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Effect of Infusion Buas-Buas Leaf (*Premna serratifolia* Linn.) on Decreasing Blood Glucose Level of Alloxan-Induced White Mice

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Abstract: The prevalence of diabetes is incresing year by year, indicates that there is a need for diabetes treatment. One alternavite treatment for diabetes melitus uses traditional medicine which has relatively small effect when compared to synthetic diabetes melitus drugs use the long term. One plant buas-buas which is utilized a natural therapy to blood glucose levels leaves buas-buas (*Premna serratifolia* Linn). that are believe have antiabetic effect. The study was to determine infusion of buas-buas leaves can decreas blood glucose levels in alloxan-induced mice. The research is experimental using purposive sampling. Method collection sample, make simplisia of buas-buas leaves, extracing buas-buas leaves and antidiabetic test on 25 male white mice. The test was conducted by divid into 5 treatments groups, namely positive control (metformin), negative control (water), and control treatment preparations with concentrations of 10, 20, and 40 %. Mice were induced alloxan with dose of 200 mg/kg BW intraperitoneally and give after blood glucose levels 200 mg/dL, then measurd blood glucose levels of mice on day 7 and day 14. The data result were analyzed using *Kruskall Wallis* Test. The results of study indicate that infusion of wild buas leaves can decreas blood glucose levels. A dose of wild buas leaf infusion with a concentration of 20 % is effective in decreasing blood glucose levels. These findings suggest that *Premna serratifolia* Linn has the potential to be developed as a treatment for diabetes mellitus.

Keywords: Buas-buas leaves, *Premna serratifolia* Linn., blood glucose level, white mice, alloxan

1. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia that occurs due to insulin hormone imbalance resulting in microvascular and macrovascular complications (DiPiro *et al.*, 2017). The increase in number of people with diabetes in 2025 that 380 million, in addition to WHO revising the incidence of diabetes mellitus in the United States which was previously estimates at 21.9 million to 30.3 million people (Suhendy *et al.*, 2021).The prevalence of diabetes in Indonesia ranks seventh highest in the world after China, India, the United States, Pakistan, Brazil and Mexico. Based on data from the International Diabetes Federation (IDF) in 2019, the Southeast Asian region, precisely in Indonesia, ranked third with a prevalence of diabetes mellitus of 11.3%. Based on Riskesdas data in 2018, there was an increase in the number of cases of diabetes mellitus at the age of ≥ 15 years from 1.5% in 2013 to 2% in

2018 (Kemenkes RI, 2018). Based on data from the East Kalimantan Health Office, the number of cases of diabetes mellitus in 2017 was 12,688 cases to 17,490 cases in 2018. This figure shows that diabetes mellitus has increased every year (RISKESDAS, 2019).

The prevalence of diabetes mellitus is increasing from year to year, indicating that diabetes treatment needs to be done. Diabetes treatment includes non-pharmacological therapy and pharmacological therapy. While non-pharmacological therapy includes lifestyle modification, weight loss, physical exercise, healthy living, reducing salt consumption, and traditional medicine, pharmacological therapy includes oral antihyperglycemic drugs and insulin therapy. Oral anti-hyperglycemia drugs have many side effects such as weight gain, hypoglycemia, and liver dysfunction. Due to the side effects, diabetic patients choose alternative or traditional medicine. One alternatives used is traditional medicine because price is relatively cheap and the side effects are relatively small when compared to synthetic diabetes mellitus drugs. One of the plants that can be used as an alternative treatment is buas-buas (Timotius *et al.*, 2018)

The buas-buas (*Premna serratifolia* Linn.) is a plant that is widely used as a vegetable or fresh vegetables by the Malay community. Buas-buas leaves are believed to have medicinal properties and used in traditional medicine as a remedy for worms, colds, increasing appetite in children, facilitating breastfeeding, decreas body, and as a food preservative (Wulandari R, 2019).Buas-buas leaves have medicinal properties such as the treatment of gastric disorders, udema, carminative, diuretic, spasmolytic, expectorant, contipation, and heart disease (Timotius *et al.*, 2018).

Based on previous research, buas-buas leaves can be used as ɑ-glucosidase inhibitors which can be utilized as antidiabetics (Timotius et al., 2018). According to research by ethanol extract of buas-buas leaves has antidiabetic activity. According to research buas-buas leaves have secondary metabolite compounds such as flavonoids, alkaloids, tannins and triterpenoids, where these secondary metabolites have activity as antidiabetics (Kasmawati *et al.*, 2020)

Infusa was chosen because it has various advantages, namely easy, cheap to use, more applicable to the community and closer to the traditional way of making by the community. People traditionally make medicine by boiling, but this method is not used if the temperature exceeds 100˚C because it can damage the content of the compounds in it (DEPKES RI, 2017). Based on the above background, the research aim to study effect infusion of buas-buas leaves (*Premna serratifolia* Linn.) on decrease blood glucose levels in alloxan-induced white mice.

2. Materials and Methods

2.1 Materials

The tools used for the production of stainless steel infusa , analytical balance (Ohaus®), banner rod, aluminum foil, mesh 60 sieve, buchner funnel, erlenmeyer (Pyrex®), measuring cup (Pyrex®), mice cage, mice drinking bottle, filter paper, oral sonde (Onemed®), syringe (Onemed®), glucometer (Autocheck®), blender (Philips®), mortar, mercury thermometer, surgical scissors (Saffa Stainless®), dropper, jar, and blood sugar strips (Autocheck®).The materials used were buas-buas leaves, alloxan monohydrate (Nitra kimia®), sodium chloride

0.9% (PT Widatra bhakti®), water (Le mineral), and metformin HCl tablets (Hexpharm jaya®). The animals used were male white mice (*Mus musculus* L.). The test criteria carried out by inclusion, namely age 2-3 months and weight between 20-40 g.

2.2 Methods

The sampling leaves of buas-buas (*Premna serratifolia* Linn.) were at Jl. Anggrek. Rt.03, Margahayu Village, Loa Kulu District, Kutai Kartanegara Regency. buas-buas is taken based on certain considerations or elements of the researcher. Considerations from researchers buas-buas leaves that are picked have good quality, green color, and are not physically damaged by parasites. Plant determination is carried out to determine the truth and type of a plant. Determination was carried out at the Tropical Forest Ecology and Biodiversity Conservation Laboratory, Faculty of Forestry, Mulawarman University, Samarinda.

Simplisia Preparation buas-buas leaves that have been collected, then wet sorted and washed with clean running water, then chopped and dried by aerating protected from sunlight, after dry sorting dry. Dry symplisia is pulverized using a blender and sieved with mesh 60.

Infusa prepation of buas leaves made 10% b/v (DEPKES RI, 2020). Weigh 10 gram, 20 gram and 40 gram of leaf simplisia buas-buas then put into an infusa pot, added water up to 100 mL, then heated for 15 minutes from the temperature reaching 90ºC, while stirring occasionally. Then filtered using filter paper. If the volume of infusion is less than 100 mL, hot water is added to the pulp of buas leaves to obtain 100 mL of infusion (DEPKES RI, 2017).

Ethical clearance from Muhammadiyah Lamongan University with number 331/EC/KEPK-S1/09/2023.Acclimatization test animals for 7 days. Every day food and drink are given *ad libitum* (unlimited). The preparation of test animals aims to allow mice to adapt to the new environment. Determination of the number of test animals is determined based on the Federer formula, namely (t-1) (n-1) >15, where n is the number needed and t is the treatment group (Kasmawati *et al.,* 2020). The test animals were grouped into 5 groups where each group consisted of 5 test animals. The groups consisted of the treatment group given the preparation of buas-buas leaf infusion with concentrations of 10%, 20%, and 40%, the positive control group (metformin 1.3 mg/20g BW) and the negative control group (water 0.5 mL).

Dose Planning infusion buas-buas leaves were given to test animals with concentrations of 10%, 20%, and 40% orally. The maximum volume requirement of the solution given i.p = 1 mL. Positive control is given metformin for mice weighing 20 gram of metformin dose in human 500 mg converted to mice 20 gram is 0.0026 x 500 mg = 1.3 mg / 20 g BW as for the negative control, 0.5 mL of distilled water was given.

Percentage of blood glucose level reduction compare the effect of reducing blood glucose levels between treatment groups, the difference between levels after alloxan administration (t0) and glucose levels at a certain measurement time was calculated, as well as the percentage reduction in blood glucose levels with the following formula (Amir *et al.,* 2019):

$$\% \text{ decrease} = \frac{blood\ glucose\ (t0) - blood\ glucose\ level\ measurement\ time}{blood\ glucose\ (t0)} \times 100\ \%$$

Antidiabetic activity test after being adapted for 7 days, mice were weighed, then 18 hours of fasting was measured for initial glucose levels. After that mice were induced alloxan 4 mg / 20 g BW intraperitoneally. How to swab 70% alcohol on abdomen of mice. After 72 hours the blood glucose levels of mice were measured using a glucometer. Mice are positive for diabetes mellitus if blood glucose levels are >200 mg/dL. After the mice's blood glucose levels have increased, the mice are grouped into 5 groups of negative control, positive control, and infusa groups with concentrations of 10, 20 and 40% given orally. After treatment blood glucose levels of mice were measured on day 7 and day 14.

The data analysis used in this study is Analysis of Variance (ANOVA) one way using the Jamovi program. Blood glucose levels were analyzed for normality and homogeneity using the normal distribution test (*Shapiro-Wilk* Test) and homogeneity test (*Levene's* Test). If the data were normally distributed and homogeneous, then continued with one way analysis of variance (ANOVA) to see whether or not there were differences between groups. If there is a significant difference, then proceed with non-parametric analysis (*Kruskall-Wallis* Test) to see whether or not there is a difference between groups.

3. Results and Discussion

Plant determination aims to determine the true identity of the plant. The determination results state that the sample used is buas-buas (*Premna serratifolia* Linn.) from the genus premna and family lamiaceae with letter number 284/UN17.4.08/LL/2022. Simplisia of buas-buas leaves (*Premna serratifolia* Linn.) is extracted using a water solvent that has an excellent ionic compound, namely the -OH group which is polar and can dissolve bioctive compounds such as phenolic compounds, alkaloids, flavonoids, tannins and streoids (Helmidanora *et al.,* 2018) .Preparations are made before giving treatment to mice and stored in the refrigerator. The infusa results obtained concentrations of 10%, 20%, and 40%.

In this study, male mice were used as test subjects because they have physiological and anthomic conditions that resemble humans such as organ completeness, metabolism, and nutritional needs. Male mice were chosen because male mice do not have the hormone estrogen. This causes hormonal conditions in male mice to be more stable compared to female mice that experience hormonal changes(Helmidanora *et al.,* 2018). After adapted for 7 days, mice were weighed, then fasted for 18 hours to measure initial glucose levels. After that mice were induced alloxan 4 mg/20 g BB intraperitoneally. The method is by rubbing 70% alcohol on the abdomen of mice. After 72 hours the blood glucose levels of mice were measured using a glucometer, to makesure mice are positive for diabetes mellitus if the blood glucose level is >200 mg/dL (Amir *et al.,* 2019).

After the blood glucose level of mice has increased, the mice are grouped into 5 and given the treatment of negative control group, positive control, and infusa group concentrations of 10, 20 and 40% given orally. After treatment, the blood glucose levels of mice were measured on day 7 and day 14. The average results of pre-induction blood glucose levels, day 0 (post-induction), day 7 and day 14 of all treatment groups. The average results of blood glucose levels are presented in the table below.

Table 1. Mean blood glucose levels of test animals based on treatment group

Treatment Group	Mean Blood Glucose Levels of Animals (mg/dL)			
	Pra Induction	Day -0	Day-7	Day-14
Negative Control	127	333	467	456
Positive Control	154	533	263	238
Infusion Concentration 10%	156	273	186	198
Infusion Concentration 20 %	156	374	183	166
Infusion Concentration 40 %	153	267	180	156

Based on the results of the average blood glucose levels, it is known that the blood glucose levels of test animals after alloxan induction showed an increase in glucose levels compared to pre-induction blood glucose levels. Blood glucose levels after alloxan induction ranged from 250 to 550 mg/dL. These variens blood glucose levels are due to differences in physiological responses in each mice to alloxan given at same dose and stress conditions experienced by test animals resulting in increased metabolic mice. Stress conditions can happen through fights between animals in cage, noise, and room temperature resulting in an increase in blood glucose levels. Increased blood glucose levels can result from stressful conditions (Morakinyo et al., 2019). Under stressful conditions humans or animals can experience various changes in physiological conditions that are often detrimental to individuals (Helmidanora et al., 2018). On day 7 measurements showed the average value of blood glucose levels for the positive control group, the buas-buas leaf infusa group experienced a decrease in blood glucose levels and the negative control experienced an increase in blood glucose levels. The results of the average measurement of blood glucose varied in all treatment groups.

The measurement of blood glucose levels on day 14 showed that the average blood glucose levels of the treatment groups varied. There was a decrease in blood glucose levels in the positive control group, buas-buas leaf infusion group, while the negative control group continued to experience an increase in blood glucose levels. The results of blood glucose level measurements on day 0 (post alloxan induction), 7, and 14 were then analyzed statistically. Statistical analysis was used to analyze and compare blood glucose levels of test animals at the three measurement times. As an initial analysis, normality test with *Shapiro-Wilk* test and homogeneity test with Levene's were conducted first. Based on *Shapiro-Wilk* normality test, the results of the blood glucose levels of the three measurement times (day 0, day 7, and day 14) were known ($p>0.05$) which means there is no significant difference between the three measurement times. Normally distributed data all treatment groups that became subjects in the study had a normal distribution. Homogeneity test with *Levene's* on day 14 showed a value of $p < 0.05$, which means the data is not homogeneity.

With data that is not homogeneity, the requirements for the One way ANOVA test (parametric test) are not met. As an alternative, the analysis continued using the Kruskall Wallis test (non-parametric). Based on the results of the *Kruskall Wallis* non-parametric test, there was a significant difference between the three measurement times (day 0, day 7, and day 14) ($p<0.05$). Based on *Kruskall Wallis* test, there was a significant difference in blood glucose levels ($p<0.05$) between the three measurement times.

The percentage decrease in blood glucose levels in the test animals was carried out to determine the difference in the decrease in blood glucose levels on day 7 and day 14 in each treatment group presented in the table below.

Table 2. Percentage decrease in blood glucose levels

Groups	Percentage decrease in blood glucose levels (%)	
	On Day -7	One Day-14
Negative Control	40.24 %	36.93 %
Positive Control	50.65 %	55.34 %
Infusion Concentration 10 %	31.86 %	27.47 %
Infusion Concentration 20 %	51.07 %	55.47 %
Infusion Concentration 40 %	32.58 %	41.57 %

Based on the results of the percentage decrease in blood glucose levels, the concentration of 20% showed the greatest decrease in blood glucose levels with a percentage of 55.47% on day 14, followed by 40% concentration and 10% concentration with a percentage of 41.57% and 27.47% respectively. This can be caused by the presence of active substances or compounds that affect the decrease in blood glucose levels. In the negative control, blood glucose levels increased because distilled water is a neutral compound that has no effect on lowering blood glucose levels so that this group does not experience a decrease in blood glucose levels (Suhendy *et al.,* 2021). Metformin works in the body to improve the sensitivity of the liver and peripheral tissues to insulin. Metformin increases glucose take by intestinal cells, thereby lowering blood glucose and inhibiting glucose absorption in the intestine after food (Nangoy *et al.,* 2019). The dose of metformin used was 1.3 mg/ 20 g. This dose is an effective oral dose in humans (65 mg/kg BW) which is converted to a dose of mice.

In the buas-buas leaf infusa group, it can decrease blood glucose levels because it has secondary metabolite compounds contained in the infusa of the buas-buas leaves. Based on previous research, the water extract of buas-buas leaves contains compounds of the alkaloid group, tannins/polyphenols, and flavonoids. Flavonoids and tannins have activity as antidiabetics with antioxidant abilities (Timotius *et al.,* 2018). Flavonoids can increase insulin sensitivity with the ability to inhibit pancreatic β-cell damage from chain perosidation reactions caused by Reactive Oxigen Species (ROS). The mechanism of action of flavonoids in healing diabetes is to increase the activity of antioxidant enzymes and be able to regenerate damaged pancreatic β-cells so that insulin deficiency can be overcome. Antioxidant activity can protect the body from damage caused by Reactive Oxigen Species, thus inhibiting degenerative diseases such as diabetes mellitus. Antioxidant activity is able to capture free radicals that cause damage to pancreatic β cells, so that pancreatic β cells still function (Nangoy *et al.,* 2019). Tannin compounds have a mechanism of lowering blood glucose levels by capturing free radicals and reducing increased oxidative stress in diabetics so as to control blood glucose levels and alocaloid compounds have imidazole rings that can stimulate insulin secretion, reduce blood glucose levels and strengthen glucose tolerance (Kasmawati *et al.,* 2020).

In this study, infusion of buas-buas leaves at a concentration of 20% was most effective in reducing blood glucose levels compared to concentrations of 10% and 40%. In previous studies on the decrease in blood glucose infusa extract concentration of 20% experienced a very high decline compared to 40% concentration, this is due to the presence of substances or active compounds that affect the decrease in blood glucose levels (Amir *et al.,* 2019). Giving increasing doses of drugs should provide effects that are proportional to the increased dose. However, with increasing doses, the increase in effect will eventually decrease. This is because a dose has been reached that can no longer increase the expected effect. This often occurs in natural or traditional medicines because the compounds contained in them are not single compounds but consist of various chemical compounds, where these components work together to cause effects. However, with an increase in dose, the number of chemical compounds contained is increasing, resulting in adverse interactions that cause a decrease in effect (Nangoy *et al.,* 2019). The number of receptors can also cause the effect obtained to be limited, because the effect provided does not increase and decrease. High concentrations do not always give greater effects than moderate concentrations. This can occur due to genetic factors and the physiological response of different test animals in responding. Another factor that can be suspected to be the cause of not optimal blood glucose reduction in mice is the effectiveness of plant extract absorption by the mice body. This can occur due to two things, the presence of a substance that inhibits glucose absorption or decrease metabolism in the diaphragm (Hikmah *et al.,* 2016). The case of the highest dose giving a smaller effect than the medium dose often occurs in testing a new drug candidate, a dose optimizer occurs which means that the maximum effect occurs at a certain dose.

4. Conclusion

Based on the results of the research that has done,it can be concluded that infusion of buas-buas leaves (*Premna serratifolia* Linn.). can decrease blood glucose levels of white male mice induced by alloxan. Infusion of buas-buas leaves (*Premna serratifolia* Linn.) concentration of 20% is the most effective dose to decrease blood glucose levels.

Conflict of Interest

All Authors declare no conflict of interest and agree with the content of the manuscript.

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