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The Effect Of Ethanol Extract From Tuba Leaves (*Derris Elliptica*) On The Decrease In Blood Glucose Levels In Male White Mice

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Abstract

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia, caused by disturbances in insulin secretion, insulin action, or both. One alternative treatment option is herbal/traditional medicine made from natural ingredients. This study aimed to determine the effect of ethanol extract of Tuba plant leaves (Derris elliptica) on blood glucose levels in male white mice induced with alloxan. This study used an experimental method in which mice were induced with diabetes using a dose of 150 mg/kgBW alloxan for 3 days. The test animals were divided into 6 treatment groups, each consisting of five mice. Group I, the normal control, was given 0.5% Na CMC; group II, the negative control, was given alloxan and 0.5% Na CMC; group III, the positive control, was given alloxan and glibenclamide; while groups IV, V, and VI were given alloxan and tuba leaf ethanol extract at doses of 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW, respectively. Blood glucose levels were measured on days 1, 7, 14, and 21. The results showed that a 400 mg/kg body weight dose of tuba leaf ethanol extract significantly reduced blood glucose levels by 125.4 mg/dl and was almost as effective as glibenclamide. A dose of 400 mg/kg body weight was the optimal dose, producing significant effects while remaining safe for use.

Keywords: Ethanol Extract Of Tuba Plant Leaves (Derris Elliptica), Blood Glucose, Diabetes, Mice, Alloxan

INTRODUCTION

According to data from the International Diabetes Federation (IDF), diabetes mellitus is among the top 10 causes of death worldwide. Since 2000, the number of adults with diabetes has more than tripled, from 151 million (4.6%) to 537 million (10.5%) in 2021. Currently, more than 10% of adults worldwide have diabetes. If this trend continues, it is estimated that there will be 643 million people with diabetes by 2030 (11.3%) and this number will jump to 783 million (12.2%) by 2045, with type 2 diabetes accounting for more than 90% of total diabetes cases worldwide. Indonesia itself is among the 10 countries in the world with the highest number of diabetes sufferers, ranking 5th with 19.5 million sufferers (aged 20-79).

Treatment for type 2 diabetes is generally oral antidiabetic medication. However, long-term use of these chemical drugs can cause various side effects, including hepatotoxicity, nephrotoxicity, hypoglycemia, and gastrointestinal issues, as well as being costly, given that diabetes mellitus requires lifelong care and treatment.

Based on the above description, the treatment of type 2 diabetes mellitus with non-pharmacological therapies such as herbal/traditional medicine from natural ingredients can be an alternative choice for people with diabetes, because it has relatively few side effects and is widely available

Several plants that have been studied have been shown to have the potential to lower blood glucose levels and contain flavonoids, tannins, saponins, and terpenoids as secondary metabolites. Among these are mangsi leaves at a dose of 1000 mg/kg BW, which are effective in improving hyperglycemic symptoms in male white mice, soursop leaves at a dose of 4.2 mg/20 g BW effectively lowered blood glucose levels in male white mice, seaweed leaves at a dose of 250 mg/kg BW had antidiabetic activity in male white mice, and yellow squash leaves at a dose of 3,200 mg/kg BW exhibit antidiabetic activity in male white mice.

According to research by Puspito et al. in 2023, the root of the Akar Tuba plant (*Derris elliptica (Roxb.) Benth*) contains a main compound called rotenone ($C_{23}H_{22}O_6$) in its roots, which can inhibit cell respiration in fish. This compound is often used as a bioinsecticide and natural fish poison.

According to research by Rahman et al. in 2024, the leaves of the Tuba root plant (*Derris elliptica*) at a dose of 400 mg/kg BW in rats can increase insulin secretion, which has the potential to lower blood glucose levels. The secondary metabolites contained in it are flavonoids, tannins, terpenoids, alkaloids, and quercetin.

This is what prompted researchers to conduct research on Tuba plant (*Derris elliptica*) leaves to examine the effect of ethanol extract from Tuba plant (*Derris elliptica*) leaves on blood glucose levels in male white mice induced with alloxan.

RESEARCH METHODS

Equipment

The equipment used was a rotary evaporator (Buchi), digital analytical balance (Shimadzu), porcelain dishes (Pyrex), glassware (Pyrex), UPLC-MS/MS data analysis software (Waters), water bath, mortar and pestle, probe, syringe (OneMed), disposable 1 cc syringe (OneMed), filter paper, funnel, glucometer (Autocheck GCU), test strips (Autocheck GCU), scissors, vials, stirring rods, gloves, masks, spatulas, spoons, dark bottles, parchment paper, aluminum foil, mouse cages.

Ingredients

The ingredients used are tuba leaves (*Derris elliptica*), distilled water, 0.5% Na-CMC (Sigma Aldrick), glibenclamide, 96% ethanol, monohydrate alloxan (Sigma Aldrick), 0.9% NaCl, and 30 healthy male white mice aged 8-10 weeks with a body weight of 20-30 g.

Sample Collection and Determination

The Tuba (*Derris elliptica*) leaf samples used were collected from plantations in the village of Seleman, Danau Kerinci Subdistrict, Kerinci Regency, Jambi Province. The determination of Tuba (*Derris elliptica*) leaves was carried out at the Jatinangor Herbarium, Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University.

Sample Preparation

6 kg of Tuba (*Derris elliptica*) leaves were collected, then wet sorting was carried out to separate the simplisia from foreign matter such as twigs, insects, damaged leaves, and soil. The leaves were then washed thoroughly with running water and chopped to facilitate the drying process. then air-dried in a place not exposed to direct sunlight for 10 days, followed by dry sorting to remove any foreign matter remaining after wet sorting, and finally dusting.

Simplisia Extraction

This study was conducted using the maceration extraction method, which is the simplest method, using 96% ethanol as the solvent. Dried Tuba (*Derris elliptica*) leaves were placed in a dark glass bottle and soaked in 96% ethanol until the simplisia was completely submerged. This was done at room temperature, away from direct sunlight, while stirring occasionally. The simplisia was soaked for 3 days, with a material to solvent ratio of 1:10. The maceration was then filtered using filter paper, then concentrated using a rotary evaporator at a temperature of 50°C, followed by a water bath until a thick extract was obtained[10]. The extract yield can be calculated using the following formula :

$$\%\ \text{Yield} = \frac{\text{Weight of concentrated extract (g)}}{\text{Initial weight of crude drug sample (g)}} \text{x} 100\%$$

The criterion for concentrated extract yield is that the value must not be less than 10%.

UPLC-MS/MS Analysis

The analysis of secondary metabolites contained in the leaves of the Tuba plant (*Derris elliptica*) was conducted at the Borobudur Museum and Cultural Heritage Laboratory, Magelang, Central Java, using Waters UPLC-MS/MS equipment.

Pharmacological Testing

Preparation of Alloxan Solution

The most commonly used dose of alloxan in research is 150 mg/kg body weight of mice administered intraperitoneally. Alloxan is weighed, then dissolved in 0.9% NaCl to a volume of 10 ml in a measuring flask[21]. The storage process until aloxan injection is carried out at a cold temperature to prevent aloxan degradation. The solution is prepared by wrapping the measuring flask with aluminum foil to avoid exposure to light.

Preparation of 0.5% Na-CMC Suspension

Weigh the Na-CMC, add it gradually to a mortar containing 70°C hot distilled water, stir until it expands and becomes homogeneous. Add distilled water until the total solution volume is sufficient. The induction dose is 1% of the mouse body weight administered orally.

Preparation of Glibenclamide Suspension

The therapeutic dose of glibenclamide for humans is 5 mg. When converted to a 20 g mouse, this becomes 0.013 mg. Weigh and grind the glibenclamide, then dissolve it in 0.5% Na-CMC, stirring until homogeneous, and adjust the volume.

Preparation of Extract Suspensions

Three variations of ethanol extract doses from Tuba (*Derris elliptica*) leaves were taken: 100 mg/kg BW of mice, 200 mg/kg BW of mice, and 400 mg/kg BW of mice. then dissolved with 0.5% Na CMC, and the volume was adjusted to 50 ml in a measuring flask.

Testing the Effect of Ethanol Extract of Tuba Plant Leaves (*Derris elliptica*) on the Reduction of Blood Glucose Levels in Male Mice

Male white mice aged 8-10 weeks, weighing 20-30 g, totaling 30 mice, were divided into 6 groups, each consisting of 5 mice. Before treatment, the test animals were acclimatized for one week. The mice were fasted for 8-10 hours, then blood glucose levels and body weight were measured before treatment. On the same day, the mice were induced with a solution of alloxan monohydrate at a dose of 150 mg/kg BW intraperitoneally. After 3 days of alloxan induction, blood glucose levels were measured. The mice used in the study were those with blood glucose levels higher than the normal fasting blood glucose (FBG) level of >126 mg/dl. Blood samples were taken from the mice's tails using a glucometer. Each group received the appropriate treatment: group 1 was the normal control group not induced with alloxan and was given a 0.5% Na cmc solution p.o., group 2 was the negative control group induced with alloxan i.p. and was given a 0.5% Na cmc solution p.o., group 3 as a positive control induced with alloxan i.p., given glibenclamide suspension p.o., and groups 4 to 6 induced with alloxan i.p., and given ethanol extract of Tuba (*Derris elliptica*) leaves at doses of 100, 200, and 400 mg/kg BW of mice. The treatment was administered for 21 days. Blood glucose levels were checked after treatment on days 1, 7, 14, and 21 after being declared hyperglycemic.

Data Analysis

Data analysis was performed using the JASP application with the Shapiro-Wilk test to determine whether the data obtained was normally distributed or not. Data was considered normally distributed if the p-value was > 0.05, after which the One-Way ANOVA test could be performed. However, if the p-value was < 0.05 in the normality test, the non-parametric Kruskal-Wallis test was used, followed by a Post Hoc test to identify differences between each treatment group

RESULTS AND DISCUSSION

Sample Determination

The results of plant determination conducted at the Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, indicate that the sample is *Derris elliptica* with identification number No. 107/HB/11/2024. The determination aims to verify the identity of the plant to avoid data collection errors.

Extraction Results

Extract preparation using the maceration extraction method with 96% ethanol solvent. The concentrated extract yielded a calculation of 12.8%.

Table 1. Extract Yield

Tuba Plant Leaves (*Derris elliptica*) (gram)	Simplisia (gram)	Thick Extract (gram)	Extract Yield (%)
6.000	3.300	424,98	12,8

Six kilograms of fresh Tuba (*Derris elliptica*) leaf samples yielded 3.3 kg of dried simplisia. The final result of maceration extraction of Tuba (*Derris elliptica*) leaf samples was 424.98 g of thick extract.

The maceration extraction method was chosen because of its easy and simple process, as well as its ability to prevent damage or degradation of active compounds by not requiring heating at high temperatures[5]. A 96% ethanol solvent was used because it is universal, non-toxic, has high selectivity, and good absorbency, allowing a thick extract to be obtained in a shorter time.

The resulting extract has a distinctive odor and is blackish-green in color. In this study, the yield value obtained was more than 10%, so it can be said that the yield value meets the criteria.

UPLC-MS/MS Analysis Results

Ultra performance Liquid Chromatography-Mass Spectrometry (UPLC-MS/MS) was performed to identify the metabolite compounds contained in the leaves of the Tuba plant (*Derris elliptica*). The results showed that the leaves of the Tuba plant (*Derris elliptica*) contain active compounds that have the potential to lower blood glucose levels, including quercetin, kaempferol, and catechin, which are flavonoids (flavonols) that play an important role in increasing antioxidant enzyme activity and regenerating damaged pancreatic β cells, overcoming insulin deficiency.

Tannin compounds (Gallic Acid, Ellagic Acid, Chlorogenic Acid) can inhibit the rate of increase in blood glucose levels[8]. Saponin compounds (Sarsasapogenin) inhibit the enzyme α-glucosidase[7], and terpenoid compounds (Beta-Sitosterol, squalene) stimulate and maintain the stability of insulin release from β cells, inhibiting the activity of the enzyme α-glucosidase.

Pharmacological Test Results

Average Blood Glucose Levels in Mice

Table 2. Average Blood Glucose Levels in Mice

Group	Mean Fasting Glucose Level in Mice (mg/dL) *Mean* ± SD			
	Day 1	Day 7	Day 14	Day 21
I (Normal)	101,00±9,51	100,00±9,05	96,80±8,70	93,20±9,41
II (Negative)	247,80±13,55	266,00±12,10	260,80±13,59	275,00±11,44
III (Positive)	245,20±15,88	214,40±29,85	172,80±36,56	107,80±14,41
IV (100 mg/Kg BW of mice)	244,20±15,22	217,40±10,01	203,80±12,17	172,60±16,41
V (200 mg/Kg BW of mice)	257,20±28,17	227,00±24,74	202,40±28,91	153,80±26,03
VI (400 mg/Kg BW of mice)	249,00±9,30	228,40±7,76	180,20±19,37	123,60±20,47

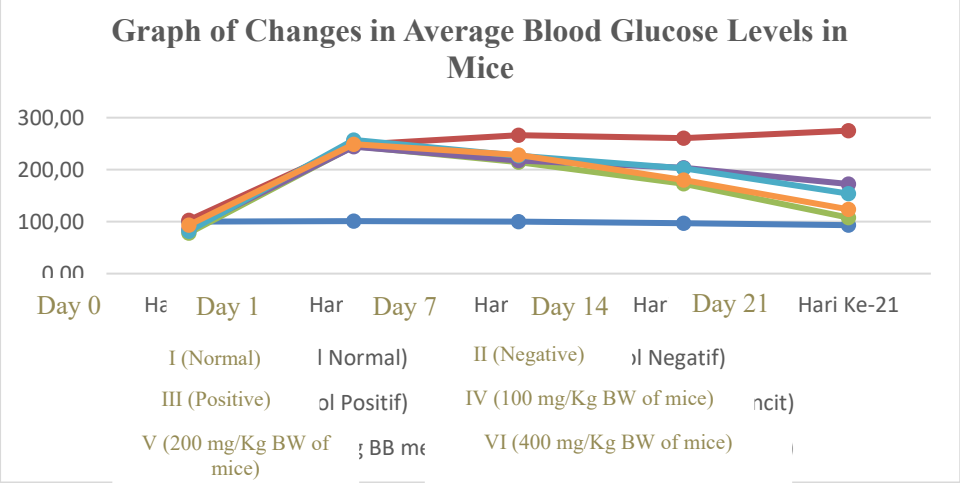
Description :

Day 1 : Day after alloxan induction
SD : Standard Deviation
mg/dL : Milligrams per deciliter
Normal blood sugar : <126 mg/dL

Table 3. Average Difference in Blood Glucose Level Decrease in Mice

Group	Average Difference in Blood Glucose Level Decrease in Mice (mg/dL) *Mean* ± SD		
	D_7	D_{14}	D_{21}
I (Normal)	1,0	4,2	7,8
II (Negative)	-18,2	-13	-27,2
III (Positive)	30,8	72,4	137,4
IV (100 mg/Kg BW of mice)	26,8	40,4	71,6
V (200 mg/Kg BW of mice)	30,2	54,8	103,4
VI (400 mg/Kg BW of mice)	20,6	68,8	125,4

Figure 1. Graph of Changes in Average Blood Glucose Levels in Mice



On average, the difference in blood glucose level reduction in mice (Table 3) shows that group I (normal) had relatively stable blood glucose levels throughout the study period, as indicated by low blood glucose level reduction values. This indicates that in healthy mice without alloxan induction, blood glucose can be maintained physiologically. In contrast, group II (negative), which was only given Na-CMC, showed an increase in blood glucose levels throughout the study period, as indicated by negative average differences in H7, H14, and H21. This indicates that Na-CMC is inert and has no pharmacological activity on blood glucose reduction.

Group 3 (positive), which was given the standard drug glibenclamide, showed the highest average difference in blood glucose levels compared to all treatment groups, up to day 21 (137.4 mg/dl). This demonstrates the effectiveness of the standard drug in consistently lowering blood glucose levels in alloxan-induced mice. Glibenclamide was chosen as the positive control because the

mechanism of glibenclamide as a sulfonylurea antidiabetic drug works by increasing insulin secretion from pancreatic β cells, thereby lowering blood glucose levels.

In the treatment group given ethanol extracts of Tuba (*Derris elliptica*) leaves at doses of 100 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW, there was also a gradual decrease in blood glucose levels from day 7 to day 21. with the 400 mg/kg BW dose showing the greatest decrease, approaching the positive control, with an average difference in blood glucose level decrease of 125.4 mg/dl on day 21. This indicates that increasing the extract dose tends to increase the ability to lower blood glucose levels. The Shapiro-Wilk normality test yielded a p-value for all treatment groups of $p>0.05$, indicating that the data in all groups were normally distributed, which shows that the normality assumption was met and can be followed by a One Way Anova test to see if there are significant differences between treatment groups.

The results of the One Way ANOVA test show that there is a significant difference between treatment groups in terms of blood glucose levels in mice ($p<0.05$), meaning that the treatment has a significant effect on blood glucose levels in mice. A Tukey Post Hoc test was then conducted to see which groups showed significant differences. The results showed that group 3 (positive) and the treatment groups given ethanol extracts of Tuba (*Derris elliptica*) leaves at doses of 100 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW had a blood glucose-lowering effect compared to the negative control group, which was indicated by a significant difference ($p<0.05$). Group 3 (positive) with the treatment group of ethanol extract of Tuba plant leaves (*Derris elliptica*) at doses of 100 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW showed no significant difference ($p>0.05$). This indicates that ethanol extract of Tuba *(Derris elliptica*) leaves has hypoglycemic activity potential that is close to the standard drug glibenclamide. This is in line with the research by Rahman et al. in 2024, which states that Tuba plant leaves (*Derris elliptica*) have therapeutic effects similar to standard glibenclamide, and that extracts at higher doses (400 mg/kg BW) are found to be more effective than lower doses (200 mg/kg BW).

Although it has not yet reached normal blood glucose levels (<126 mg/dl), as indicated by a significant difference ($p<0.05$) between the normal control group and the treatment group given ethanol extracts of Tuba (*Derris elliptica*) leaves at doses of 100 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW. This is because the dosage variations used are still not optimal; further research with increased dosage variations is needed to maximize the therapeutic potential.

Active compounds such as flavonoids (quercetin), terpenoids, tannins, saponins, and steroids in the ethanol extract of Tuba (Derris elliptica) leaves play a role in lowering blood glucose levels, which can protect pancreatic beta cells from oxidative damage and increase insulin sensitivity, thereby minimizing damage.

CONCLUSIONS

Based on the results of the study, it can be concluded that ethanol extract of Tuba (*Derris elliptica*) leaves has been proven to reduce blood glucose levels in male white mice induced by alloxan, as indicated by a significant difference ($p<0.05$) in blood glucose levels between the negative control group and the groups treated with doses of 100 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW. The most effective dose in lowering blood glucose levels in male white mice was 400 mg/kg BW.

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