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

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Abstract

Background: Hyperglycemia is when blood sugar levels increase excessively from normal sugar levels. High blood sugar levels in the body that exceed normal limits occur when the random blood sugar test value is ≥200 mg/dl and fasting blood sugar is ≥126 mg/dl. One natural ingredient known to lower blood sugar levels is red betel leaf (*Piper crocatum*). **Methods:** The method used in this research is the extraction and fractionation of n-hexane, ethyl acetate fraction, n-butanol fraction, and residual water fraction. **Results:** The results of research that has been carried out by giving fractionated n-butanol is effective in reducing blood sugar levels in male white mice (*Mus musculus*) that have been induced by alloxan. **Conclusions:** The positive group was obtained, and the results of administering the n-butanol fraction at a dose of 100 mg/KgBW affected reducing the blood sugar levels of male mice (*Mus musculus*) which had been induced by alloxan on days 2, 4, and 6, which decreased the most.

Keywords: Alloxan; Blood Sugar Levels; ethyl Acetate Fraction; N-butanol Fraction; N-hexane Fraction; Residual Water Fraction

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Introduction

Research related to epidemiology conducted in Indonesia shows an increase in the problem of high blood glucose from year to year, with as much as 13% of the population diagnosed with hyperglycemia compared to 2013 (Idris et al., 2017). Hyperglycemia can cause damage to the blood vessels and 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 the random blood sugar test value is ≥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 autonomic system side effects and neuroglycopenia. The rate of decrease in hypoglycemia blood glucose levels is <70 mg/dl, with or considering side effects, such as decreased blood glucose levels or side effects due to treatment (Rusdi, 2020). Factors influencing a person's glucose levels can occur due to the individual's active work. Over an extended period, when glucose levels are uncontrolled, they can cause discomfort (Herwanto & Rumampuk, 2016). Many plants have properties and are believed to be medicines for lowering glucose levels. One plant that can reduce glucose levels is red betel leaves. Red betel leaves can be found in Sumatra and Kalimantan (Astuti & Munawaroh, 2011).

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

Methods

This research was carried out at the Pharmacology Laboratory, STIKES Harapan Ibu Jambi research laboratory, and the Jambi Provincial Health Laboratory Center, which was carried out from March to June 2023. This study used an experimental method with a randomized block design using white mice as research objects, totaling 72 experimental animals divided into eight groups. In this study, searches were conducted, and surveys were conducted to determine the activity of red betel leaves (*Piper crocatum*). The tools in this research 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), evaporating cup, aluminum foil, surgical scissors, scalpel (allee), surgical forceps, squid (one med), oral probe, alcohol swab, pin. The materials in this research were test animals using 72 male mice, alloxan, glucose, ethanol, N-hexane, ethyl acetate, N-butanol, Aquadest, and alcohol swabs. The way this research works is determination. Samples are taken from Merangin district, maceration, extraction, and fractionation. Then, phytochemical testing (alkaloids, flavonoids, saponins, tannins, yesterday, triterpenoids, steroids, animal treatment, and blood sugar level analysis) will be performed. The data was calculated using the %RBO value (organ weight ratio) to determine the effect of the dose given and analyzed statistically using the SPSS non-parametric Kruskal Wallis test.

Discussion

This research used red betel leaves (*Piper crocatum*) taken in Merangin Regency, Bangko City, Bangko District, Pasar Atas Bangko Village, Jambi Province. After that, a determination was carried out at the Plant Systematics Laboratory, Gadjah Mada University number 0326/S.Tb./V/2023, the results of the determination of red betel leaves (*Piper crocatum*), namely the species *Piper crocatum* Ruiz & Pav. A total of 5 kg of dried leaves were then chopped. After drying, the samples were chopped for maceration (Hermes et al., 2021). A sample of dry red betel leaves obtained 1 kg after chopping obtained 900 grams. After that, extraction is carried out using the maceration method using 70% ethanol (1:10 w/v). After the extract is filtered, the extract and solvent are separated using a rotary evaporator at 500C to obtain a thick extract (Dechayont et al., 2021; Khan & Islam, 2012). The resulting extract was then separated between the extract and the solvent using a water bath at a temperature of 800C. After obtaining a thick extract, fractionation was carried out from n-hexane, ethyl acetate, n-butanol, and the remaining water.

Phytochemical screening was carried out to determine the secondary metabolite content found in red betel leaves (*Piper crocatum*). The results obtained in Table 1 of red betel leaves are positive for containing alkaloids, flavonoids, saponins, coumarins, triterpenoids, and steroids. This research does not align with research (Rezeki et al., 2017) because red betel leaves contained negative triterpenoids in this study. Meanwhile, research has been conducted on red betel leaves containing positive triterpenoids.

In this study, the animals used were 72 male mice weighing 20-30 grams and 2-3 months old (Sinata & Arifin, 2016). Mice were grouped into eight groups (group 1 was negative, group 2 was positive, group 3 was given the comparison drug metformin, group 4 was given samples 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). Before the test animals are used as experimental animals, the animals are acclimated for seven days or one week to adapt to the new environment and are still given food and drink. Acclimation is done so the animals used for experiments do not experience stress. After acclimation was completed for seven days, the glucose levels were tested first by cutting the tails of the mice and pressing slightly so that blood would come out. Then, the blood was put into a strip to determine the blood sugar levels in the mice before alloxan induction, and the blood sugar levels were measured using Multicheck/Nesco. Before being induced, the test animals were fasted for 18 hours but still given plain water to drink.

After the blood sugar level was significantly different from the usual sugar level, alloxan induction was given at 150 mg/kg BW twice intraperitoneally, and a 20% w/v glucose solution was also given to the test animals to help increase the blood sugar levels in the test animals and blood sugar levels. In mice, it did not decrease drastically. In this study, the increase in blood sugar in male mice occurred three days after being induced by alloxan. After the blood sugar levels that had been caused by alloxan were different from the sugar levels before being induced by alloxan in the blood of male mice on day one and day 3, group 3 was given the comparison drug metformin, group 4 was given samples of red betel leaf extract, group 5 was given fractionated n-hexane, group 6 was given ethyl acetate, group 7 was given the n-butanol fraction, and group 8 was given the remaining water fraction, each of which was given a dose of 100 mg/KgBW. On the 2nd, 4th, and 6th days of male mice, blood was taken by dislocation/taking it from the neck vein. The blood obtained ± 1 ml was 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 the GOP-PAP kit (Subiyono et al., 2016; Triandita et al., 2016).

The results of glucose levels were obtained using the SPSS test in Table 3. The cheerful collection and the collection given the distribution of n-butanol obtained a value ($p<0.05$) that was not too big, meaning there was a difference. Meanwhile, the other groups were considered critical ($p>0.05$), meaning no difference existed. Since then, the Kruskal-Wallis test has been performed as a non-parametric test. The results of the Kruskall-Wallis test on day 4 showed no difference in glucose levels between all groups with a p-value >0.05, meaning it was huge. There were no differences on days 2, 4, and 4. 6. In the quality diagram of glucose levels in Table 5, the correlation collection of metformin and excess water can reduce the glucose levels of mice. Still, the most persuasive collection shows a natural decrease in glucose levels on days 2, 4, and 6, specifically in the group positive and cleavage of n-butanol-containing saponins and tannins (Setyawati et al., 2023). Saponin has an antioxidant effect that will protect β cells and reduce the amount of insulin degranulation slightly compared to before (Yin et al., 2004). Saponins significantly improve clinical symptoms of diabetes, including high blood glucose levels, and mimic the mechanism of action of α-glucosidase enzyme inhibitors (Deng et al., 2012). Tannins are known to inhibit glucose transport loss, producing insulin. Tannins are also thought to induce phosphorylation of the insulin receptor by forming glucose transporter 4 (GLUT-4) (Liu et al., 2005).

In Table 7, the organ weight ratio results show that the organs that experience differences are the liver, pancreas, lungs, and stomach because the p-value is <0.05, meaning it is insignificant and there is a difference. Next, a non-parametric test was carried out, namely the Kruskal-Wallis test for all organs. There were no differences with a p-value >0.05, which means it was significant. This means that the mouse organs (pancreas, kidneys, liver, heart, stomach, and lungs) did not affect the organs after being given each

treatment to each group. In the Kruskal-Wallis test on the results of all organs, there was a difference in the p-value <0.05, which means it was insignificant.

Conclusions

The results of the blood sugar level test that had been carried out and tested were positive for the 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 experienced the most significant decline.

Declaration statement

The authors reported no potential conflict of interest.

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