

Testantidiabetic activity of a combination of 70% ethanol extract of meniran leaves (*Phyllanthus Niruri* L.) and glibenclamide in male white Wistar rats induced by alloxan

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ARTICLE INFO	ABSTRACT
<p>Article history:</p> <p>Received Apr 17, 2024 Revised Apr 27, 2024 Accepted Apr 29, 2024</p>	<p>Diabetes mellitus is a metabolic disease that causes hyperglycemia due to impaired insulin function (insulin resistance). The use of glibenclamide has side effects in the form of hypoglycemic and weight gain. This study aims to determine the activity of a combination of 70% ethanol extract of meniran leaves (EEDM) and glibenclamide in reducing blood glucose and body weight of rats against the use of single glibenclamide. A completely randomized design method was used involving 28 rats divided into 7 groups. Normal control (KN) was given distilled water; negative control (K(-)) was given distilled water; positive control (K(+)) was given glibenclamide 1 mg/kgBW, EEDM treatment group doses of 5; 10; 20 mg/kgBW and a combination group of glibenclamide and EEDM 5 mg/kgBW. Alloxan 150 mg/kgBW intraperitoneally (ip) and 30% glucose solution orally were used to induce the hyperglycemic rat model. Blood sugar levels and body weight were checked on days: 0, 7, 14 after treatment. Based on the results of statistical tests showed that EEDM all doses and combinations have antidiabetic activity ($p < 0.05$). The use of a combination of glibenclamide 1 mg/kgBW and ethanol extract of meniran leaves 5 mg/kgBW can also overcome the side effects of weight gain from the use of glibenclamide alone.</p>
<p>Keywords:</p> <p>Antidiabetic Alloxan Glibenclamide Meniran leaf</p>	

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INTRODUCTION

Diabetes Mellitus (DM) is a disease or disorder of carbohydrate, fat and protein metabolism characterized by high blood sugar levels as a result of decreased insulin action or secretion. (Rahmasari and Wahyuni, 2019). Diabetes mellitus is often associated with an increased risk of death. The prevalence of diabetes mellitus continues to increase throughout the world, including in Indonesia. Diabetes mellitus causes functional limitations and multiple morbidities, so diabetes mellitus sufferers need to learn to maintain lifelong self-management behaviors, such as self-care. (Swarjana et al., 2023).

Various therapies have been used to reduce the incidence of hyperglycemia by using anti-diabetic drugs regularly to keep blood sugar levels under control. Glibenclamide is a sulfonylurea antidiabetic drug that works by increasing insulin secretion by pancreatic β cells.(Soelistijo, 2021). If used in the long term, this sulfonylurea drug causes a decrease in the effectiveness of insulin secretion, this is due to a decrease in sulfonylurea receptors on pancreatic β -cells.(Grant and Graven, 2016). Apart from hypoglycemia, the side effect of using glibenclamide is weight gain(Utomo et al., 2023). Trust in herbs and synthetic drugs when combined can reduce the side effects or toxicity of these synthetic drugs. This combination is aimed at lowering blood sugar levels, so it is hoped that it will get better(Iswahyudi et al., 2018). Research on the efficacy of meniran extract (*Phyllanthus niruri* L.) has been widely carried out, methanol extract from all parts of *Phyllanthus niruri* has great potential as an antidiabetic(Okoli et al., 2010). Another benefit of the meniran plant is that it can reduce weight(Krisyanella et al., 2013). The mechanism of action of meniran leaf extract as an antihyperglycemia in reducing glucose levels is mainly played by the flavonoid compound quercetin through the release of insulin by pancreatic β -cells or changing glucose metabolism. The flavonoid quercetin plays a role in increasing insulin secretion by pancreatic β -cells through a mechanism that maintains functioning beta cells and improves the work of the pancreas, so that insulin secretion by Langerhans' β -cells increases.(Wahjuni, 2017).

Glibenclamide metabolism occurs in the CYP3A4 enzyme and meniran herb is a non-competitive inhibitor of the CYP3A4 enzyme (Zhou et al., 2010). An in-vitro test of a combination of ethanol extract of meniran leaves and glibenclamide has been carried out by(Iswahyudi et al., 2018) which showsThe interaction between treatment of meniran herb extract and glibenclamide resulted in decreased expression of CYP3A4, so that its antidiabetic activity would increase. Therefore, in this study we wanted to determine its antidiabetic activityjoint administration of meniran leaf ethanol extract and glibenclamidein lowering blood glucose in-vivo. And to see whether combination therapy can overcome the side effects of weight gain from using glibenclamide alone.

RESEARCH METHOD

Explaining This research is experimental, using the Completely Randomized Design (CRD) method. This research was conducted at the Pharmacology and Clinical Pharmacy Laboratory, Faculty of Pharmacy, Muhammadiyah University, Surakarta

Research variable

Independent Variable: Dosage of meniran leaf extract and the combination of meniran leaves and glibenclamide. Dependent Variable: Alloxan-induced changes in rat blood sugar levels and body weight. Control Variable: Healthy male Wistar strain rats, 2-3 months old, weighing 150-200 grams.

Tools and materials

Equipment: Animal cage, Triple Beam Balance Scale 2610 g (Chinese Lark), analytical balance (Ohaus), drying cabinet, maceration vessel, rotary evaporator, porcelain cup, water bath, oral probe, 1 mL and 3 mL injection equipment, glassware, microcapillary (Vitrex), mini spin centrifuge (Eppendorf), semi micro disposable cuvette, 5-50 μ L micropipette, 100-1000 μ L micropipette (Acura), yellow tip (OneMed), blue tip (OneMed), visible spectrophotometry (Star Dust MC*15). Ingredients: Meniran leaves purchased at the Testing Laboratory - UPF Tawangmangu Traditional Health Services, 70% ethanol (technical), glibenclamide powder (Pharmaceutical Grade), alloxan monohydrate (Sigma Aldrich), 0.9% saline solution (WIDA), distilled water, mice white male Wistar strain aged 2-3 months (weight 150-200 grams), BR comfeed feed, husks, mineral water, Mg powder (Sigma Aldrich), HCl, Dragendroff reagent, FeCl₃ and Glucose GOD FS kit reagent (Diasys).

This research test protocol has been approved by the Health Research Ethics Commission (KEPK) with No. 1.701 / IX / HREC / 2023. Identification of meniran leaf fruit (*Phyllanthus niruri* L.) was carried out by testing at the Tawangmangu Traditional Health Services UPF Testing Laboratory with number TL.02.04/D.XI.6/2772.276/2024. 2 kg of meniran leaves were wet sorted by removing dirt and sorting the leaves that were suitable for use. The leaves are washed under running water to ensure that no dirt remains. Next, the leaves were dried in a drying cupboard at a temperature of 60°C. Next, dry sorting is carried out to select leaves that are suitable for obtaining meniran simplicia and then crushed to obtain dry powder. The dry powder of meniran leaves was then soaked in 70% ethanol for 3 days with a ratio of 1:5 while stirring occasionally. The soak was filtered using a Buchner funnel to produce filtrate 1 and residue 1. Residue 1 was re-macerated with 70% ethanol in a ratio of 1: 4 for 2 days. The filtrate was concentrated using a rotary evaporator at a temperature of 60°C and a speed of 100 rpm. Next, it is thickened in a water bath at a temperature of 60°C. 1 mL of extract was put into a test tube, some Mg powder and concentrated HCl solution were added. A positive result if the color of the solution turns tomato red or orange indicates the presence of flavonoids(Raudah et al., 2020). 1 g of extract was put into a test tube and 1 mL of 2N HCl and two drops of Dragendroff's reagent were added. A positive result is indicated by the presence of an orange or brown precipitate in the test tube indicating the presence of alkaloids(Raudah et al., 2020). 0.5 mL of the extract was put into a test tube and 5 mL of hot distilled water was added, then cooled and shaken vigorously for 10 minutes. The result is positive if foam forms which indicates the presence of saponin(Raudah et al., 2020). The extract was put into 2 test tubes, 1 ml each, then 2 ml of 2% FeCl₃ was added. A positive result if a blackish blue or green color forms indicates the presence of tannin(Raudah et al., 2020).

The test animals used in this research were male Wistar rats aged 2-3 months and weighing 150-200 grams. 28 test animals were selected. The mice used were mice with the characteristics of having clean fur, clear red eyes, and normal behavior(Nurhayati and Rahmawati, 2023). Test animals were allowed to adapt to the new environment for 1 week to avoid stress. During the research process, mice were given food in the form of pellets twice a day and drinking water ad libitum. The mouse rearing area uses a rectangular plastic box measuring 40 x 30 x 22 cm and is covered with wooden husks. The room temperature was set at 25±1°C with light-dark cycle lighting (12 hours each). The box is cleaned every 2-3 days so that the test animals remain comfortable and protected from bacteria and disease.

Test animals were divided into 7 treatment groups (each group consisted of 4 mice): Group I. Normal controls were given distilled water, Group II. Negative controls were given distilled water, Group III. Positive controls were given glibenclamide 1 mg/KgBW, Group IV. EEDM dose 5 mg/KgBW, Group V. EEDM dose 10 mg/KgBW, Group VI. EEDM dose 20 mg/KgBW, Group VII. Glibenclamide 1 mg/kgBW and EEDM 5 mg/KgBW. On day 0, the test animals were fasted for 14 - 18 hours. After that, positive control group, positive control, single dose EEDM 5 treatment group; 10; 20 mg/KgBW and the combination group of glibenclamide and EEDM 5 mg/KgBW were induced by alloxan at a dose of 150 mg/kgBW intraperitoneally, 4 hours after being induced they were given 2 mL of 30% glucose orally. On the 3rd day after induction, blood sugar levels were measured using a visible spectrophotometer. The results of measuring blood sugar levels were compared with the results of blood glucose levels on the first day before alloxan was induced. If there is an increase in glucose levels in the rat's blood, namely ≥ 200 mg/dL, the rat is considered a diabetic rat(El Qahar, 2020). Blood sugar measurements were carried out on the day before induction, days 0, 7, and 14 after treatment by taking 0.5 mL of blood through the capillary vein in the conjunctiva of the eye. The blood samples taken were placed in a microtube and then centrifuged at a speed of 13,400 rpm for approximately 10 minutes until serum was obtained.

Determination of blood glucose levels using the GOD-PAP method and the GOD FS Glucose kit reagent. Determination of glucose levels is carried out first by making blanks, standards and samples. A blank solution was made using 5 μ L distilled water and added with 500 μ L Glucose

GOD FS kit reagent, then a standard solution was made by mixing 5 μ L standard solution serum and added with 500 μ L Glucose GOD FS kit reagent. The sample solution was made by mixing 5 μ L of sample solution and 500 μ L of Glucose GOD FS kit reagent. After that, the blank solution, standards and samples were incubated for 10 minutes at 37°C. Absorbance was read on a visible spectrophotometer at a wavelength of 546 nm. Monitoring the body weight of test animals was carried out by weighing the mice with the aim of monitoring changes in body weight before and after treatment. The body weight of the test animals was checked on the day before alloxan induction (baseline), post alloxan, day 7 and day 14 after treatment. Data analysis of blood glucose levels and body weight of mice was carried out on 7 groups using SPSS with the parametric One Way ANOVA test to see significant differences between groups and continued with Post Hoc LSD.

RESULTS AND DISCUSSIONS

Determination Results

Plant determination was carried out to identify and ensure that the plants used in the research were meniran plants. Plant determination was carried out at the Testing Laboratory - UPF Tawangmangu Traditional Health Services. The results of the determination stated that the plant used in this research was correct, namely meniran leaf simplicia. The results of determining meniran plants are as follows:

Family: Phyllanthaceae

Species: *Phyllanthus niruri* L.

Synonym: *Disaperus niruri*(L.) Kuntze

Extraction

The extraction process is carried out using the maceration method or what is often called the soaking method. This maceration extraction has the principle that the filter fluid will penetrate the cell wall and enter the cell cavity which contains the active substance. This active substance will later dissolve because there is a difference in the concentration of the active substance solution inside and outside the cell, so the concentrated solution will be forced out. The events repeat so that concentration equilibrium occurs between the solution inside the cell and outside the cell (Nurhayati and Rahmawati, 2023).

Some of the advantages of the extraction process using the maceration method are that it is a simple process, produces a fairly large yield, and the possibility of damage to compounds contained in a natural material can be avoided because it is not accompanied by the application of heat. In addition, a remaceration process is carried out with the aim of pulling back the compounds that remain after the previous maceration process which is caused by the saturation point reached by the solvent used. So, after remaceration, it is hoped that the yield will increase significantly. The solvent in this extraction process is 70% ethanol. 70% ethanol solvent is a solvent with a polarity level that is closest to the polarity of bioactive compounds. The closer the polarity of the solvent to the substance contained in the extract, the more components of the substance that can be extracted and the yield of the extract obtained will increase. (Guna et al., 2020).

The yield was carried out to determine the comparison between meniran leaf simplicia and extract. The dry weight of the initial simplicia meniran leaves used was 1000 grams, the extract yield obtained was 213.3 grams, so the extraction yield was obtained at $\frac{213.3}{1000} = 0.213$ grams = 21.3%. The parameters obtained were determined to be both specific and non-specific, and the results obtained were compared with relevant literature.

Table 1. Results of examination of meniran extract parameters

No	Inspection	Test results	Indonesian Herbal Pharmacopoeia
1	Color	Black	Black
2	Smell	No smell	No smell

3	Flavor	Bitter	Bitter
4	Yield (%)	21.33	≥ 19

From the data generated on Table 1. Results of examination of meniran extract parameters obtained a yield of 21.3%. The results obtained are in line with the research results Luliana et al (2019) who extracted meniran leaves using 70% ethanol obtained a yield of 25.18%. Yield analysis has significant importance because it can act as an indicator to assess the level of success of the extraction process. The higher the yield value, the higher the possibility that the number of compound components produced in extraction will also increase. The yield calculation results in this research and previous research are in accordance with the standards stated in the Herbal Pharmacopoeia Edition II, where the resulting yield is not less than 19% (RI, 2017).

Phytochemical Test of 70% Ethanol Extract of Meniran Leaves

Identification of compound content in this study aims to determine the compounds contained in the 70% ethanol extract of meniran leaves.

Table 2. Results of examination of meniran extract parameters

No	Testing	Reactor	Observation	Results	Reference
1	Flavonoids	Extract + Mg powder + concentrated HCl	Orange colored solution	+	Results (+) tomato red or orange (Raudah et al., 2020)
2	Alkaloids	Extract + 2N HCl + Dragendroff Reagent	A brown precipitate forms	+	Result (+) orange or brown precipitate (Raudah et al., 2020)
3	Saponins	Extract + hot distilled water + shake vigorously	There is stable foam	+	Results (+) stable foam (Raudah et al., 2020)
4	Tannin	Extract + FeCl 2%	Dark green solution	+	Result (+) blackish green (Raudah et al., 2020)

Information :

The sign (+) indicates that a substance is present

Based on Table 2. Results of examination of meniran extract parameters above, the results of the phytochemical screening test in this study are in accordance with the research Dimas et al (2022) as well as research Auliani and Ridho (2023) which shows that the ethanol extract of meniran leaves contains flavonoids, alkaloids, saponins and tannins. Flavonoids are natural phenolic compounds that have potential as antioxidants and have bioactivity as medicines. The flavonoids contained in the extract reduce blood glucose levels in mice by stimulating the release of insulin in pancreatic beta cells to be secreted into the blood. The alkaloid content can reduce blood glucose levels by inhibiting the α -glucosidase enzyme in the duodenal mucosa so that the breakdown of polysaccharides into monosaccharides can be hampered. In this way, the glucose released is also slower and its absorption into the blood is reduced and lower so that peaks in blood sugar levels can be avoided (Akuba et al., 2022).

Saponins can reduce blood glucose levels by inhibiting sugar transport in the digestive tract and stimulating the secretion of insulin, astringents or chelators which can shrink the epithelial membrane of the small intestine, thereby reducing the absorption of food essence and ultimately lowering blood glucose levels. (Wulandari et al., 2022). The tannin content is also known to reduce blood glucose by inhibiting the work of α -glucosidase which can delay the absorption of glucose after eating, so that sugar absorption and the rate of increase in sugar in the digestive system are still not too high. (Pratiwi et al., 2021). Thus, meniran leaves have an effect that can reduce blood glucose levels, the effect of which can possibly be obtained from the active components of flavonoids, alkaloids, saponins and tannins.

Measurement of Blood Sugar Levels

The results of research testing the antidiabetic effectiveness of a combination of meniran leaf extract (*Phyllanthus niruri* L.) and glibenclamide in reducing blood sugar levels in mice are shown in Table 3. Mean blood sugar levels (KGD) ($n=4$)

Table 3. Mean blood sugar levels (KGD) ($n=4$)

No	Group	Mean KGD (mg/dL) \pm SD				Percentage Change in KGD (%)
		Baselines	Induction	7th day of treatment	Treatment day 14	
1	Normal Control (aquadest)	116.5 \pm 37.81*	109.8 \pm 36.17*	103.3 \pm 37.26*	89.3 \pm 32.66*	↓ 18.67
2	Positive Control (Glibenclamide 1mg/KgBW)	102.5 \pm 12.40	281.8 \pm 87.88	179.0 \pm 56.54*	112.5 \pm 24.13*	↓ 60.07
3	Negative Control (aquadest)	89.3 \pm 6.85	256.5 \pm 23.61	271.5 \pm 24.91	286.5 \pm 23.46	↑ 11.70
4	EEDM 5 mg/kgBW	106.8 \pm 7.93	302.8 \pm 21.87	251.3 \pm 27.33	185.0 \pm 11.70*	↓ 38.90
5	EEDM 10 mg/kgBW	117.0 \pm 8.83*	347.5 \pm 40.94*	272.0 \pm 36.81	168.8 \pm 23.79*	↓ 51.42
6	EEDM 20 mg/kgBW	115.3 \pm 16.68*	406.5 \pm 36.57*	285.3 \pm 56.29	136.8 \pm 13.02*	↓ 66.35
7	Combination of Glibenclamide and EEDM 5 mg/kgBW	112.8 \pm 4.50	408.5 \pm 34.68*	337.7 \pm 29.35*	205.3 \pm 45.27*	↓ 49.74

Information :

* = there is a significant difference in the negative group ($p < 0.05$)

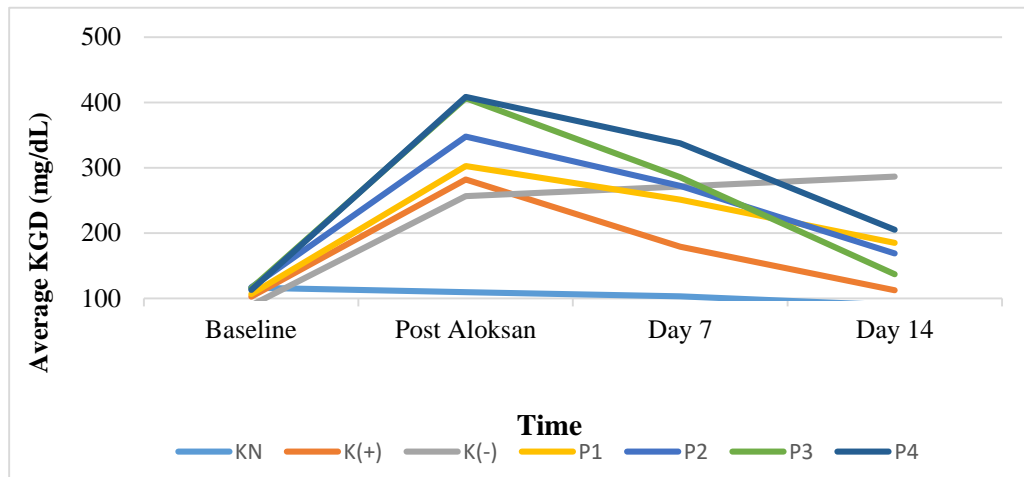
↑ = experienced an increase in KGD

↓ = experienced a decrease in KGD

$$\% \text{ reduction in KGD} = \frac{\text{Mean blood sugar level Day 14} - \text{Mean blood sugar level Post Alloxan}}{\text{Mean blood sugar level Post Alloxan}} \times 100\%$$

In this research, the diabetogenic substance used was alloxan at a dose of 150 mg/KgBW to induce mice to experience an increase in KGD which was then used to test the antidiabetic activity of the ethanol extract of meniran leaves. Alloxan in the blood binds to GLUT-2 (glucose transporter) which facilitates the entry of alloxan into the cytoplasm of pancreatic beta cells. Alloxan will cause excessive depolarization in the mitochondria as a result of the influx of Ca^{2+} ions which is followed by excess energy use resulting in a lack of internal energy in the pancreatic beta cells. This mechanism causes damage to both the number of cells and the mass of pancreatic cells resulting in a decrease in insulin release which results in diabetes mellitus (Sari, 2020). The hypoglycemia phase generally lasts 4 to 8 hours after alloxan induction. Hypoglycemia conditions can also cause seizures and even death if sugar is not given, so it is necessary to administer additional 30% sugar solution orally. Blood sugar levels were measured after 72 hours (day 3) to check hyperglycemia (Puspitasari and Choerunisa, 2021).

From the data listed in Table 3. Mean blood sugar levels (KGD) ($n=4$) There was a difference in the average KGD in mice before and after induction with alloxan. The data results are in accordance with research Lolok et al (2020) as well as research Natalia and Sara (2023), which indicates a successful increase in KGD after administering alloxan 150 mg/KgBW. On research Saree (2020) It was also proven that alloxan at a dose of 150 mg/KgBW (ip) was able to increase blood glucose levels and damage to rat pancreatic β cells. This proves that alloxan at a dose of 150 mg/kgBW is successful in creating a diabetic mouse model. Data on changes in rat blood sugar levels in this study are explained by: Picture 1. Graph of average blood sugar levels



Picture 1. Graph of average blood sugar levels

Information: KN = Normal Control, K(+) = Positive Control, K(-) = Negative Control, P1 = EEDM 5 mg/kgBW, P2 = EEDM 10 mg/kgBW, P3 = EEDM 20 mg/kgBW and P4 = Combination glibenclamide+EEDM 5 mg/kgBW.

In the antidiabetic test on days 7 and 14 after treatment, the control group was positive and the test control showed a gradual decrease in blood glucose. In the results of this study, the positive control given glibenclamide appeared to have experienced a significant decrease. Mice that have experienced hyperglycemia after being induced with alloxan will experience damage to pancreatic beta cells, so that insulin production and secretion is hampered. So the choice of glibenclamide as a comparative antihyperglycemia drug is considered appropriate, because glibenclamide has a mechanism of action that can stimulate insulin production and increase insulin secretion from pancreatic beta cells. (Widiana and Marianti, 2022).

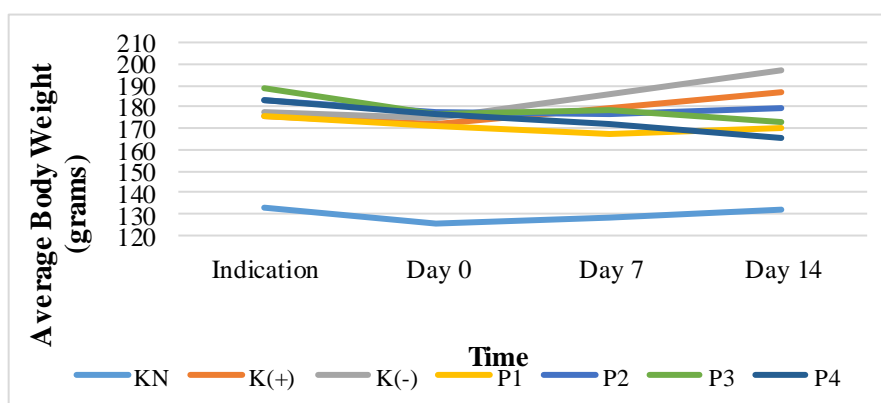
The treatment group given a single dose of EEDM of 5 mg/kgBW, 10 mg/kgBW, and a dose of 20 mg/kgBW also experienced a decrease in blood glucose levels. This shows that meniran leaf extract can reduce blood glucose levels in mice that have been induced by alloxan. Meanwhile, the results from the negative control group did not show a decrease in blood glucose levels because distilled water is neutral, does not contain any substances so it does not have the effect of lowering blood glucose levels.

The statistical results of the research in Table 3 show that the administration of a combination of glibenclamide and EEDM at a dose of 5 mg/kgBW had an effect in reducing blood sugar levels and provided optimal results that were almost the same as the positive group in reducing blood sugar levels as evidenced by a significant value that was not significantly different ($p > 0.05$). Apart from that, the statistical results also showed that there was no significant difference ($P > 0.05$) between the administration of a combination of glibenclamide+EEDM 5 mg/kgBW compared to the administration of glibenclamide EEDM at a single dose of 5 mg/kgBW, 10 mg/kgBW, and a dose of 20 mg. /kgBW. The treatment group given a combination of glibenclamide and EEDM at a dose of 5 mg/kgBW resulted in a lower % reduction in blood glucose levels, namely 49.74% compared to administration of glibenclamide alone. Meniran extract is a non-competitive inhibitor of the CYP3A4 enzyme, while the metabolism of glibenclamide itself occurs in the CYP3A4 enzyme. The expected results should be that with this combination, the antidiabetic activity will increase, but the results of this study show that giving a combination of glibenclamide and EEDM at a dose of 5 mg/kgBW is effective in reducing blood sugar levels but not as much as compared to giving a single dose of glibenclamide, EEDM at a single dose of 10 mg/kg. kgBW and 20 mg/kgBW, this may occur due to drug interactions so that the efficacy can be reduced, the condition of the test animals or because the timing of the combination is not appropriate. Therefore,

it is necessary to investigate further regarding the mechanism of antidiabetic activity, the time interval between administration of glibenclamide and ethanol extract of meniran leaves, and it is necessary to carry out further research regarding the histopathological picture of the pancreas of test animals that have been given glibenclamide and meniran leaf extract to see whether there is any improvement in pancreatic cells after alloxan administration.

Measurement of Rat Body Weight

The results of measuring the body weight of mice during treatment in this study can be seen in Picture 2.



Picture 2. Graph of changes in mouse body weight

Information: KN = Normal Control, K(+) = Positive Control, K(-) = Negative Control, P1 = EEDM 5 mg/kgBW, P2 = EEDM 10 mg/kgBW, P3 = EEDM 20 mg/kgBW and P4 = Combination glibenclamide+EEDM 5 mg/kgBW.

Based on picture 2 this shows that there were changes in the mice's body weight from the results before induction to the 14th day of treatment, namely a decrease and an increase. The mice's body weight was measured in a fasting state so that blood glucose was stable and there were no changes in blood glucose levels due to the influence of food intake. Weight loss occurred in mice after being induced with alloxan. Apart from increasing sugar levels, signs of diabetes mellitus can be seen from weight loss in mice, where there is a difference in the average body weight of mice before and after induction.(Datu et al., 2023). Changes in mouse body weight occurred in each treatment group at each measurement. The normal control group, positive control group, negative control group experienced an increase in body weight. Meanwhile, the test group given a single dose of EEDM 5 mg/kgBW, 10 mg/kgBW, and 20 mg/kgBW experienced weight loss. Results from research Kosnayani et al (2021) showed that administration of meniran water extract could suppress weight gain in obese mice and reduce fasting blood glucose levels. In this study, the group with a combination of glibenclamide and EEDM at a dose of 5 mg/kgBW also experienced weight loss. It is possible that using glibenclamide together with meniran can prevent the side effects of using glibenclamide alone, namely weight gain.

Table 4. Body weight of white mice after treatment with ethanol extract of meniran leaves (EEDM) (n=4)

Group	Treatment day 0 (Post Alloxan)	Treatment day 14	Change in Body Weight Per Day
Normal Control	125.3 ± 8.18	131.8 ± 24.31	↑ 0.46*
Positive Control	171.8 ± 8.66	187.3 ± 15.52	↑ 1.11*
Negative Control	175.0 ± 12.19	197.0 ± 20.83	↑ 1.57*
EEDM 5 mg/kgBW	171.3 ± 2.50	170.3 ± 2.22	↓ 0.07
EEDM 10 mg/kgBW	177.5 ± 5.20	179.5 ± 9.11	↓ 0.14
EEDM 20 mg/kgBW	176.3 ± 7.00	172.5 ± 8.58	↓ 0.27

Group	Treatment day 0 (Post Alloxan)	Treatment day 14	Change in Body Weight Per Day
Combination of Glibenclamide and EEDM 5 mg/kgBW	176.3 ± 8.06	165.5 ± 7.23	↓ 0.77

Information :

* = there is a significant difference in the combination group ($p < 0.05$)

↑ = experiencing weight gain

↓ = experiencing weight loss

Based on Table 4. Body weight of white mice after treatment with ethanol extract of meniran leaves (EEDM) ($n=4$) The results of changes in mouse body weight per day showed that there was an increase in the body weight of mice in the group given glibenclamide and distilled water from day 0 to day 14 after treatment. In the group given EEDM at a dose of 5 mg/kgBW, dose of 10 mg/kgBW, dose of 20 mg/kgBW and a combination of glibenclamide and EEDM 5 mg/kgBW showed a decrease in the body weight of the mice per day. This is in accordance with the results of statistical tests, namely that there is a significant difference ($P < 0.05$) between giving a combination of glibenclamide and EEDM 5 mg/kgBW and giving glibenclamide alone. So from the results above it can be concluded that the combination of glibenclamide and EEDM 5 mg/kgBW can overcome the side effects in the form of weight gain from using glibenclamide alone.

CONCLUSION

Giving a combination of glibenclamide 1 mg/kgBB and ethanol extract of meniran leaves 5 mg/kgBW can reduce blood sugar levels in mice but the effect is still lower compared to giving glibenclamide alone, a single dose of EEDM 10 mg/kgBB, and a dose of 20 mg/kgBW. The combination use of glibenclamide 1 mg/kgBW and ethanol extract of meniran leaves 5 mg/kgBW can also overcome the side effects of weight gain from using glibenclamide alone.

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