	6	3	1	85	6300	Toxic
12	Dextrorphan	257	2	1	2,8	75	-	Toxic
13	Schaftoside	564	14	10	-1,9	130	823	Toxic
14	2,2,6,6-tetramethyl-1-piperidinol (TEMPO)	157	2	1	2,3	45	139	Toxic
15	-(-) caryophyllene oxide-	220	1	0	3,9	66	5000	Toxic
16	Galaxolidone	272	2	0	3,9	79	3200	Toxic
17	Corymboside							Toxic

Molecule mass < 500 Da; LogP < 5; H donor < 5; H acceptor < 10; molar refractivity 40-130.
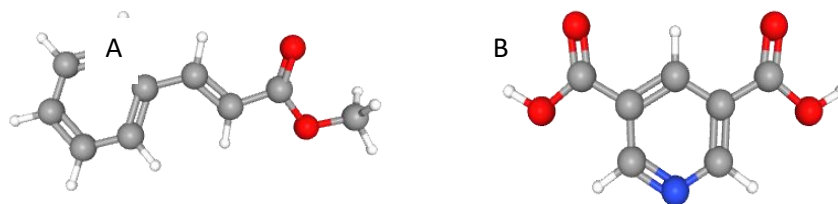
Figure 2. 3D Structure of methyl cinnamate(A) dan 3,5-pyridinedicarboxylic acid (B)

Result of screening protein target for potentially secondary metabolites from beta-beta leaves

Methyl cinnamate has 3 anti-ancer and antibacterial target proteins, namely NFκβ P105, ADAM 10, Catephsin. D For 3,5-pyridinedicarboxylic acid, no target proteins were found for anticancer and antibacterial. The results of the target protein screening are shown in Table 3.

Table 3. Result of target protein from methyl cinnmate and 3,5-pyridinedicarboxylic acid

Secondary metabolites	Super pred
Methyl cinnamate	NFκβ P105
	ADAM10
	Catephsin D
	-

Discussion

The use of beta-beta leaves in treating diseases has not been maximized. This is because the potential of beta-beta leaves is not widely known. Not only that, traditional medicine has used more beta-beta stems and roots because they are believed to have better benefits than leaves. Based on the results of the study, it was found that the leaves of the beta-beta plant contain various secondary metabolites that are pharmacologically important. The results of screening using LCHR-MS showed that beta-beta leaves contain 139 secondary metabolites, especially from the steroid, ester, flavonoid, coumarin, alkaloid, and terpenoid groups (Figure 1). There are 10 secondary metabolites each that have the potential as anticancer and antibacterial based on screening using Passonline (Pa> 0.7). Secondary metabolites with the highest activity as anticancer are gibberellic acid and (-) caryophyllene oxide (Pa = 0.95) while the highest activity as an antibacterial is shown by nobiletin (Pa = 0.91).

Passonline is a tool that can predict the bioactivity of a compound using the QSAR principle, namely the quantitative relationship between structure and compound.[18] Currently, passonline has been able to predict thousands of activities of secondary metabolites with an

accuracy level of more than 95% (https://www.way2drug.com/passonline/predict.php). This is certainly very useful in the search for natural-based drugs because it can save time, energy and costs. The results of Passonline analysis are expressed in predicted activity (Pa). A high Pa value (> 0.7) describes the bioactivity of a secondary metabolite based on the results of experimental tests. The higher the Pa value of a secondary metabolite, the higher its bioactivity value. However, if the Pa value <0.7, it can be ascertained that the secondary metabolite has a low probability of pharmacological activity However, not all potential secondary metabolites from beta-beta leaves have similarities to drugs and are safe for the body.

The results of the analysis using Lipinski's rule of five found that schaftoside had no similarities to drugs. This is because the properties of schaftoside do not comply with the Lipisnki rule of five, namely molecular mass> 500 Da, H acceptor> 10, H donor> 5. The size of the molecular mass> 500 Da causes the ligand to be impermeable to penetrate the lipid bilayer in the digestive tract and the blood-brain barrier.[19] Likewise, the number of H donors and H acceptors that are too many makes it difficult for the ligand to pass through the cell membrane.[20,21] This of course inhibits the absorption process of the ligand in the digestive tract. Based on the results of the toxicity prediction, only 3,5-pyridine dicarboxylic acid and methyl cinnamate were declared safe and non-toxic. To be used as a drug ingredient, it is necessary to search for target proteins from 3,5-pyridine dicarboxylic acid and methyl cinnamate.

Other target proteins owned by methyl cinnamate are NFκβ P105, ADAM10, and Catephsin D. NFκβ is one of the proteins involved in the process of cell transcription, stimulating cell proliferation, preventing apoptosis and increasing tumor angiogenesis and metastasis.[22] One of the subunits of NFKb is NFκβ P105 which is a transcription factor in the development of canine oral melanoma cells. The ADAM 10 protein is reported to support cancer cell growth by mediating cell division on several substrates that can promote the growth of malignant tumors.[23] In addition, ADAM10 is also involved in the development and chemoresistance of triple-negative breast cancer (TNBC),[24] and prostate cancer metastasis.[25] In cancer patients, exosomal ADAM10 expression increased so that ADAM10 can be used as a potential biomarker to detect cancer. Not only in cancer, ADAM 10 is also related to *Staphylococcus aureus* infection. When *Staphylococcus aureus* infection occurs, ADAM10 becomes a receptor for the cytotoxin α-hemolysin (Hla) which triggers the body's immune response. Currently, ADAM10 is used as a candidate target protein in antibiotic treatment due to *Staphylococcus aureus* infection. Cathepsin D is a protein found in

lysosomes and is involved in angiogenesis, proliferation, carcinogenesis, and pathogenesis of several types of cancer (breast, ovarian, and gastric). Recent studies have found that the mechanism of cathepsin D in promoting macrophage polarization and tumor-related metastasis is through TGFBI-CCL20 signaling.[26]

Methyl cinnamate and its derivatives are derivatives of cinnamic acid which has been known for its bioactivity as a potential anticancer and antimicrobial. Previous studies have found that methyl cinnamate is cytotoxic to RAW264.7 cells.[27] Based on in vitro studies, it is known that cinnamic acid derivatives, namely phenyl amide cinnamate, have strong cytotoxic activity against the MCF-7 cell line.[28] The anticancer ability of cinnamic acid and its derivatives is likely due to the presence of α,β-unsaturated bonds in its chemical structure.[29] As an antimicrobial, methyl cinnamate has been reported to be able to inhibit biofilm formation, thereby inhibiting the growth of *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeroginosa, Heliobacter pylori* and *Candida albicans* colonies.[30,31,32] Through this research, valuable insights were obtained regarding the anticancer and antibacterial potential of secondary metabolites from beta-beta leaves which have not yet been revealed. By knowing the activity of methyl cinnamate, we can further explore its potential therapeutic benefits and identify it as a source of valuable potential bioactive compounds. In the next stage, it is necessary to conduct in vitro activity tests of beta-beta leaf extracts to see the extent of the antimicrobial and anticancer effects caused.

Conclusion

Ethyl acetate extract from beta-beta leaves contains secondary metabolites that are anticancer and antibacterial based on Passonline server analysis. Methyl cinnamate is a potential secondary metabolite as an anticancer and antibacterial with target proteins such as NFκβ P105, ADAM10, and Catephsin D.

Conflicts of Interest

We have no conflicts of interest to disclose

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ORIGINAL ARTICLE   **Open Access**

The Efficiency of Utilizing The Physioex® Application Through E-Learning Among Students in The Field of Health at Universitas Negeri Gorontalo

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ABSTRACT

Introduction: Physiology is a study of the normal functions of the human body. However, the traditional lecture-based approach to teaching this subject has been observed to cause disengagement and boredom among students. Universitas Negeri Gorontalo was exploring the use of PhysioEx®, an interactive e-learning laboratory to studying physiology. This study aims to identify effective teaching methods to enhance student learning outcomes in physiology courses.

Method: A true experimental study was carried out at the Faculty of Medicine in Universitas Negeri Gorontalo in October 2023 with a pretest and posttest group. Each test comprised of nine questions and had a time limit of nine minutes. The study population consisted of 200 Public Health students and 69 medical students from the year 2023. A sample of 161 random individuals was selected for analysis using the Slovin formula, and the data was analyzed using the T-test analysis.

Results: Based on the statistical analysis of the pretest and posttest scores, it was observed that both the lecture method and the PhysioEx® method resulted in a significant increase in scores ($p<0.05$). However, no substantial difference was observed in the learning outcomes between the two methods ($p > 0.05$).

Conclusion: The comparative effectiveness of the lecture method and the PhysioEx® method in the instruction of physiology has been analyzed. The findings indicate that both methods are similarly effective when employed in educational contexts pertaining to physiology. The results of this analysis underscore the utility and potential of both pedagogical approaches.

Key words: Learning effectiveness, e-learning, physiology, physioEx®

Introduction

Physiology is the study of how the body works under normal circumstances.[1] Physiology is a very important basic science, by understanding how the body normally works, it will be easier for students to know and understand everything that is abnormal in the body, making it easier for them to learn the pathophysiology of various diseases. [2]

Physiology is semi-abstract but how it works is very real because people do it in everyday life, but in reality most students still have relatively difficulty understanding it. This physiology course requires students' ability to understand, connect and analyze the functions of each body system through metacognitive activities.[3]

Research results have shown that the final semester exam scores for physiology courses are still relatively low. The final semester exam results of Biology Education students at Brawijaya University for human physiology courses are still relatively low with an average score of 66.[4] Only 15% of students from the Nursing Professional Education Study Program Batch 16, Faculty of Medicine and Health Sciences, UIN Alauddin Makassar for the 2019-2020 academic year had good knowledge of physiology, while 45% were in the sufficient group and the other 40% were in the poor group.[5]

Data from the Gorontalo State University Academic System for 2022 has also shown the same thing, where Biomedical Science scores are still relatively low among students from the class of 2022, both in the Department of Public Health and in the Faculty of Medicine. The Department of Public Health with a total of 176 students showed an average score of B and there was only one student who got an almost perfect score (with an A-). The same thing was also found in students from the Faculty of Medicine, namely the physiology score in Biomedik 2 which showed that the average Physiology score of 59 students was 49.4 with the highest score being 72 and the lowest being 28. In Biomedicine 3 it showed the same thing. namely, the average value is 44, with the highest value being 69 and the lowest value being 25.

The low understanding of students in Biomedical Sciences, especially physiology, is

thought to be because human physiology courses are generally synonymous with learning using seminar methods and rote memorization, causing students to become bored quickly.[6] Biomedical Science books, especially physiology books, are usually very thick with a complicated writing style and content, making it difficult for students to understand them.[2]

The importance of physiology has triggered the emergence of several new methods to support students' understanding of biomedical science subjects, especially physiology. In the world of education, the role of technology is a new breakthrough that must be mastered to welcome the era of rapidly increasing globalization. In a study of the use of technology in the world of education in America, Alavi and Gallupe found that the use of technology can improve the quality of learning and teaching, reduce operational costs, and develop new products and service quality.[7] In responding to this, all elements of global governance, including Indonesia, are taking strategic steps in responding to the new era of education in order to guarantee and improve the quality of education, which must even be of higher quality than in previous times.[8] Health and Medical Education must also adapt to current conditions where digital-based technology continues to be developed. One breakthrough in technology-based physiology learning media is through applications *PhysioEx®.* Application *PhysioEx®* created with the hope of changing the method of learning physiology courses from difficult and boring to easy and fun.[9]

PhysioEx® is an application that provides sensation *learning by doing*, which offers laboratory experience *online* and allows students to practice in an environment that focuses on critical thinking and understanding. Through simulations, students can read an overview of the exercise, find the purpose of the laboratory, and undergo an introduction to the laboratory and laboratory equipment before diving into the virtual laboratory itself. Students can also take pre-lab and post-lab quizzes, do an overall review, and create a lab report. *Physioex®* is also an application that can be downloaded and installed on a laptop or computer easily.[9] Students can carry out laboratory and learning activities repeatedly according to their wishes, making it easier for them to learn and re-understand what they still don't understand.

PhysioEx® has the advantage of making it easier for students to carry out experiments that are difficult to carry out in a wet laboratory environment due to time, cost, or safety issues, and carrying out experiments without endangering live animals. In addition, the virtual laboratory presented by *PhysioEx®* Minimize the occurrence of equipment damage in wet laboratories.[9] However, so far there has been information about the effectiveness of learning methods using applications *PhysioEx®* This has not been widely publicized and is

rarely carried out among students, especially health students at Gorontalo State University.

This research aimed to identify effective learning methods for physiology courses at Gorontalo State University. It is hoped that the results of this research can provide a solution to overcome the low grades in human physiology courses, especially at Gorontalo State University.

Methods

This research was carried out at the Faculty of Medicine, Gorontalo State University, in October-November 2023. The type of research used was quantitative with a design.*experiment* with a plan *two groups pretest-posttest design*. The population in this study were students from the Faculty of Medicine and students from the Department of Public Health, first year student, namely class of 2023. There were 200 students from the Department of Public Health, and 69 students from the Faculty of Medicine, so the total population was 269 people. Sampling was carried out using the method *Simple Random Sampling*. Meanwhile, the number of samples was determined using the Slovin formula, and was obtained as many as 161 people.

The research variables consist of the dependent variable, namely the level of student understanding, the independent variable, namely the learning method, and the control variable, namely the duration of the material provided. The level of student understanding is how capable the student is of capturing and understanding the material being taught, and is assessed based on the results *Pretest* and *posttest* using Google forms. The pretest and posttest consist of 9 questions, in accordance with those available in the *PhysioEx*® application. Both groups were given the same questions and had 9 minutes to complete them. The data obtained was then carried out using univariate and bivariate tests *T-Test* with the SPSS application®. Learning methods are provided with two different methods, namely *e-learning* using the application *PhysioEx*® and conventional learning, namely using the lecture method.

Result

Table 1 shows that respondents from the control group (A) have the characteristics of 65 female respondents and 12 male respondents. Meanwhile, respondents from the treatment group (B), namely 16 male respondents and 62 female respondents. Most respondents in both groups were 18 years old.

Table 2 shows the analysis of the value results *pretest* and *posttest* in the control group

(A) and treatment group (B). in the control group (A) there was an increase in the average value before (*pretest*) and after (*posttest*) intervention was carried out on 77 respondents in the control group amounting to 15.0. The standard deviation value of 17.7 is higher than the average difference value. In the treatment group (B), from 78 respondents it was found that the minimum value obtained before the intervention was 0, and after the intervention it was 11.1. Meanwhile, the maximum value before the intervention was 77.8 and after the intervention it rose to 88.9. This shows an increase before and after the intervention in both groups.

Table 1. Characteristics of control and treatment group respondents

Characteristics	Control Group N = 77	%	Treatment Group N = 78	%	p value
Gender					
Male	12	15.6	16	20.5	0.475
Female	65	84.4	62	79.5	
Age (years)					
16	1	1.3	1	1.3	
17	7	9.1	5	6.4	0.777
18	53	68.8	56	71.8	
19	15	19.5	14	17.9	
20	1	1.3	2	2.6	

Chi-square test

Table 2. Analysis of results of *pretest-posttest* control and treatment group

Statistical Value	*Pretest*	*Posttest*
Control Group		
Mean	33.2	48.2
Median	33.3	44.4
Mode	33.3	33.3
Standard Deviation	16.4	17.3
Minimum	0	11.1
Maximum	88.9	100.0
Range	88.9	88.9
Treatment Group		
Mean	33.0	44.1
Median	33.3	44.4
Mode	44.4	55.6
Standard Deviation	16.9	18.3
Minimum	0	11.1
Maximum	77.8	88.9
Range	77.8	77.8

Based on Table 3, a comparison of values is obtained *pretest* and *posttest* control group and treatment group. For the control group (A), the average difference value before and after

intervention in the control group was -15.0, which means that on average there was an increase from *pretest* and *posttest*. Meanwhile, for the significance test value *paired sample t-test* namely 0.000. This shows that there is a significant difference between the average values before and after the intervention in the control group (*p-value*<0.05). In other words, learning using the lecture method influences the grades of UNG Medical and Public Health Students class of 2023.

Table 3. Comparison of values before and after intervention control group and treatment group

Group	Pretest (Mean ± SD)	Postttest (Mean ± SD)	Difference	p-value
Control (N = 77)	33.2 ± 16.4	48.2 ± 17.3	-15.0	0.000
Treatment(N = 78)	33.0 ± 17.0	44.2 ± 18.3	-11.1	0.000
Difference	0.2	4.0		
p-value	0.928	0.160		

The average difference between the treatment group (B) before and after the intervention was -11.1. This value indicates or gives meaning that on average there has been an increase in results *pretest-posttest* by using the test *paired sample t-test* with a significance value of 0.000 (*p-value*<0.05). In other words, learning by method *PhysioEx*® influence on the grades of UNG Medical and Public Health Students class of 2023.

Significance test results *independent sample t-test* shows a value of 0.928 which means that there is no significant difference between the average values *pretest* in the control group with the treatment group before the intervention (*p-value*>0.05). Meanwhile, the value of the significance test results is between values *posttest* control group and treatment group use *independent sample t-test* of 0.1. This shows that there is no significant difference between the average values *posttest* control group (A) with treatment group (B) after the intervention (*p-value*>0,05).

Discussion

Based on value data *pretest* control group, shows that the results *pretest* the control group was still relatively low, namely with a minimum value of 0 and a maximum value of 88.9. Reasons for low grades *pretest* can be caused by the respondent himself. Several factors that can influence include the respondent having never been exposed to physiology material before, the respondent's lack of interest and concentration in completing *pretest*, the respondent's inability to understand the questions, or due to low intellectual abilities. This is in line with research by Widayat, et al., the factor that influences student learning outcomes

is the student's initial abilities. Students who have good initial abilities have the potential to be able to think critically or think higher than those who have a low level of initial abilities, because they already have a good foundation for thinking.[10]

Minimum value of *posttest* in the control group, it was 11.11 and the maximum score reached a perfect score, namely 100. This shows that the lecture method is effective in increasing student understanding. This lecture method is a conventional method, or you could say it is a traditional method that has been used for a long time. Students feel more accustomed to receiving material using the lecture method, resulting in significant improvement.

The effectiveness of using this lecture method is thought to be influenced by the mentor who uses language that is simple and easy for respondents to understand so that it is easy for respondents to understand the material being taught. Sari stated that the lecture method is a method of delivering learning material orally and directly, participants are only required to see, hear and record important information from the teacher which is always considered correct among students and there is a psychological mechanism that allows them to reject and obtain information from the teacher.[4] A comfortable classroom and a learning process that is not too long can cause respondents to remain focused and comfortable in class. This is in line with Fatah et al. which states that the teaching and learning process, the use of simple language, and the atmosphere in learning also influence students' ability to understand the material being taught.[11] The research results have shown that there was a significant increase in learning outcomes before and after the intervention was given to the control group (A). This is in line with Nisa's research, which states that the level of respondents' understanding of learning material is said to increase if the results *posttest* which is greater than the average value*pretest*.[12]

Pretest results in the treatment group (B) was still relatively low with an average score of 33.0. This may be because health students still consider physiology to be a difficult subject to understand. This is in line with Miranto and Wardani who stated that many health students still have difficulty understanding human physiology due to the large amount of material that students have to study and memorize.[2] Other factors are thought to be the cause of the low scores *pretest* is the condition of the samples/respondents taken. The students who were used as respondents were the first year students who were still relatively new students and had never been taught physiology material before. Therefore, value *pretest* Low levels could also be because the sample has never been exposed to physiological material before.

Results of value analysis results *post-test* treatment group after exposure to the application *PhysioEx*[®] indicates the increased value of the results*pretest* average 33.04 to 44.15. This can provide an illustration of learning physiology using applications *PhysioEx*[®] can help students improve their understanding of physiology material. Students are presented with applications that require them to learn by doing. Apart from that, the animations displayed by the application *PhysioEx*[®] It is also clear and detailed, so that students can easily understand the material being taught. This is in line with research conducted by Moya-Salazar, application use *PhysioEx*[®] effective in learning physiology because this application uses simple language so it is easy to understand and also if students don't understand, they can try experiments or study repeatedly without limit until they understand the material.[9] But the results are value *posttest* shows that there are no samples/respondents who have received a perfect score in this study.

Several things may be the cause of the relatively short learning process, namely 45 minutes per material with a total of 2 (two) materials only 90 minutes. The researcher's assumption is that students still need more time to study. Learning methods use applications *PhysioEx*[®] which is still relatively new can also be the cause of the low value *posttest* respondents.[13] The assumption that students still consider physiology to be a difficult subject also influences the respondents' test results. This is in line with Tiraini's research, which proves that instilling the suggestion "Mathematics is easy" is effective in improving mathematics exam results.[14] Students who are used to using the lecture method during their learning from kindergarten to high school still need time to adapt to the application first. The optimal time for someone to understand true learning is 5 to 10 hours.[15] Meanwhile, this study only used 90 minutes, so it was still not enough to be able to understand 2 (two) learning materials at once.

Analysis results *pretest* and *posttest* in the treatment group (B) has shown that there is a significant average difference between before and after the intervention (*p-value*<0.05). In other words, learning using the application method *PhysioEx*[®] influence on the grades of 2023 UNG Faculty of Medicine and Public Health students.

Mark *pretest* control group (A) and treatment group (B) there is no significant difference as indicated by a value of 0.9 (*p-value*). Mark *pretest* which can still be said to be relatively low with an average of 33 also shows that both groups do not have adequate initial knowledge about physiology. In other words, both groups have the same initial knowledge.

Posttest results between the two groups has a relatively different average difference, namely 4.0. The control group (A) had a higher mean value compared to the mean value of

the treatment group (B), but the T-test result showed that the average value *posttest* between the control group (A) and the treatment group (B) there was no significant difference. Research results have shown that the use of applications *PhysioEx*® effective for use in studying physiology as shown by an increase in the average value *posttest*, but based on the criteria for determining learning completeness it is still in the poor category.

The habit of students who are more accustomed to learning using the lecture method, causes students to need time to adjust to new learning methods when changes are made to learning methods. The conventional method or lecture method remains the most effective method because students are often exposed to this method, so they are more accustomed to and more comfortable with the lecture method.[16] According to Fatah et al, social interaction has an effect on increasing students' understanding of the material being taught. An interactive mentor will certainly build greater social interaction compared to learning using applications.[6] This could have an effect on increasing respondents' understanding regarding the physiology material being taught.

The limitation in this research is the application *PhysioEx*® In use, it is only available in one language, namely English, so mentors need more time because they have to explain more than once. Apart from that, there were several respondents who filled in *pretest* and *posttest* more than once, and still need a companion.

Conclusion

Based on the research results, it was concluded that the two methods, both the lecture method and *PhysioEx*® significantly effective in learning physiology, there is no significant difference between the two methods. Suggestions from researchers, applications are expected *PhysioEx*® This can be used as a supporting application in learning physiology and still applies the lecture method, with the hope of increasing students' understanding of physiology.

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ORIGINAL ARTICLE   **Open Access**

Characteristics of Lower Urinary Tract Symptoms (LUTS) in the Chronic Disease Management Program (Program Pengelolaan Penyakit Kronis, PROLANIS) Patients: A Pilot Study in Gorontalo Province

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ABSTRACT

Introduction: Lower Urinary Tract Symptoms (LUTS) are common in older adults and significantly impact individuals, caregivers, and the broader healthcare system. As the elderly population with various comorbidities increases, the burden of LUTS will increase. The chronic disease management program (Program Pengelolaan Penyakit Kronis, PROLANIS) is a government program generally followed by the elderly. This study aims to describe the characteristics of LUTS in PROLANIS patients in Gorontalo Province.

Method: The analytical observational study design, using a cross-sectional approach, was conducted on PROLANIS patients living in rural and urban areas in Gorontalo Province is between July - October 2023. An incidental sampling technique was conducted on eligible patients and areas. LUTS symptoms were measured using the International Prostate Symptom Score (IPSS) questionnaire and categorized into mild, moderate, and severe. LUTS symptoms were considered significant if the IPSS score was ≥ 8.

Results: Of the 52 participants, 38.50% experienced significant LUTS symptoms. The majority of symptoms felt were nocturia (score two, as many as 23.10%) and mild symptoms (53.80%). Geographical differences, gender, education level, and age were not associated with LUTS symptoms ($p > 0.10$).

Conclusion: The prevalence of LUTS in patients undergoing PROLANIS is relatively high. The majority of LUTS patients have mild symptoms, with nocturia being the most common complaint compared to other symptoms. Further studies with a larger sample size and involving multiple sites are needed to obtain a more valid description of the characteristics of LUTS epidemiology in the PROLANIS population.

Keywords: Aged, disease managemen, lower urinary tract symptoms, nocturia

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Introduction

Lower urinary tract symptoms (LUTS) represent a prevalent clinical concern among the elderly population, exerting significant impacts on patients, caregivers, and the healthcare infrastructure at large. With demographic shifts favoring an aging population characterized by an increasing prevalence of multiple chronic diseases, the incidence and associated healthcare burden of LUTS are projected to rise notably. The European Association of Urology (EAU), alongside the American Urological Association (AUA) categorically delineates LUTS into storage (irritative) symptoms, including daytime frequency, urgency, and nocturia; voiding (obstructive) symptoms, featuring straining, weak stream, intermittency, incompleteness of flow and voiding; and postmicturition symptoms such as postmicturition dribbling, all of which cumulatively afflict the lower urinary tract.[1]

Lower urinary tract symptoms are classified into storage and voiding categories initially delineated by the International Continence Society (ICS). This framework was further refined in 2002 when the ICS expanded the classification to include a novel category — post-micturition symptoms, thereby providing a more comprehensive understanding of LUTS.[2,3]

The etiological contributors to LUTS are multifaceted and intricate. The elevated prevalence of LUTS correlates significantly with the surge in degenerative pathologies that predispose individuals to LUTS complications. Notable among these are benign prostatic hyperplasia (BPH), diabetes mellitus (DM), obesity, and hypertension, in addition to socio-psychological determinants including educational attainment and socioeconomic status.[4] Furthermore, while the concurrent increase in the incidences of LUTS and hypertension with advancing age is observed, the causative linkage between these two conditions has historically been underexplored.[5]

Through their effects on the detrusor muscle and urinary sphincter function, several categories of prescription drugs can worsen LUTS, including antidepressants, antihistamines, bronchodilators, anticholinergics, sympathomimetics, and diuretics. Diuretics disrupt fluid volume balance and are increasingly used in elderly patients, contributing to the development of LUTS. The prevalence of these medications increases with age, contributing to the increase in age-related LUTS.[6]

The Chronic Disease Management Program (Program Pengelolaan Penyakit Kronis, PROLANIS), which is generally followed by the elderly population, is a health service and proactive approach that is implemented in an integrated manner involving participants and health facilities in order to maintain health in patients with chronic diseases (especially hypertension and type 2 diabetes) to achieve optimal quality of life. PROLANIS activities

include preventing ongoing complications and improving public health, including medical consultation activities, PROLANIS clubs, home visits, and health screening.[7] On the one hand, LUTS dramatically affects a person's quality of life, including PROLANIS patients with various comorbidities.[8] Therefore, this study aims to describe the characteristics of LUTS sufferers in patients who follow PROLANIS. The results of this study can be used as a basis by PROLANIS managers in screening and following up on LUTS symptoms in the patients they manage so that it can help improve their quality of life.

Methods

This research will be conducted in two different Community Health Centers in Gorontalo Province, each representing the characteristics of rural and urban areas, namely Bulango Ulu Community Health Center and Kota Tengah Community Health Center. The research implementation time is in July - October 2023. This study used an analytical observation research design with a cross-sectional approach.

The population of this study was all PROLANIS patients registered in both health centers, totaling 100 people. The sampling technique used in the present study was accidental sampling. The minimum sample size was calculated based on the Harry-King normogram. Based on a 90% confidence level, the minimum sample size was 35% of the total 100 people, which was 35 respondents.

The LUTS variable is defined as a set of lower urinary tract symptoms experienced by PROLANIS patients. The international Prostate Symptom Score (IPSS) questionnaire was used to assess LUTS symptoms in Indonesian and has been previously validated by Monoarfa and Mochtar.[9] Data collection was carried out directly by visiting the health center during the PROLANIS activity, and all respondents were accompanied during the questionnaire filling process. Respondents were categorized as LUTS sufferers if they got an IPSS score ≥ 8.

Upon completion of data acquisition, the subsequent phase entails processing the acquired data utilizing SPSS statistical software, version 13.0, compatible with the Windows operating system. The data encapsulating the prevalence of LUTS were articulated as percentages and delineated according to their specific attributes. The discrepancy in the distribution of attributes among individuals afflicted with LUTS versus those unafflicted is evaluated through the employment of the Chi-Square or Fisher's Exact Test, depending on the suitability of the dataset's characteristics. Concurrently, the Mann-Whitney U test is deployed to analyze disparities in the median values of quantitative attributes between the two aforementioned groups. A p-value threshold of less than 0.10 is adopted to denote statistical significance.

Result

The study was conducted at two Community Health Centers in the Gorontalo region, representing the characteristics of urban areas (Puskesmas Kota Tengah) and rural areas (Puskesmas Bulango Ulu). In the initial screening, the total number of study participants was 52 people, consisting of 24 people at Puskesmas Kota Tengah and 28 at Puskesmas Bulango Ulu. All respondents completed the IPSS questionnaire, so no data was excluded.

The characteristics of the study participants, in general, can be seen in Table 1. Most participants came from rural areas, were female, and had an elementary school education or equivalent. The study participants had an average age of 57 years, with the lowest age being 23 years and the highest age being 81 years. The prevalence of significant LUTS symptoms (IPSS score >7) in PROLANIS patients in this study was 38.50%, with the majority in the mild symptom category followed by moderate and severe symptoms.

Table 1. Baseline characteristics of study participant

Parameter	Frequency (n)	Percentage (%)
Origin of Puskesmas		
Kota Tengah (Urban Area)	24	46.20
Bulango Ulu (Rural Area)	28	53.80
Gender		
Male	14	26.90
Female	38	73.10
Educational Level		
Elementary School	26	50.00
Junior High School	5	9.60
Senior High School	10	19.20
Higher Education	11	21.20
Age (years)[a]	57.12 ± 13.69	
Significant LUTS		
Present	20	38.50
Absent	32	61.50
LUTS Severity		
Normal	4	7.70
Mild	28	53.80
Moderate	14	26.90
Severe	6	11.50
Total	**52**	**100**

[a]Mean±standard deviation

LUTS: Lower urinary tract symptoms

The characteristics of LUTS scoring based on each symptom can be seen in Table 2. This study's median total IPSS score was 6, with the lowest total score of 0 and the highest of 27. Most participants were classified as having a score of 0 in all categories of LUTS

symptoms except for nocturia symptoms. In nocturia symptoms, most participants were classified as having a score of 2, followed by a score of 5, a score of 1, and a score of 0. In this study, the quality of life-related to LUTS symptoms in PROLANIS patients had a median value of 1, with the lowest score being 0 and maximum scores of 6.

Table 2. Detailed sharacteristics of LUTS in the study participants

IPSS Parameter	Score 0		Score 1		Score 2		Score 3		Score 4		Score 5	
	n	%	n	%	n	%	n	%	n	%	n	%
Total Score[a]					6 (0-27)							
Score at Each Symptom (N=52)												
Incomplete emptying	25	48.10	4	7.70	1	1.90	9	17.30	3	5.80	10	19.20
Urinary frequency	17	32.70	11	21.20	2	3.80	8	15.40	8	15.40	6	11.50
Urinary intermittency	42	80.80	1	1.90	3	5.80	2	3.80	1	1.90	3	5.80
Urinary urgency	41	78.80	1	1.90	4	7.70	4	7.70	0	0.00	2	3.80
Weak stream	40	76.90	4	7.70	1	1.90	5	9.60	1	1.90	1	1.90
Straining	41	78.80	2	3.80	2	3.80	7	13.50	0	0.00	0	0.00
Nocturia	9	17.30	9	17.30	12	23.10	5	9.60	6	11.50	11	21.20
Quality of life scores[a]					1 (0-6)							

[a]Median (Min-Max)
LUTS: Lower urinary tract symptoms

Differences in demographic characteristics based on significant LUTS symptoms experienced by participants can be seen in Table 3. Both groups classified as not experiencing and experiencing significant LUTS symptoms had similar characteristics regarding region of origin, gender, education level, and age.

Discussion

The prevalence of significant LUTS symptoms in this study was 38.50%. This is relatively higher than previous studies that found the prevalence rate of LUTS around 20% .[8,10] Compared to the results of studies in Japan, the prevalence rate obtained in this study is relatively lower. Of the 6,210 participants, it was reported that 77.90% of subjects aged ≥20 years suffered from LUTS. On the other hand, 82.50% of subjects aged ≥40 years suffered from LUTS in the study.[11] Another study also found similar results, where the prevalence of LUTS was 69.80% in Poland.[12] This difference in prevalence could be due to the way LUTS symptoms were interpreted. When the study reported only significant LUTS symptoms

(IPSS score ≥8), the reported prevalence was relatively lower.[8,13] Conversely, when the study reported existing LUTS symptoms without considering the threshold of significant score, the reported prevalence was relatively higher.[8]

Table 3. Relationship between demographic characteristics and LUTS

Characteristics	Significant LUTS				p-value
	Present (N=20)		Absent (N=32)		
	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)	
Origin of Puskesmas (N=52)					
Kota Tengah (Urban Area)	9	17.31	15	28.85	0.895[b]
Bulango Ulu (Rural Area)	11	21.15	17	32.69	
Gender (N=52)					
Male	5	9.62	9	17.31	0.805[b]
Female	15	28.85	23	44.23	
Educational Level (N=52)					
Elementary School	10	19.23	16	30.77	0.922[c]
Junior High School	2	3.85	3	5.77	
Senior High School	3	5.77	7	13.46	
Higher Education	5	9.62	6	11.54	
Age (years)[a]	54.05 ± 14.45		59.03 ± 13.05		0.205[d]

[a]Mean±standard deviation [b]Chi-Square test [c]Fisher-Exact tesr [d] Independent T test

The present study indicates a predominance of mild symptoms of LUTS among the respondents, who were participants in the PROLANIS program, primarily afflicted with chronic conditions including hypertension, diabetes mellitus, dyslipidemia, and cardiac diseases. It was observed that the focus of these individuals predominantly lay on the management and monitoring of their pre-existing chronic diseases, consequently sidelining the attention towards LUTS symptoms.[14] This observation aligns with prior research findings indicating that individuals suffering from LUTS frequently neglect the mild symptoms experienced, delaying the pursuit of medical intervention until the symptoms coexist with multiple comorbidities and significantly impair quality of life.[8,14]

The most common LUTS symptom experienced by respondents in the recent study

was nocturia. Other symptoms were mostly at a score of 0. In addition, respondents generally did not feel that their quality of life was disturbed by these LUTS symptoms. These results are different from previous studies when compared with the characteristics of women who predominantly suffered from LUTS in this study. Mitsui et al. (2024) found that urgency and stress urinary incontinence were the most common complaints of women with LUTS.[11] Likewise, a study by Przydacz et al. (2020) reported that women with LUTS mostly complained of urinary incontinence of any type.[12]

Geographical differences (urban vs. rural), gender, education level, and age were not associated with LUTS symptoms in this study. In general, LUTS can be caused by many factors, such as drug consumption, age factors, gender, and diseases that can cause LUTS.[1] Geographical differences were also found to be unrelated to LUTS symptoms. In addition, the difference in proportion between men and women was also relatively small.[12] Education level was also not associated with LUTS symptoms.[8] As for age, the present study had discrepancies from the other studies, which found an increasing trend in the prevalence of LUTS with increasing age.[12,15]

This study has several limitations. The pilot project study design had a relatively small sample for a descriptive study. Therefore, further research with a larger sample and multisite is needed to obtain a more convincing description. In addition, other factors such as comorbidities, medical history, and certain medications have yet to be explored, even though these factors may play a role in the prevalence of LUTS in this study.

Conclusion

The prevalence of LUTS in the patient population undergoing PROLANIS is quite high. The majority of symptoms experienced are nocturia and are mild symptoms. Geographical differences, gender, education level, and age are not related to LUTS symptoms.

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ORIGINAL ARTICLE   **Open Access**

The Incidence of Anemia and Its Relationship With Nutritional Status (A Study on Female Students at SMA Negeri 3 Gorontalo)

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ABSTRACT

Introduction: The calibre of its human resources dramatically impacts the development of a nation. However, specific health concerns, such as malnutrition, specifically anemia, continue to impede the progress of women of reproductive age, particularly teenage girls, due to the increasing requirement for iron during puberty and menstruation. This study aims to explore the correlation between nutritional status and the prevalence of anemia in female students, specifically focusing on high school students.

Method: This cross-sectional study examined the correlation between the nutritional status of female students in grade XI at SMA Negeri 3 Gorontalo from September to November 2023. Microtoise and digital scales will be employed to obtain the student's body mass index (BMI) values to evaluate their nutritional status. Anemia will be detected using a digital strip-test Hb measuring instrument, and purposive sampling will be used as the sampling technique. The Spearman rank analysis test will be used to determine the significance of the associative hypothesis.

Results: The study primarily consisted of young women, with the majority being 15 years old (41.2%), possessing normal nutritional status (76.5%), and not experiencing anemia (66.7%). An intriguing correlation was discovered between nutritional status and anemia incidence among female students at SMA Negeri 3 Gorontalo (r=-0.364, p= 0.009).

Conclusion: The prevalence of anemia among female students at SMA Negeri 3 Gorontalo is related to their nutritional status. It is recommended that healthcare professionals provide counselling and education on anemia, particularly for teenage girls.

Keywords: Anemia, teenage girl, nutritional status.

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Introduction

A country's progress in development is influenced by the quality of available Human Resources (HR). However, several obstacles hinder the achievement of this hope, especially those related to health, such as nutritional problems such as anemia. Anemia affects various age groups and genders. However, the most vulnerable group is women of childbearing age, especially adolescent girls. This is related to the increased need for iron during puberty and menstruation. Blood loss during menstruation can cause a decrease in iron levels in the body, which can cause anemia if not balanced with sufficient iron intake through food or supplements. This health issue remains a significant concern in society. Adolescence for girls is considered a crucial period because it is an essential stage in preparation for becoming a mother-to-be. Special attention is required to meet nutritional needs.[1]

According to the United Nations (UN), around 800 million people worldwide are malnourished, especially in developing countries.[2] The prevalence of overnutrition is around 16%, with an overweight rate of around 11.2% and obesity of around 4.8%.[3] According to the World Health Organization, since 2008, around 2.8 million people have died each year due to overnutrition and obesity. The prevalence of malnutrition in the world reaches 14.9%, and Southeast Asia has the highest rate at 27.3%.[4] In 2018, in Indonesia, the prevalence of malnutrition in adolescents aged 13-15 years was around 8.7%, consisting of a very thin level of 1.9% and a thin level of 6.8%.3 According to data from the Gorontalo Provincial Health Office in 2022, in junior high school/MTs adolescents, 78.6% had normal nutritional status, 2.4% were very thin, 9.8% were thin, 69.9% were obese, 1.6% were obese, and 6.5% were stunted. The risk of anemia was recorded in male students as much as 2.4% and female students as much as 5.6%. In high school/MA adolescents, 73.12% had normal nutritional status, 3.6% were very thin, 11.6% were thin, 14.9% were obese, 3.5% were obese, and 0.35% were stunted. The risk of anemia was recorded in male students at 1.07% and female students at 5.9%.

Anemia refers to a condition in which the amount of hemoglobin in the blood is below normal limits.[5] The dangers of anemia in adolescent girls include delayed physical growth and behavioral and emotional disorders. This condition can affect the development of brain cells, affecting the immune system, impaired concentration, fatigue, decreased academic achievement, and low work productivity.[6]

The prevalence of anemia in adolescents in various countries ranges from 40% to 88%. According to the World Health Organization, in 2018, the incidence of anemia in adolescent girls in developing countries reached around 53.7% of the total adolescent girls.[4] Based on

the 2018 Basic Health Research (RISKESDAS), the prevalence of anemia in Indonesia by gender was 20.35% in males and 27.2% in females. In the age range of 5-14 years, the prevalence rate of anemia reached 26.8%, while in the age range of 15-24 years it reached 32.0%.[6] From data from the Gorontalo Provincial Health Office in 2023, the number of adolescent girls suffering from anemia was 0.3% in 2022, and only 0.7% consumed iron supplement tablets. Therefore, this study aimed to prove whether it was confirmed that only 0.3% of adolescent girls in Gorontalo suffered from anemia and the other 99.07% did not.

Thin condition is a nutritional problem that is often found in adolescent girls. They often view thinness as something beautiful, so dieting without expert guidance and direction causes the need for essential nutrients to be unmet.[7] If this condition is not corrected, it can cause nutritional problems such as excess nutrition, malnutrition, inadequate nutrition, Iron Deficiency Anemia, vitamin A deficiency, and Iodine Deficiency Disorders.[8] The impact of anemia in adolescence, if not treated, can be carried over to marriage, pregnancy, and childbirth. The long-term impact can increase the risk of Iron Deficiency Anemia (AKI) and Iron Deficiency Anemia (IDA).[9]

Therefore, researchers are interested in investigating the relationship between nutritional status and the incidence of Anemia in adolescent girls, particularly high school students. Researchers chose SMA Negeri 3 Gorontalo as a research location because, in the initial survey, it was found that of 18 female students aged 16-17 years, there were 16.7% of female students who had a height of <150 cm, 11.1% of female students weighed 30-39 kg, 5.6% weighed >59 kg. In addition, in the last month, 50% of female students felt paler than before, 55.6% who sometimes had headaches, 22.2% who felt they often had headaches, and 11.1% felt they always had headaches. In the last month, 38.9% of female students had difficulty sleeping, and 33.3% of female students often had difficulty sleeping. From some symptoms, there is an impact, namely difficulty concentrating for female students where the results obtained are 55.6% of female students who sometimes find it difficult to concentrate, 16.7% of female students who often find it difficult to concentrate, and 22.2% of female students who always find it difficult to concentrate. Therefore, researchers hope this study can identify the incidence of Anemia in Female Students at SMA Negeri 3 Gorontalo and how Nutritional Status can affect the incidence of Anemia. By knowing this, prevention and screening efforts for Anemia can be carried out and treated early.

Methods

Sampling Techniques

The study employed a purposive sampling method to select participants, ensuring that

only those who met specific inclusion criteria were chosen. The criteria included students from SMA Negeri 3 Gorontalo, specifically those in class XI, who were available and willing to participate in the research. This approach was selected to focus on a specific subset of the population most relevant to the study objectives—students at a critical developmental stage where nutritional status and anemia risk are particularly significant. By using purposive sampling, the study ensured a more targeted and efficient data collection process, allowing for in-depth analysis of the relationship between nutritional status and anemia within this specific group.

Data Measurement

Anemia was assessed using a digital hemoglobin (Hb) strip-test, a reliable and non-invasive tool for measuring hemoglobin levels. Prior to the study, the digital Hb strip-test was calibrated according to the manufacturer's guidelines to ensure accuracy. Validation of the instrument was performed by comparing test results with standard laboratory methods in a preliminary trial. The process involved collecting a small blood sample from each participant, which was then applied to the Hb strip. The device provided an immediate readout of hemoglobin concentration. For the purposes of this study, anemia was defined as an Hb level of ≤ 12 g/dL, while levels ≥ 12 g/dL were considered normal. This method was chosen for its ease of use in field settings, rapid results, and minimal discomfort to participants, ensuring the reliability and validity of the anemia assessments. This research was conducted at SMA Negeri 3 Gorontalo between September and November 2023, utilizing a cross-sectional design with an analytical observational approach. The population comprised 246 students from class XI, determined using the Slovin formula. Nutritional status was assessed using Body Mass Index (BMI), calculated from measurements obtained with a microtome and digital scales. BMI results were classified as follows: normal (z-score $\geq$ -2 SD to 1 SD), thin (z-score $\geq$ -3 SD to < -2 SD), very thin (z-score < -3 SD), obese (z-score >1 SD to 2 SD), and very obese (z-score >2 SD). Anemia was assessed using the digital Hb strip-test, with results categorized as 'yes' if Hb levels were ≤ 12 g/dL, and 'no' if Hb levels were ≥ 12 g/dL.

Data Analysis

Data collected from each respondent were coded and entered into SPSS for analysis. The software was used to conduct frequency analyses of both dependent and independent variables. The results were presented in frequency distribution tables, enabling a clear understanding of the data. To determine the relationship between anemia (the dependent variable) and nutritional status (the independent variable), the Spearman rank correlation test was applied. This statistical method was chosen due to its suitability for analyzing ordinal

data and identifying potential correlations between the studied variables.

Result

In Table 1, it is observed that the majority of respondents at SMAN 3 Gorontalo were 15-year-old female students. A significant portion of these students had normal nutritional status and were not affected by anemia. This trend suggests a possible link between adequate nutrition and the absence of anemia in this age group.

Table 1. Frequency distribution of respondent characteristics based on age, nutritional status, and anemia status

Category	Frequency	Percentage (%)	Means	Standar Deviations
Age				
14	6	11.80		
15	21	41.20		
16	18	35.30		
17	5	9.80		
18	1	2.00		
Nutritional Status				
Overweight	1	2.00		
Normal	39	76.50		
Underweight	11	21.60		
Anemia Status				
Anemia	17	33.30		
No Anemia	34	66.70		
Anemi Levels				
Anemia	17	33.30	10.182	1.1669
No Anemia	34	66.70	13.897	1.4809

Table 2 shows results between nutritional status and anemia incidence using the Spearman Rank correlation test, which produces p value = 0.009. This shows a significant relationship between nutritional status and anemia incidence (p-value <0.05).

The negative correlation value of -0.364 suggests that as nutritional status improves, the incidence of anemia decreases, albeit with a weak strength of association. This finding may point to a broader pattern where better nutritional practices among the students could

potentially lower the risk of anemia. However, the weak correlation implies that other factors may also play a role in anemia incidence, which could be explored further in future studies

Table 2. The relationship between nutritional status and the incidence of anemia

Nutritional Status	Anemia Incidence				Total		p-value	Correlation Coefficient
	Present		Absent					
	n	%	n	%	n	%		
Overweight	1	2.00	0	0	1	2.00		
Normal	8	15.70	31	60.80	39	76.50	0.009	-0.364
Underweight	8	15.70	3	5.90	11	21.50		
Total	**17**	**33.30**	**34**	**66.70**	**51**	**100**		

Discussion

The study results showed that most respondents at SMAN 3 Gorontalo were students with normal nutritional status, namely 39 students (76.5%). This was followed by students who had underweight nutritional status, as many as 11 (21.6%). The remaining one student (2%) was a student who had an overweight nutritional status.

Nutritional status reflects a certain balance in the form of variables or a representation of nutrition in specific variables.10 In children and adolescents, the measurement of Body Mass Index (BMI) is closely related to age due to changes in body proportions and body density that occur with age.[4] Therefore, in children and adolescents, the BMI used is BMI/U, which adjusts for age. Assessment of nutritional status is grouped according to the WHO Child Growth Standards classification for the age range of 0-5 years and the WHO Reference 2007 for the age range of 5-18 years.[3]

According to the study conducted by Ferdian (2024), most female students have normal nutritional status because their family's economic situation and parents' knowledge are able to meet the nutritional needs of their children.[10] Female students with underweight nutritional status can be affected by the economy and the knowledge of parents, who are still lacking.[11] The environment also dramatically influences teenage girls, who highly desire a slim and smooth appearance. Many teenagers eat irregular diets by skipping meals and preferring snacks.[7]

From the research results, it was found that there was one student with overweight nutritional status; in this case, the researcher assumed that overweight nutritional status

could be caused by excessive fat consumption but not paying attention to the nutrients in food. Research conducted by Nafiah supports on the Relationship between Education Level and Family Economy to the Nutritional Status of Toddlers in Mirigambar Village, Sumbergempol District, Tulungagung Regency, where according to the Spearman rank test, there is a significant correlation between the family's economic status and the nutritional condition of toddlers.[20] The study conducted by Ali (2024) also found a significant correlation between family economic status and the nutritional condition of adults.[21] This is also followed by the journal by Ilmirh (2015), who states that external factors that cause nutritional status are income, knowledge, work, and culture.[11] Another supporting factor is, according to Sunita (2010), that thin conditions are a nutritional problem often found in adolescent girls. They often view thinness as something beautiful, so dieting without expert guidance and direction causes the need for essential nutrients to be unmet.[7]

This is also in line with the argument that improvements in the economy and technology have led to improvements in nutritional status compared to previous decades.[12] Adolescent diets significantly affect adolescent health, one of which is important for nutritional status to be in adequate condition to ensure adolescent growth and development in the future. Lack of physical activity in adolescents can be one of the causes of being overweight. A sedentary lifestyle, such as smoking, lack of sleep, and fast food consumption, can worsen this condition. Preventive efforts to maintain body health involve physical activity. However, most adolescents prefer to walk around the mall rather than do physical activities such as walking or light exercise, even though both activities can be a fun way to be physically active.[13]

The research data shows that most of the respondents at SMAN 3 Gorontalo are female students who do not have anemia, totaling 34 students (66.7%), while the remaining 17 students (33.3%) do have anemia. The research data shows that most female students do not have anemia because their body's nutritional needs have been met. This is proven by the fact that out of 34 female students who do not have anemia, 31 female students have normal nutritional status. On the other hand, female students who experience anemia may be caused by an imbalance in the nutrients absorbed, as shown by the research results. Out of 17 female students who experience anemia, eight have an underweight nutritional status, and one female student has an overweight nutritional status.

The findings of this study may be related to the perception of body image and supplement consumption with the incidence of anemia.[14] They emphasized that adolescent body image significantly impacts various behavioral changes, especially those related to

nutritional status. These changes include changes in portion, time, and type of food consumed. Negative body image, which is often influenced by comments and responses from the surrounding environment, can affect adolescent eating behavior, which can ultimately play a role in the incidence of anemia.

Findings from the study indicate that the quality of nutritional status is closely related to the types of food consumed daily.[15] Nutritional status tends to improve When dietary patterns meet the criteria for good nutrition. On the other hand, if food intake is of poor nutritional quality, this can cause nutritional deficiencies and lead to anemia.

The study results showed that the correlation analysis between nutritional status and the incidence of anemia in female students of SMA Negeri 3 Gorontalo using the Spearman Rank correlation test produced a p-value = 0.009. This value indicates a significant relationship between nutritional status and the incidence of anemia in female students of SMA Negeri 3 Gorontalo because the p-value <0.05. Nutritional status refers to the balance between nutrient intake or consumption, absorption, and utilization of these nutrients. Micronutrients and vitamins are elements that produce red blood cells or Hb. If one of the micronutrients is lacking, Hb formation will not be good, and vice versa. Lack of iron in the human body can cause a decrease in Hb formation.[16]

Iron, which is difficult for the body to absorb, can cause anemia. If the body does not get enough iron, the body will not work optimally. The formation of hemoglobin requires many nutrients so that the formation can be optimal, not only the formation but also as a body metabolism. If there are no iron reserves in the body and iron absorption from food is low, the body will produce fewer red blood cells with little hemoglobin. This is what causes anemia.[17]

From the study results with 51 respondents, it was recorded that one student had an overweight nutritional status and anemia. Of the 39 students with normal nutritional status, eight (around 15.7%) had anemia, while 31 (around 60.8%) did not. Of the 11 students who had an underweight nutritional status, eight students (around 15.7%) had anemia, while the other three students (around 5.9%) did not have anemia.

Participants in this study showed various characteristics. Some had normal nutritional status but experienced anemia, while others had abnormal nutritional status but did not experience anemia. Here, it should be noted that factors that influence the incidence of anemia are nutritional status, age, family income, mother's knowledge, diet, menstrual cycle, and knowledge of the adolescents themselves. Although the nutritional status is good, the knowledge about anemia of the mother and adolescents themselves could be better.

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Insufficient family income, a poor diet, and disturbed menstruation can cause anemia.[18]

These results support the findings of research by Shara and colleagues (2017), who found a significant relationship between nutritional status and the incidence of anemia in adolescent girls at SMAN 2 Sawahlunto in 2014, and research by A. Muhayati & D. Ratnawati (2019) showed a relationship between nutritional status and diet with the incidence of anemia in adolescent girls has been studied. These findings confirm that nutritional status is closely related to daily diet.[11,15] If the food intake is varied and nutritious, the nutritional status tends to be good. However, if the food eaten has a low nutritional content, this can result in malnutrition and anemia.

The negative correlation between nutritional status and anemia illustrates a complex relationship influenced by various factors, including dietary intake, BMI, and socioeconomic conditions.[22] While poor nutritional status often leads to higher rates of anemia due to deficiencies in essential nutrients like iron and vitamin B12, the relationship is not always straightforward. Anemia can occur across different BMI categories, with underweight individuals typically having higher rates and overweight individuals potentially affected by factors such as inflammation.[23] Additionally, socioeconomic status does not always correlate with better nutritional outcomes, indicating that knowledge and access do not necessarily lead to improved nutritional status. Thus, addressing anemia requires a holistic approach that considers these diverse factors.

This study has a few limitations. Firstly, it only gathered data on nutritional status and Hb levels, which means that other potential causes of abnormal nutritional status, apart from anemia, were not explored. Additionally, there were issues with the statistical data processing; specifically, the chi-square test resulted in a minimum percentage exceeding 20%, which did not meet the test's requirements. Consequently, the Spearman rank statistical test was used instead. Despite these limitations not obstructing the completion of data management, it is advised that future studies include additional factors such as socio-economic status, dietary habits, and access to healthcare to achieve a more thorough understanding of the variables affecting nutritional status and Hb levels.

Conclusion

There is a relationship between nutritional status and the incidence of anemia among female students at SMA Negeri 3 Gorontalo. Based on these findings, it is recommended that health workers implement educational programs and counseling on anemia, specifically targeting female adolescents. Additionally, future research should explore the effectiveness of these educational interventions and examine other contributing factors to anemia, such as

socio-economic status and dietary habits, to inform more comprehensive health policies and strategies..

Conflicts of Interest

There is no conflict of interest in this research

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ORIGINAL ARTICLE   **Open Access**

Utilization of Nanotechnology in Metformin Delivery: The Morphometric Study of Pancreatic Islets of Diabetic Rat Model

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ABSTRACT

Introduction: In diabetic conditions, damage of β cells and changes the structure of pancreatic islets was exhibited. Metformin can improve this condition. The nanoparticle form of metformin can improve bioavailability and accelerate cell regeneration, and pancreatic islets can be repaired. The aim of this study to know the effect of nanoparticles metformin on fasting blood glucose levels and pancreatic islet morphometry in diabetic rat models.

Method: An experimental research with posttest-only controlled group design was conducted on 16 white male Wistar rats. The streptozotocin (STZ) 40 mg/kgBB were injected i.p. Rats were divided into four groups: K1: normal control; K2: negative control (diabetes model); K3: diabetes model treated with metformin 100mg/kgBB; K4: diabetes model treated with nanoparticle metformin 100mg/kgBB. The body weight and fasting blood glucose levels were measured periodically. The histology of pancreatic islets was performed with hematoxylin-eosin staining and quantified using ImageJ software. The data were analyzed with GraphPad Prism 8.0.0 using nonparametric Kruskal-Wallis test.

Results: Metformin therapy decreased the fasting blood glucose levels in K3 starting on day 21 and K4 starting on day 7, but there was no statistical difference (p=0.0597). Pancreatic islet morphometry showed the pancreatic islet area was found to be statistically different (p=0.026), and the perimeter was not statistically different (p=0.115).

Conclusion: Metformin nanoparticle form decreased the fasting blood glucose levels and effectively improved the area and perimeter of pancreatic islets of the diabetic rats model, but the perimeter of the pancreatic islets is not statistically significant.

Keywords: Diabetes, metformin, nanoparticles, pancreatic islets, morphometry.

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Introduction

Diabetes mellitus (DM) is a chronic disease caused by abnormalities in insulin secretion, action, or both, which is characterized by increased glucose levels.[1-3] This increase in insulin levels is caused by the impairment of pancreatic cells, the dominant cells in the pancreatic insula. The hyperglycan usecemic condition due to the destruction of cells triggers the inflammation, stimulating the formation of connective tissue or fibrosis.[4-5] This can be followed by changes in the overall structure of the pancreatic islets. The use of animal model such as rat has been widely used in diabetes research. To obtain hyperglycemia conditions in rat, streptozotocin (STZ) the most prominent diabetogenic chemical that is widely used.

Metformin is an oral antidiabetic drug recommended as first-line treatment in type 2 diabetes mellitus.[6] Metformin induces glucagon-like peptide-1 (GLP-1)[7] and enhances insulin actions.[8] The bioavailability of metformin in the gastrointestinal system is only about 50-60% with conventional preparations. This leads to unfavorable effects in glycemic level control, resulting in low improvement of insulin resistance and secretion in pancreatic cells.[9] Reducing the particle size in its delivery system improves the bioavailability of a drug. Reducing the particle size of drug can leads to increase in surface area, this condition can increases in rate of dissolution and rate of diffusion (absorption). In many studies the use of nanotechnology to make the metformin preparations has been carried out with good results. Metformin with nano size has a higher bioavailability, leading to more effective pancreatic cell repairment.[10] Improvement or regeneration in pancreatic islets cells will be able to improve their structure. The aim of the study was to determine the effect of metformin nanoparticles on changes in fasting blood glucose levels and pancreatic islets morphometry in diabetic rats.

Methods

Animal Samples

This experimental study was designed with posttest only controlled group. This study using resource equation' approach the sample size, that is included a total of 16 male Wistar rats, 8 weeks of age, with body weight of 250 -350 grams. Intraperitoneal injection are the way to administer the streptozotocin (STZ) at a dose of 40 mg/kg/BW. The rats were assigned into 4 treatment groups; K1: normal control; K2: negative control (diabetic model); K3: diabetes model + 100mg/kg/BW metformin single dose daily; K4: diabetes model + 100mg/kg/BW metformin in nanoparticles single dose daily. Formulation of the chitosan metformin nanoparticles preparation using the ionic gelation method. The evaluation of the particle size by scanning electrone microscopy (SEM) using particle size analyzer and

measured with ImageJ software. The body weight and fasting glucose levels (the rat were fasted overnight) were assessed with a glucometer on a regular basis every week until the 28th day. This study has obtained approval from the Medical and Health Research Ethics Commission, Faculty of Medicine, Universitas Tadulako (No.6507/UN 28.1.30/KL/2020).

Histology Analysis

The rats were terminated on day 28 and their pancreatic tissue was necropsied. The tissues were embedded in paraffin blocks and cut to a thickness of 5 μm. The tissues were stained with hematoxylin eosin (HE). Five randomly selected fields of view were observed with 400× magnification and 1.25 numerical aperture (NA) Olympus CX23 light microscope and optilab software. The images were quantified using ImageJ software to measured the area and perimeter of the Langerhans islets.

Statistical Analysis

Data were analyzed by GrapPhad Prism 8.0.0 using Kruskal-Wallis non-parametric test.

Result

The Wistar rats were assigned into four groups and treated until the 28th day post-hyperglycemia. The body weights are illustrated in Figure 1. The mean of the body weight of rats at the beginning of the study (before STZ induction) was, ranging from 294 ± 43,64 to 331.5 ± 37,72 gram, in the K1 group body weight of the rats tended to be stable from the beginning to the end of the study (H+28). The K2, K3 and K4 group that induced by STZ showed a decrease in weight at the H0 or the day of diabetes diagnosed. The mean body weight decreases in K2 to 281.25 ± 16,01 grams, K3 decrease to 264.67 ± 30,08 grams, and K4 decrease to 276 ± 16,25 grams. The body weight in K2 group continued to decrease until the end of the study to 272.25 ± 56,01 grams. The K3 and K4 groups showed stable body weight until the end of the study with mean body weight of 305 ± 15,55 grams and 283 ± 7,54 grams, respectively. One-way Anova test for the mean weight showed a statistically significant mean weight differences for each group at each measurement time ($p = 0.0001$).

The fasting blood glucose levels of the Wistar rats are describe in Figure 2. At the beginning of the study (before STZ induction), the mean fasting blood glucose levels ranged from 82.75 ± 6,7 to 90.5 ± 12,79 mg/dL. The STZ-induced K2, K3 and K4 group showed an increase in fasting blood glucose levels above normal level (reference value ≤126 mg/dL) at day 7 after STZ induction. The mean fasting blood glucose levels of K2, K3 and K4 were 105.5 ± 29,56, 202.33 ± 48,19, and 239 ± 190,6 mg/dL, respectively. The K1 group showed stable, normal level at each measurement time with fasting blood glucose level of 116.25 ± 25,61 mg/dL at day 28. The K2 group showed continuously high, above the reference level,

up to 184.75 ± 76,02 mg/dL at day 28. The K3 and K4 group showed a decrease in the mean blood glucose level began on day 7. The decline in fasting blood glucose levels in the K3 group were gradual and reached the normal at day 21 and day 28 that is 122 ± 11,31. The K4 group started to show the decrease at day 7 and were stable at day 28 with a mean fasting blood glucose of 79 ± 15,39 mg/dL. Kruskal-Wallis statistical test was performed on the mean level of fasting blood glucose, yielding no statistically significant differences in each group at each measurement time (p = 0.0597).
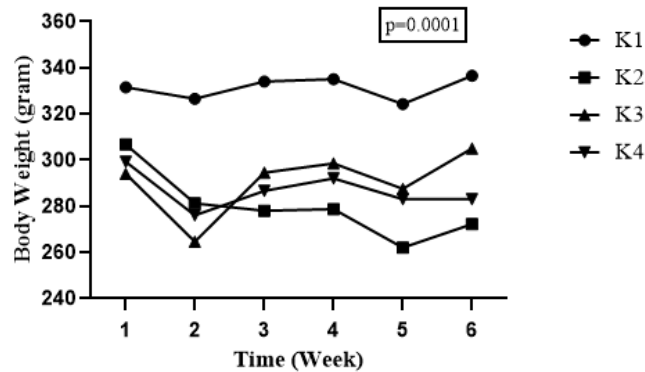

Figure 1. The mean body weight of the Wistar rats. Note: K1: Normal rat control group; K2: Group of diabetic rats; K3: Group of diabetic rats treated with metformin; K4: Group of diabetic rats with metformin in nano preparations. 1: STZ Pre Induction; 2: the establishment of diabetes diagnosis; 3: 7 days post diabetes; 4: 14 days post diabetes; 5: 21 days post diabetes; 6: 28 days post diabetes.
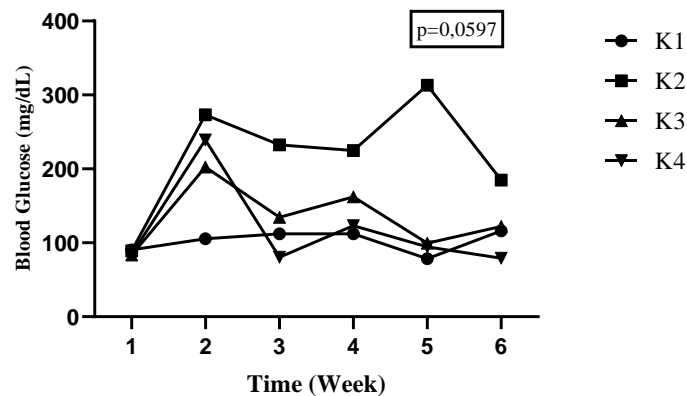
Figure 2. The mean fasting blood glucose levels of the Wistar rats. Normal rat control group; K2: Group of diabetic rats; K3: Group of diabetic rats treated with metformin; K4: Group of diabetic rats with metformin in nano preparations. 1: STZ Pre Induction; 2: the establishment of diabetes diagnosis; 3: 7 days post diabetes; 4: 14 days post diabetes; 5: 21 days post diabetes; 6: 28 days post diabetes.

Pancreatic Islets Morphometry

This research was carried out by measuring the area and perimeter of the pancreatic islets of a diabetic rat model treated with nanoparticle metformin. The pancreas was preparations using HE staining. The results of the examination showed differences in area and perimeter in each treatment group. area The histopathological appearance of the pancreatic insula in all groups can be seen in Figure 3.
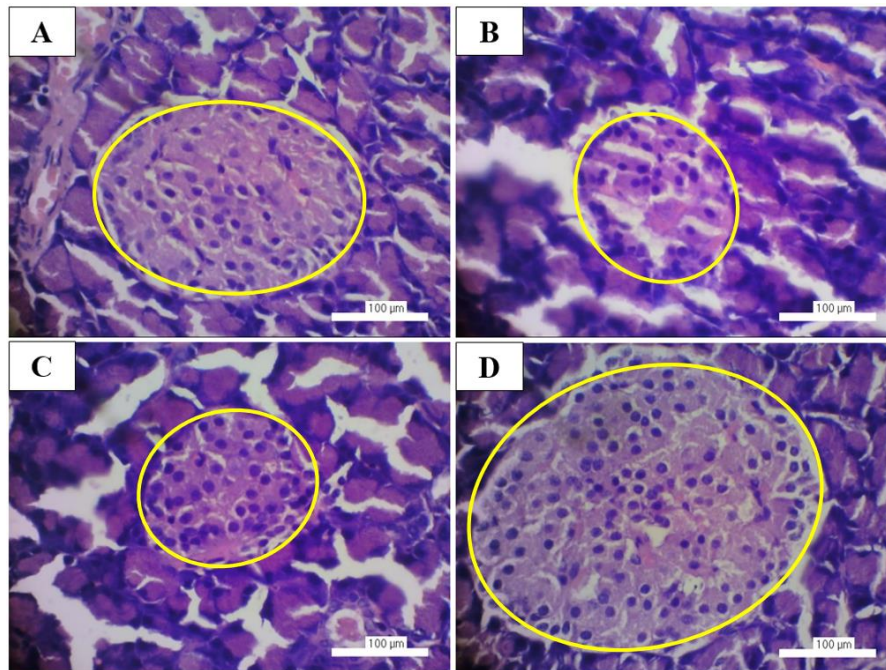
Figure 3. Histopathological of the pancreatic islets of a diabetic rat model at D+28 with HE staining. Magnification 400 times. Description: (A) Group K1, (B) Group K2, (C) Group K3, (D) Group K4. The perimeter is measured along the entire yellow line on the pancreatic islets, while the area is a calculation of the entire shape within the yellow line.

Pancreatic Islets Area

The data on the mean area of the pancreatic islets can be seen in Figure 4. The mean pancreatic islets area of K1 $53,13 \pm 10,78 \mu m^2$, K2 has the smallest area that is $23,09 \pm 8,29$ μm^2 respectively. The K4 group showed comparable pancreatic islets area with K1 $57,79 \pm 21,39 \mu m^2$, while the K3 showed comparable pancreatic islets area with K2 group $24,37 \pm 3,29$ μm^2. Kruskal-Wallis non-parametric test was performed on the pancreatic islets area, yielding a statistically significant difference (p=0.026). a Mann Whitney test was further performed, resulting in a statistically significant difference between K1 against K2, and K2 against K4
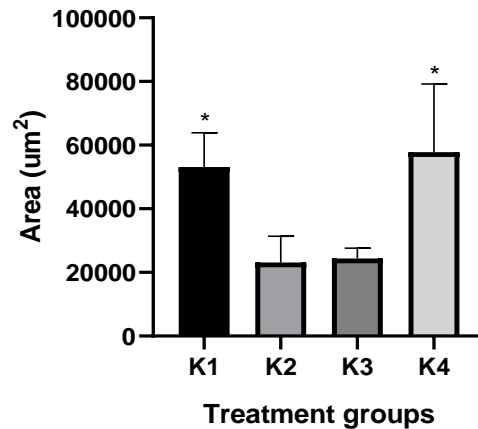
Figure 4. The mean area of the pancreatic islets. Kruskal Wallis test p=0.026. Mann Whitney post hoc test: K1 with K2 (p=0.02), K1 with K3 (p=0.06), K1 with K4 (p=0.724), K2 with K3 (p=0.355), K2 with K4 (p=0.034), K3 with K4 (p=0.085). *=p<0.05 vs K2.

Pancreatic Islets Perimeter

Figure 5 shows the perimeter measurement of the pancreatic islets. The K1, K2, K3, and K4 pancreatic islets perimeter were 957,01±65,39, 622,06±305,15, 708,89±127,12 and 960,00±185,20 μm, respectively. The K2 perimeter is the smallest than the other, K1 pancreatic islets perimeter was comparable to K4, while the K2 pancreatic islets perimeter was comparable to K3. The Kruskal Wallis test on the pancreatic islets perimeter showed no statistically significant differences (p = 0.115).
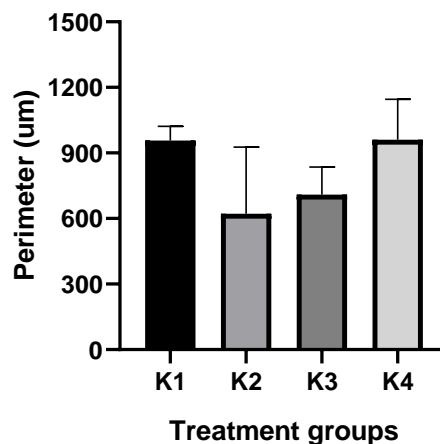
Figure 5. The mean perimeter of the pancreatic insula. Kruskal Wallis test p=0.115.

Discussion

This study was conducted by inducing STZ intraperitoneally in Wistar rats assigned to K2, K3, and K4 group. Streptozotocin is a diabetogenic substance that damage the

pancreatic cells directly on the nitrosourea group and induce an increase in reactive oxygen species (ROS).[11] The STZ is widely used in animal test because it increases blood glucose level by triggering the excess free radical production.

The mean fasting blood glucose level in K1 showed normal results in all measurement times. The K2 showed high, above normal level fasting blood glucose levels started from day 0 to day 28. This is because STZ injures the pancreatic islets cells that causes an increase in blood glucose level.[12] Treatment with 100 mg/kg/BW (K3) metformin and 100 mg/kg/BW (K4) nano-metformin were able to affect the fasting blood glucose levels in diabetic rat models. The diabetic rats treated with 100 mg/kg/BW metformin showed a decrease in fasting blood glucose levels started at day 21, while diabetic rats treated with 100 mg/kg/BW nano-metformin showed a decrease in fasting blood glucose levels started at day 7.

In addition to conventional metformin administration, the animal tests were given nano-metformin to induce hypoglycemic effect. This effect improves pancreatic function by regenerating pancreatic cells. Treatment with nanoparticles has shown its effectiveness in diabetes mellitus. The STZ-induced diabetes causes degeneration in the islets of Langerhans causing injuries. Metformin increases the glucose uptake via insulin and is widely used because it can reduce oxidative stress that increase hyperglycemia.[13]

The area of the pancreatic insula from each group was different based on the treatment. The K1 group as the control group had round or oval pancreatic islets and well-appearance of cells. The K2 group showed smaller, irregular shape pancreatic islets area. The K3 group that received 100mg/kg/BW metformin showed wider area of pancreatic islets area than the K2 group. The K4 group that received 100mg/kg/BW nano-metformin showed wider, uniform shape, comparable to the K1 group. Statistical analysis showed statistically significantly differences in the pancreatic islets area. Mann Whitney's post hoc test showed that there were statistically significant differences between K1 and K2 and K2 and K4. This indicates that nano-metformin given to K4 improved the pancreatic islets area in diabetic rats.

The K1 showed comparable pancreatic islets perimeter to K4. In contrast, the K2 as the untreated diabetic rat group showed a small perimeter. The perimeter size of K3 is not much different from that of K2. This indicates that metformin preparations did not rapidly improve the size of the pancreatic islets perimeter in diabetic conditions. The state of hyperglycemia can be caused by damage to pancreatic β cells leading to inability to produce insulin optimally. Changes in the structure of the pancreatic islets were correlated with

pancreatic function and the amount of insulin produced. Injuries to pancreatic islets cells affect the changes in morphometry in terms of the area and perimeter.[14]

Nanoparticles increases bioavailability with minimal side effects due to their various natural, synthetic, and semi-synthetic polymers that are useful in the formulation delivery. This study showed that nano-metformin reduces the fasting blood glucose levels. Thus, it improves the pancreatic islets structure. Metformin has lower bioavailability leading to poorer glycemic control and subsequent lower improvement in insulin resistance and pancreatic cell secretion impairment.[15] Nano-metformin has a small particle size, triggering an increase in bioavailability and rapid improvement in pancreatic islets.[16]

Conclusion

The nano-metformin preparation reduced the fasting blood glucose levels in diabetic Wistar rat model although the difference was not statistically significant. The nano-metformin preparation improved the area and the perimeter of the pancreatic islets, but the perimeter of the pancreatic islets is not statistically significant.

Conflicts of Interest

There is no conflict of interest in this research

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Acknowledgments

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