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TESTING THE ANTIHYPERGLYCEMIC POTENTIAL OF THE ETHANOL EXTRACT OF PAPUAN ANTS' NESTS (*Myrmecodia pendans*) ON MENCIT (*Mus musculus*)

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Abstract :
The use of synthetic drugs has potential pose a risk of complications in sufferers of hyperglycemia. WHO recommends the use of traditional medicine to prevent & treat chronic diseases. The problem formulation includes the effectiveness of the original Papuan EESS test in treating hyperglycemia in mice, with a comparison of results at doses of 100.8 mg/kgBB, 50.4 mg/kgBB, and 25.2 mg/kgBB. This research aims to prove effectiveness of native Papuan EESS in treating hyperglycemia with mice & compare the effectiveness at different doses. The research uses an experimental laboratory design. Male mice aged 2 – 3 months with body weight of 20 – 30 grams, divided into five groups: control + glibenclamide 13.39 mg/kgBW, control - NaCMC 1%, native Papuan EESS treatment dose 100.8 mg/kgBW, 50.4 mg/kgBB, and 25.2 mg/kgBB. Alloxan 4.54 mg/kgBW was administered to create hyperglycemia in mice. Extraction used the maceration method with 70% ethanol solvent. The research results showed that EESS suspension was effective in treating hyperglycemia. The dose of 100.8 mg/kgBB had an average decrease in blood sugar of 122.17 mg/dL, higher than the dose of 50.4 mg/kgBB (decrease of 155.00 mg/dL) and 25.2 mg/kgBB (decrease 200.33mg/dL). In the control group, control + with 13.39 mg/kgBW glibenclamide showed a greater reduction (121.87 mg/dL) than control - with 1% NaCMC (205.83 mg/dL reduction). EESS is native to Papua antihyperglycemia is effective in mice with hyperglycemia. Comparison of the effectiveness of EESS doses showed a significant difference in response.

Keywords: Ant Nest (*M. Pendans*), Antihyperglycemia, Male Mencit

INTRODUCTION

Hyperglycemia is a condition where the level of sugar in the blood exceeds normal limits. Typically, blood sugar levels range between 70 - 100 mg/dL.(Mouri & Badireddy, 2024) The common cause of hyperglycemia is diabetes mellitus, and if this condition persists for a long time, it can lead to complications in various organs of the body, such as the eyes, kidneys, and heart.(PERKENI, 2021)(Wild et al., 2004) According to the International Diabetes Federation (IDF), the number of cases of hyperglycemia in people aged 20 to 79 worldwide has reached at least 463 million cases.(Cahyaningrum, 2023)

The North African-Arab region has the highest number of cases with 56.4 million sufferers, while Indonesia ranks third with 52.3 million sufferers. The projection states that the number of hyperglycemia sufferers is estimated to increase to 700 million by 2040 worldwide (Tim Kelompok

Kerja SDKI DPP PPNI, 2018). The high number of cases of hyperglycemia patients requiring long-term treatment and significant costs potentially increase the risk of complications due to the side effects of synthetic drugs used.(Saputri et al., 2016) As an alternative, the use of traditional medicines has been recommended by the World Health Organization (WHO) in efforts to prevent and treat chronic and degenerative diseases. (Yuliarisma, 2018)

One of the natural riches in Sorong Regency is the ant nest (M. pendens), which is considered by local residents as one of the ways to lower blood glucose levels. This plant has been empirically proven to have healing properties for various diseases naturally and with a relatively high level of safety (Dirgantara et al., 2018). "Sarang semut" (Myrmecodia pendans) contains various active compounds such as tannins, polyphenols, flavonoids, calcium, magnesium, tocopherol, iron, zinc, sodium, and phosphorus.(Ahmad & Lestari, 2011) This active compounds provide various benefits, including antimicrobial, anti-inflammatory, antioxidant, antidiabetic, and anticancer properties (Subroto & Saputro, 2019).

Based on research Subroto & Saputro (2019) It is known that ant nests (M. pendens) contain around 85% complex type sugars which have medicinal potential. Research conducted by Retnowati & Satyabakti (2015) it shows that ant nests contain flavonoids, tannins, and polyphenols, which have the ability to address various diseases. These compounds act as antioxidants in the body. (Supriatna et al., 2019). In addition, research was carried out Raya et al. (2016) as well as Rosyadi & Hariono (2018) evaluating the effectiveness of ant nest tuber extract (M. pendens) in reducing blood glucose levels in diabetic (Sprague Dawley) rats and proven effective in demonstrating blood sugar levels.

Although previous studies have shown the potential of ant nest (M. pendens) in reducing blood glucose levels, most of these studies used Sprague Dawley rats as experimental animals. There has been no specific research evaluating the effectiveness of Papua's native ant nest ethanol extract (M. pendens) in addressing hyperglycemia in mice (Mus musculus). Mice have different metabolism from rats, thus requiring specific research to determine the effectiveness of this extract on mice experiencing hyperglycemia.

This study aims to test the effectiveness and compare the differences in the anti-hyperglycemic effects of Papua's native ant nest ethanol extract (M. pendens) at doses of 100.8 mg/kg body weight, 50.4 mg/kg body weight, and 25.2 mg/kg body weight against hyperglycemic mice (Mus musculus). This study will be one of the few studies examining the effectiveness of Papua's native ant nest ethanol extract (M. pendens) in reducing blood glucose levels in mice (Mus musculus) with hyperglycemia conditions. Additionally, this research will also explore the specific active compound contents in Papua's native ant nest ethanol extract that play a role in lowering blood glucose levels. These findings may provide new insights into the mechanism of action of active compounds in ant nests in addressing hyperglycemia.

The results of this study are expected to provide scientific evidence of the potential of Papua's native ant nest ethanol extract (M. pendens) as an alternative therapy for addressing hyperglycemia. If proven effective, these findings could support the development of safer and more affordable herbal medicines based on ant nests for the community. Furthermore, the identification of active compounds in ant nest extracts may open opportunities for the development of new synthetic drugs that are more effective in addressing hyperglycemia and other related diseases.

RESEARCH METHOD

The experimental laboratory design was used with male mice (Mus musculus) as the research subjects. The research population consists of 25 male mice (Mus musculus) aged 2-3 months with a body weight of 20-30 grams, divided into 5 groups: positive control group of glibenclamide 0.013 mg/kgBW, negative control group of 1% NaCMC, treatment group of original Papua ant nest ethanol extract (M. pendans) at doses of 100.8 mg/kgBW (high dose), 50.4 mg/kgBW (medium dose), and 25.2 mg/kgBW (low dose). The sample in this study is ant nests (Myrmecodia Pendans).

The experimental laboratory research design was used with male mice (Mus musculus) as the research subjects divided into positive control group glibenclamide 0.013 mg/kgBW, negative control group 1% NaCMC, treatment group of ethanol extract of ant nest (M. pendans) native to Papua at doses

of 100.8 mg/kgBW (high dose), 50.4 mg/kgBW (medium dose), and 25.2 mg/kgBW (low dose). The research was conducted at the Natural Materials Laboratory and Pharmacology Laboratory of the Faculty of Applied Sciences, Muhammadiyah Sorong University, from August to October 2023.

The materials used included ant nest tubers (M. pendans), 70% ethanol, distilled water, 4.54 mg/kgBW alloxan, 1% NaCMC, 0.9% NaCl, and glibenclamide. The working procedures included extraction and anti-hyperglycemia testing. In the extraction process, it started with cleaning the ant nest tubers (M. pendans) with running water, then cutting them into small pieces and drying them for 2-3 days covered with black cloth. After drying, the tubers were blended into crude powder which was then macerated with 70% ethanol for 3x24 hours. The maceration extract was filtered and evaporated with a rotary evaporator, then concentrated using a water bath. Then, a 1% NaCMC suspension base was made for the five treatment groups with the addition of different doses of extract and glibenclamide for the positive control.

In the anti-hyperglycemia test, male mice were divided into 5 treatment groups and adapted for 7 days. The mice were fasted for 8-12 hours, fasting blood sugar levels were checked, fed for 3 hours, normal blood sugar levels were checked, then induced with 4.45 mg/kgBW alloxan. After 5 days, blood sugar levels were checked again. If hyperglycemia occurred, the mice were treated according to the group, namely positive control glibenclamide 0.013 mg/kgBW, negative control 1% NaCMC, and extract doses of 100.8; 50.4; and 25.2 mg/kgBW. Blood sugar levels were checked again on days 7, 14, and 21 to obtain research results. Data will be analyzed using one-way ANOVA to see significant differences between groups, and the least significant difference (LSD) test will be used. If the collected data do not meet normal distribution or show non-homogeneous variances, the Kruskal-Wallis method will be used as an alternative, and the results will be tested using the Mann-Whitney method.

RESULTS AND DISCUSSION

Table 1. Average Blood Glucose Level Examination in Mice

KGD examination	Blood sugar levels (mg/dL)						Average (mg/dL)
	Fast (mg/dL)	Pre induction (mg/dL)	Post induction (mg/dL)	H-7 (mg/dL)	H-14 (mg/dL)	H-21 (mg/dL)	
K + Glibenklamid 13,39 mg/kgBB	117	122	220	103	100	68	121.87
K – NaCMC 1%	195	231	247	206	183	173	205.83
Dosis 100,8 mg/kgBB	125	143	197	144	84	70	122.17
Dosis 50,4 mg/kgBB	75	155	227	171	146	143	155.00
Dosis 25,2 mg/kgBB	107	161	226	238	222	187	200.33

Based on table 1, it is known that the examination of blood sugar levels in mice (Mus musculus) after being averaged from 6 examinations, namely control + Glibenclamide 0.013 mg/kgBW, is 121.87 mg/dL, control - 1% NaCMC is 205.83 mg/dL, EESS dose 100.8 mg/kgBW is 122.17 mg/dL, EESS dose 50.4 mg/kgBW is 155.00 mg/dL, EESS dose 25.2 mg/kgBW is 200.33 mg/dL. This shows that ethanol extract of ant nest has anti-hyperglycemic effects on mice (Mus musculus) experiencing an increase in blood sugar levels. Although not as much as the control + using Glibenclamide 0.013 mg/kgBW, the ethanol extract of ant nest in this study has proven its role in lowering blood sugar levels in mice (Mus musculus).

Table 2. Data Normality Test

Treatment group	Shapiro-Wilk		
	Statistic	Df	Sig.
K+ Glibenklamid 13,39 mg/kgBB	0.826	6	0.099
K - NaCMC 1%	0.951	6	0.749
Dosis EESS 100,8 mg/kgBB	0.956	6	0.792
Dosis EESS 50,4 mg/kgBB	0.757	6	0.023
Dosis EESS 25,2 mg/kgBB	0.903	6	0.392
Data is normally distributed p > 0.05 SIGNIFICANT			

In Table 2, the normality test used in this study is the Shapiro-Wilk test. Since the sample size is less than 50, the data is considered to have a normal distribution, and the condition for conducting a One-Way ANOVA test is met if p > 0.05.

The results of the normality test indicate that all treatment groups have normal data. The control + treatment group has p = 0.099, the control - group has p = 0.749, the EESS Dose 100.8 mg/kgBW group has p = 0.792, the EESS Dose 50.4 mg/kgBW group has p = 0.023, and the EESS Dose 25.2 mg/kgBW group has p = 0.392. All treatment groups are considered to have normal data because their significance value is p > 0.05.

Table 3. Homogeneity Test

Decline	Test of Homogeneity of Variances			
	Levene Statistic	df1	df2	Sig.
Based on Mean	4.306	4	25	0.009
Based on Median	1.662	4	25	0.190
Based on Median and with adjusted df	1.662	4	11.404	0.226
Based on trimmed mean	4.249	4	25	0.009
Data is homogeneously distributed p > 0.05 SIGNIFICANT				

Based on Table 3, the significance value or probability of the homogeneity test of variances shows p > 0.05, so it can be concluded that the data comes from populations that have equal variances.

The results of the normality and homogeneity tests indicate that the data is normally distributed and homogeneous. Subsequently, the entire data set is analyzed using a one-way analysis of variance (ANOVA) hypothesis test.

Table 4 ANOVA test

ANOVA					
Decline	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	39889,667	4	9972,417	2,060	0,006
Within Groups	121020,333	25	4840,813		
Total	160910,000	29			
P > 0,05 SIGNIFICANT					

Table 4 shows the results of the ANOVA test, which is 0.006, meaning H0 is rejected, thus the hypothesis is accepted, indicating differences in the effectiveness of antihyperglycemic activity in ethanol extract of ants' nests. In the decrease of H-21, there is at least one pair of groups that significantly differ. To determine which groups differ, the analysis continues with the Post Hoc LSD statistical test.

Table 5. LSD Test

Multiple Comparisons			
DependentVariable: respon			
LSD			
Control	Decline	Mean Difference (I-J)	Sig.
K + Glibenklamid	K - NaCMC 1%	-84.167*	0.046
	Dose EESS 100,8	-0.500	0.990
	Dose EESS 50,4	-33.333	0.414
	Dose EESS 25,2	-78.667	0.061
K - NaCMC 1%	K + Glibenklamid	84.167*	0.046
	Dose EESS 100,8	83.667*	0.048
	Dose EESS 50,4	50.833	0.217
	Dose EESS 25,2	5.500	0.892
Dosis EESS 100,8 mg/kgBB	K + Glibenklamid	0.500	0.990
	K - NaCMC 1%	-83.667*	0.048
	Dose EESS 50,4	-32.833	0.421
	Dose EESS 25,2	-78.167	0.03
Dosis EESS 50,4 mg/kgBB	K + Glibenklamid	83.333*	0.414
	K - NaCMC 1%	-50.833	0.217
	Dose EESS 100,8	32.833	0.421
	Dose EESS 25,2	-45.333	0.270
Dosis EESS 25,2 mg/kgBB	K + Glibenklamid	82.666*	0.061
	K - NaCMC 1%	-5.500	0.892
	Dose EESS 100,8	78.167	0.063
	Dose EESS 50,4	45.333	0.270

Table 5 shows the Post Hoc LSD test aimed at determining the presence of significant differences between one group and another. The results of the Post Hoc LSD test show asterisks (*) indicating that all groups have significant differences from each other.

The uniqueness possessed by ant nests (M. pendans) lies in the interaction of ants that turn bulb tunnels into nests and form colonies within them.(Reski, 2017) Over a long period of time, such interactions will lead to the occurrence of chemical reactions naturally between the compounds emitted by ants and the substances contained within them (Hertiani, 2010) (Sitorus, 2017).

To test the effectiveness of this antihyperglycemic agent, male mice (Mus musculus) were used as test animals because they are more active in their activities and are not influenced by hormones as female mice are (Herrera et al., 2011)(Oktiansyah, 2015). Male mice are also not susceptible to stress, so it does not interfere during testing (Ariyanti et al., 2007)(Mu'nisa et al., 2022). To obtain hyperglycemia conditions, all mice (Mus musculus) were given alloxan after fasting for 8-12 hours.(Hasim et al., 2020).

To induce hyperglycemia conditions in mice (Mus musculus), it is done by administering alloxan compound after the mice have been fasted for 8-12 hours beforehand.(Samsul et al., 2020) The administration of alloxan is a rapid method to induce hyperglycemic conditions due to its selective toxic nature towards pancreatic beta cells that produce insulin. Alloxan is administered intravenously and accumulates specifically through the glucose transporter GLUT2.(Irdalisa et al., 2015) Mice are considered hyperglycemic if their blood sugar level exceeds 200 mg/dL, while the normal level is between 62-175 mg/dL. Blood sugar levels are checked using a glucometer by taking a small amount of blood from the tip of the mouse's tail. (Safna et al., 2021).

This research used 25 mice (Mus musculus) divided into 5 groups. The first group served as the positive control, administered with 0.013 mg/kgBW of glibenclamide as a reference for blood sugar reduction effect. The second group served as the negative control, administered with 1% NaCMC to ensure the effectiveness testing method for anti-hyperglycemic was correct. The remaining three groups were experimental groups administered with ethanol extract of ant nest (M. Pendans) at different doses,

namely 100.8 mg/kgBW, 50.4 mg/kgBW, and 25.2 mg/kgBW.(Rosina et al., 2022)(Sarker & Nahar, 2009).

Ethanol extract of ant nest is given in the form of suspension with 1% NaCMC suspending agent orally using a cannula. Evaluation of blood sugar levels was performed on the 7th, 14th, and 21st days. Blood sugar levels on the 5th day post-alloxan induction were used as the initial blood sugar levels to observe the decrease in hyperglycemic mice. The positive control group given glibenclamide experienced a decrease in blood sugar levels with an average of 121.87 mg/dL over 21 days because glibenclamide stimulates insulin release and secretion. Meanwhile, the negative control group given 1% NaCMC did not experience significant decrease with an average of 205.83 mg/dL because 1% NaCMC is an inert compound as a negative control.(Suena, 2020).

In the test group, the administration of ethanol extract of ant nest at doses of 100.8 mg/kgBW, 50.4 mg/kgBW, and 25.2 mg/kgBW respectively experienced a decrease in blood sugar levels with averages of 122.17 mg/dL, 155.00 mg/dL, and 200.33 mg/dL. Compounds suspected to play a role in regulating blood sugar levels are tannin, tocopherol, and flavonoids, which function as antioxidants to neutralize free radicals and prevent damage to pancreatic beta cells, thus allowing beta cells to regenerate and secrete insulin again.(Murtihapsari et al., 2022). Using the SPSS program, statistical analysis was conducted using the one-way analysis of variance (ANOVA) method followed by the LSD Post Hoc Test. The LSD test results showed statistically significant comparisons (differences not occurring by chance) between the control + and control - groups. In the first comparison, the P/Sig value was 0.046, smaller than the significance level of 0.05, indicating a statistically significant difference between the two groups. This suggests that control + and control - have significant differences in their ability to lower blood sugar levels. In the second comparison, between the control - group with a dose of 100.8 mg/kgBW, the P/Sig value was 0.048, also smaller than 0.05, indicating a statistically significant comparison between the two groups. This indicates that the control - group with a dose of 100.8 mg/kgBW has a significant difference in its ability to lower blood sugar levels.

The third comparison showed a non-significant statistical comparison (differences occurring by chance) between the dose of 50.4 mg/kgBW and the control + group, with a P/Sig value of 0.414, greater than 0.05. This indicates that the dose of 50.4 mg/kgBW with the control + group has no significant difference in its ability to lower blood sugar levels. And the fourth comparison showed a statistically significant comparison (differences not occurring by chance) between the dose of 25.2 mg/kgBW and the control + group, with a P/Sig value of 0.061 smaller than 0.05. This indicates that the dose of 25.2 mg/kgBW with the control + group has a significant difference in its ability to lower blood sugar levels.

These research findings are consistent with previous studies examining the effectiveness of ant nest tuber extract (M. pendans) in reducing blood glucose levels in diabetic rats (Sprague Dawley). (Raya et al., 2016)(Rosyadi & Hariono, 2018). Although using different trial animals, both studies confirm the potential of ant nest extract as a natural anti-hyperglycemic agent. However, this study is more specific, utilizing ethanol extract from native Papua ant nests and laboratory mice (Mus musculus), which have different metabolism from rats.

In general, it can be generalized that ethanol extract from native Papua ant nests (M. pendans) has anti-hyperglycemic effects on experimental animals such as mice and rats. These effects are believed to stem from active compounds such as tannins, tocopherols, and flavonoids, which act as antioxidants to prevent damage to pancreatic beta cells that produce insulin. Thus, pancreatic beta cells can regenerate and secrete insulin back into the blood, thereby reducing blood glucose levels.(Alam et al., 2022).

The implication of this research is the potential development of ethanol extract from ant nest (M. pendans) native to Papua as a herbal medicine for hyperglycemia or diabetes mellitus therapy. If further developed, this extract could become a safer and more affordable alternative therapy compared to synthetic drugs. However, further research is needed to identify specific active compounds responsible for the anti-hyperglycemic effects, as well as their mechanisms of action in more detail. Limitations of this study include only using laboratory mice (Mus musculus) as test subjects, so the results cannot be directly generalized to humans. Additionally, this study did not identify specific active

compounds responsible for lowering blood glucose levels, but only speculated based on previous research findings. Therefore, for future research, it is recommended to conduct clinical trials on humans and identify specific active compounds along with their mechanisms of action in lowering blood glucose levels.

CONCLUSION

Based on the research results, it can be concluded that the effectiveness test of ethanol extract of native Papua ant nests (M. pendans) in overcoming hyperglycemia in mice (Mus musculus) was conducted by dividing the mice into 5 groups: positive control (0.013 mg/kgBW glibenclamide), negative control (1% NaCMC), and 3 test groups with extract doses of 100.8 mg/kgBW, 50.4 mg/kgBW, and 25.2 mg/kgBW. The research results indicate that the ethanol extract of ant nests has an effect in lowering blood sugar levels in hyperglycemic mice, although not as effective as glibenclamide. In the LSD test, the dose of 100.8 mg/kgBW showed a significant difference from the negative control in its ability to lower blood sugar levels, while the dose of 50.4 mg/kgBW did not differ significantly from the positive control, and the dose of 25.2 mg/kgBW differed significantly from the positive control.

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