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Effectiveness Test of Red Betel Leaves (*Piper crocatum*) on The Reduction of Blood Sugar Levels of Male White Mice (*Mus musculus*) Induced By Alloxan

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Abstract

Background: Hyperglycemia is when blood sugar levels increase excessively from normal blood sugar levels. High blood sugar levels in the body that exceed normal limits occur when random blood sugar test values are ≥200 mg/dl and fasting blood sugar is ≥126 mg/dl. One of the natural ingredients known to lower blood sugar levels is red betel leaves (*Piper crocatum*). This study aimed to determine the effectiveness of red betel leaves (*Piper crocatum*) in reducing blood sugar levels. **Methods:** The methods used in this study were extraction and fractionation of n-hexane, ethyl acetate fraction, n-butanol fraction, and residual water fraction. **Results:** The results of the research that has been carried out show that administering fractionated n-butanol is effective in reducing blood sugar levels in male white mice (*Mus musculus*) that have been induced by alloxan. **Conclusions:** The results of the blood sugar level test that had been carried out and tested obtained a positive group, and the results of administering the n-butanol fraction at a dose of 100 mg/KgBW had an effect on reducing the blood sugar levels of male mice (*Mus musculus*) that had been induced by alloxan on days 2, 4, and 6, which experienced the greatest decrease.

Keywords:
Alloxan; Blood sugar levels; ethyl acetate fraction; n-butanol fraction; n-hexane fraction,

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Introduction

Epidemiological research conducted in Indonesia shows that there has been an increase in high blood glucose problems from year to year by 13% of the population diagnosed with hyperglycemia compared to 2013 (Idris *et al.*, 2017). Hyperglycemia can cause damage to blood vessels and the nervous system, causing heart disease, stroke, and kidney failure (*Bohari et al.*, 2021; Chen *et al.*, 2018). High blood sugar levels in the body that exceed normal limits or hyperglycemia occur when random blood sugar test values are ≥200 mg/dl and fasting blood sugar is ≥126 mg/dl (Alza, 2013). Meanwhile, hypoglycemia is a condition of reduced glucose focus, characterized by the absence or presence of side effects of the autonomic system and neuroglycopenia. Hypoglycemic blood glucose levels are <70 mg/dl, with or considering side effects, such as decreased blood glucose levels or side effects that decrease due to treatment (Rusdi, 2020).

Factors that can affect a person's glucose levels can occur due to the active work done by the individual. In the long term, when glucose levels are not controlled, it can cause discomfort (Herwanto & Rumampuk, 2016). Many plants have properties and are believed to be drugs that lower glucose levels. One of the plants that can lower glucose levels is red betel

leaves. Red betel leaves can be found in the regions of Sumatra and Kalimantan (Astuti & Munawaroh, 2011).

Red betel leaves have many benefits, one of which is that people believe they can lower blood sugar levels or as an antihyperglycemic (Kohar & Kartini, 2016). Based on the results of a study conducted by Listiana (2019) regarding the effectiveness of boiled red betel leaf water on lowering blood sugar levels in diabetic patients it states that there is a difference in blood sugar levels before and after giving boiled red betel leaf water. This study focused on reducing blood sugar levels in male white mice (*Mus musculus*) induced by alloxan using red betel leaf samples (*Piper crocatum*) and determining the type of fraction that can reduce blood sugar levels.

Methods

This research was conducted at the Pharmacology Laboratory, STIKES Harapan Ibu Jambi Research Laboratory, and the Jambi Provincial Health Laboratory Center, which was carried out in March-June 2023. This study used an experimental method with a randomized block design using white mice as research objects. As many as 72 experimental animals were divided into eight groups in this study, which carried out tracing/survey of the activity of red betel leaves (*Piper crocatum*). The tools in this study were Rotary evaporator (IKA RV 10), centrifuge (EBA 200), clinical photometer (autolyze), analytical balance (Fujitsu), separating funnel (pyrex), microtube (oxygen), multi check (Nesco), micropipette (dragon lab), styrofoam, test tube rack, test tube (pyrex), evaporator cup, aluminum foil, surgical scissors, scalpel (allee), surgical tweezers, syringe (one med), oral sonde, alcohol swab, pin. The materials in this study were test animals using 72 male mice, alloxan, glucose, ethanol, N-hexane, ethyl acetate, N-butanol, Aquadest, and alcohol swabs. The method of work in this study was Determination; samples taken from Merangin district, maceration, extraction, and fractions were then tested for phytochemicals (alkaloid, flavonoid, saponin, tannin, quinine, yesterday, triterpenoid, steroid, animal treatment, and blood sugar level analysis. The data was calculated for the %RBO value (organ weight ratio) to determine the effect of the dose that had been given and analyzed statistically using the SPSS nonparametric Kruskal Wallis test.

Result

This study used 72 male mice weighing 20-30 grams and aged 2-3 months (Sinata & Arifin, 2016). Mice were divided into eight groups (group 1 negative, group 2 positive, group 3 given metformin drug comparison, group 4 given red betel leaf extract sample, group 5 given n-hexane fractionation, group 6 given ethyl acetate, group 7 given n-butanol fraction, and group 8 given residual water fraction). Before the test animals were used as experimental animals, the animals were acclimated for 7 days or one week to adapt to the new environment and were still given food and drink. Acclimation was carried out so that the animals did not experience stress when used for the experiment. After acclimation was completed for 7 days, glucose levels were tested by cutting the mouse's tail and pressing it slightly so blood came out. Then, the blood was put into a strip to determine the blood sugar levels in the mice before being induced by alloxan, and the blood sugar levels were measured using multi-check/Nesco. Before induction, the test animals were fasted for 18 hours but were still given plain water. After the blood sugar levels were significantly different from the usual sugar levels, Alloxan induction was given 150 mg/kg BW twice intraperitoneally, and 20% b/v glucose solution was also given to the test animals to help increase sugar levels in the test animals and blood sugar levels in the mice did not drop drastically. In this study, the increase in blood sugar in male mice occurred 3 days after alloxan induction. After the blood sugar levels that had been induced by alloxan were different from the blood sugar levels before alloxan induction in the male mice on day 1 and day 3, group 3 was given a comparison drug, metformin, group 4 was given a sample of red betel leaf extract, group 5 was given n-hexane fractionation, group 6 was given ethyl acetate, group 7 was given n-butanol fraction, and group 8 was given the remaining water fraction, each given a dose of 100 mg/KgBW. Day 2, 4, and 6 of male mice

were taken by blood dislocation/taken from the neck vein. The blood obtained was ±1 ml, then put into a microtube and centrifuged for 20 minutes at a speed of 3000 rpm. Then, blood sugar levels were analyzed using a clinical photometer instrument with the help of a GOP-PAP kit (Subiyono *et al.*, 2016; Triandita *et al.*, 2016).

The results of the study of red betel leaf extract (*Piper crocatum*) as a reducer of blood glucose levels in male white mice (Mus mucus) can be seen in Graph 1 below.

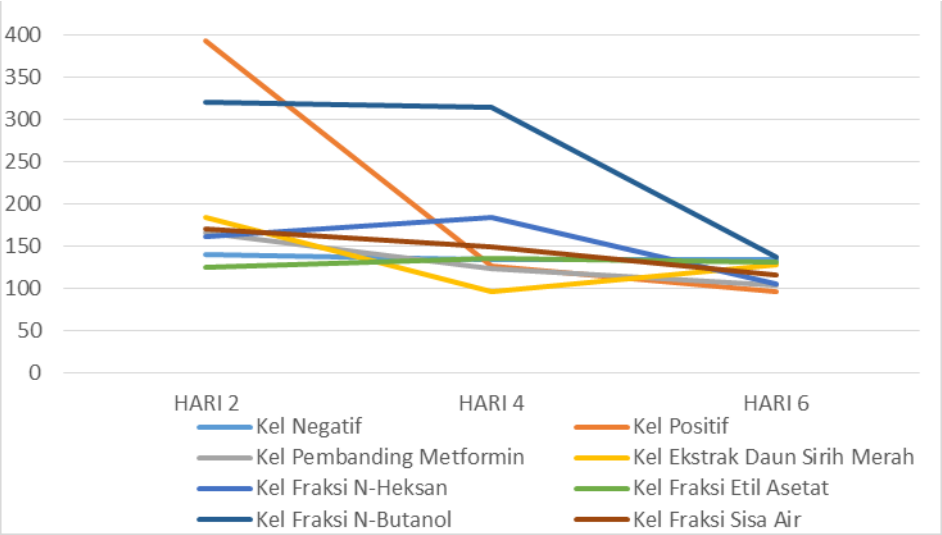

Figure 1. Blood sugar levels of male white rats on days 2, 4 and 6

The graph above explains the group that showed the most decrease in blood sugar levels, namely the positive group and the n-butanol fraction group, which means that this group can reduce blood sugar levels on days 2, 4, and 6. In the graph of blood sugar levels, the comparison group metformin and the remaining water fraction can reduce blood sugar levels in mice. Still, the most influential group showed decreased blood sugar levels on days 2, 4, and 6, namely the positive and n-butanol fraction groups containing saponins and tannins (Setyawati *et al.*, 2023). Red betel leaves (*Piper crocatum* Ruiz & Pav.) contain phytochemical compounds. These compounds include alkaloids, flavonoids, carvacrol, eugenol, saponins, and tannins. Alkaloid and flavonoid compounds have hypoglycemic activity or reduce blood glucose levels (Mardiana, 2012) Saponins have an antioxidant effect that will protect β cells and reduce the amount of insulin degranulation slightly from before (Yin *et al.*, 2004). Saponins significantly improve clinical symptoms of diabetes, including high blood glucose levels, and resemble the mechanism of action of α-glucosidase enzyme inhibitors (Deng *et al.*, 2012). Tannins are known to inhibit the loss of glucose transport that produces insulin. Tannins are also thought to induce phosphorylation of insulin receptors by forming glucose transporter 4 (GLUT-4) (Liu *et al.*, 2005).

Alloxan induction in the peritoneum of experimental animals can cause selective damage to pancreatic β cells. Alloxan is a causative agent of diabetes mellitus. In vitro, alloxan causes pancreatic β cell necrosis by stimulating intracellular H2O2. Alloxan causes permanent hyperglycemia within 2-3 days. Alloxan also disrupts cell homeostasis, which is the beginning of cell death due to the disruption of the cell oxidation process. Increased calcium ion concentration accelerates damage to pancreatic ß cells. When β cells are damaged by alloxan, insulin secretion is disrupted, reducing insulin levels. Decreased insulin secretion has resulted in the body's inability to use glucose as an energy source (Dewi et al., 2014).

Conclusions

The blood sugar level test results that had been carried out and tested obtained a positive group. The results of administering the n-butanol fraction at a dose of 100 mg/KgBW had an effect on reducing the blood sugar levels of male mice (*Mus musculus*) that had been induced by alloxan on days 2, 4 and 6, which experienced the most significant decrease.

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Declaration statement

The authors reported no potential conflict of interest.

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