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Original Article

Antidiabetic effect of *Psychotria malayana* Jack in induced type 1 diabetic rat

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A B S T R A C T

Background: Therapies for hyperglycemic treatment, including insulin and oral diabetes medications, have been confirmed to cause several side effects. Thus, finding new drugs with fewer side effects is of high importance. Salung leaf herb (*Psychotria malayana* Jack) reported used in traditional societies as a treatment for diabetes. However, the scientific proof of this plant for diabetes treatment is still lacking.

Objective: To evaluate the antidiabetic effect of the *P. malayana* jack in induced type 1 diabetic rats by assessing blood glucose level and pancreatic cells in white rats.

Methods: Alloxan used to induce type I diabetes. Rats randomly divided into six groups. A Group P1 received 250 mg/kg BW; group P2 received 500 mg/kg BW, group P3 received 1000 mg/kg BW. While group 4 basal received no treatment, group 5 received distilled water as a negative control, and group 6 received glibenclamide as a positive control. Medications are given for six days. Glucose levels were measured, and observation of pancreatic Langerhans cell damages.

Results: A decrease in blood glucose levels observed in all treatment groups. The most significant reduction (49.76%; 1000 mg/kg BW) occurred in the P3 group. Morphological features of pancreatic Langerhans cell damage were slightly high in the P1 group.

Conclusion: *P. malayana* Jack can consider having an antidiabetic effect in a type 1 diabetic rat by reducing blood glucose levels.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder that features hyperglycemia. Hyperglycemia in diabetes results from defects in insulin secretion, insulin action, or, most commonly, both. According to the International Diabetes Federation, in 2012, the national prevalence of diabetes in Indonesia was 4.8%, compared to 5.1% internationally [1-3]. Diabetes mellitus can cause the impaired quality of life due to its various complications. The therapeutic approach to diabetes, in general, is to restore healthy blood glucose metabolism and prevent complications [1,4]. The available methods for treating diabetes, including oral diabetes medications and insulin injections, have been found to cause several side effects [5,6].

Indonesia is a country that has a vast biodiversity. Of the 26% that have cultivated, 940 types of plants have used as traditional medicine. One of the traditional medicinal plants derived from the family Rubiaceae is *Psychotria malayana* [7]. This plant grows to a height of 1–4 m and mostly distributed in the west Indonesian archipelago [8]. *P. malayana* jack spread in the area of flora malesiana consisting of regions of Malaysia, Indonesia, the Philippines, and Papua New Guinea. Sumatra is the closest area of kinship to the flora of the field of Malaysia compared to the vegetation found on the island of Java. In Sumatra, this type of plant found in the provinces of Jambi, Riau, and South Sumatra.

The family Rubiaceae has long known as a source of traditional Indonesian medicinal plants. Several types have used as conventional medicine. They thought to have an

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activity as an antidote to free radicals, reactive oxygen species, and superoxide, namely natural, free radicals that have one unpaired electron [9]. Because of its effects in treating diseases, *Psychotria* herb species are used by traditional people to treat diabetes.

In several studies reported the presence of anti-inflammatory, antioxidant, antimicrobial, and anti-inflammatory properties on the leaves of *Psychotria*, which used to treat infectious diseases in the female reproductive system, abdominal pain or infections in other digestive systems [10-13]. However, the scientific proof of this plant in diabetes treatment is still unknown. This study aimed to evaluate the antidiabetic activity of the plant extract in the induced type 1 diabetic rat.

METHOD

Study Design

This is an experimental animal study with the randomized post-test control only group design.

Study Site

All the studies carried out in the Biomedical Laboratory, Faculty of Medicine and Health Science, Jambi University.

Plant Material and Extraction

The fresh leaves of the plant obtained from the Sarolangun District in Jambi, Indonesia. The leaves were cleaned with water and then dried at room temperature (28+ 1^{o}C) for 12 days. The dried leaves were ground and stored at 50–60°C for 24 hours before further treatment into powdered form. Then, 4000 ml Ethanol 70% used as a solvent for extraction. The mixture was kept in the dark bottle at room temperature for three days and shaken occasionally. The filtrate filtered with Whatman no.1 filter paper. Then, the filtrate was processed in a rotary evaporator at 60°C for about 8 hours per day for seven days to get a thick extract [14].

Animals Preparation

Animals were maintained consistently with the approved animal care operating procedures. Male Sprague Dawley rats (150–300 g) were used in this study and acclimated to the laboratory facility for seven days.

Experimental Procedure

Diabetes induced using alloxan purchased from Sigma-Aldrich, USA. Alloxan is to produce experimental diabetes in various animal models and can be used intravenously, intraperitoneally, and subcutaneously. At doses below 140 mg/kg BB, it induces diabetes and lasts about one week. At a dose of 160 mg/kg BB, it can cause a stable diabetes condition that can last for one month [15,16]. The diabetogenic characteristic of alloxan has reported in much research. The mechanism of alloxan in damaging pancreatic β is oxidative stress, which generates (ROS) [17,18]. The rats fasted overnight and were injected intraperitoneally with 140mg/kg BW alloxan in 0.5 mL saline. The rats divided into six groups of four rats each:

Group 1 (P1), alloxan induced diabetic rats: extract of P. malayana (250mg/kg).
Group 2 (P2), alloxan induced diabetic rats: extract of P. malayana (500 mg/kg).
Group 3 (P3), alloxan induced diabetic rats: extract of P. malayana (1000 mg/kg).
Group 4 (Basal control): non-alloxan induced rats
Group 5 (negative control), alloxan induced diabetic rats: normal saline
Group 6 (positive control), alloxan induced diabetic rats: standard drug (glibenclamide 10 mg/kg)

The variable, Instrument, and Measurement

Rats' blood glucose levels were measured enzymatically using a glucometer. A 10 µL blood sample taken from the rat femoral vein. The rat will fast for 8 hours before the blood is drawn. Blood glucose levels measured on the first day before alloxan induction given, then checked on the third day after alloxan induction and the seventh day after treatment.

Histological Analysis

After sacrificing the animals by cervical dislocation, the pancreas was removed and immediately placed in 10% formaldehyde. The pancreas tissue embedded in paraffin sectioned 5 µm sections with a microtome for histological studies. Sections stained with hematoxylin-eosin stain. The slides were kept under room temperature (28 + 1^{o}C) for at least 24 hours before the observation of pancreatic Langerhans cell damage under a light microscope, observed at 40x19 [19].

Statistical Analysis

The experimental results expressed as mean ± standard error of mean and data assessed using the One-way ANOVA test [20].

Ethical Consideration

The Ethics Committee approved the study design of the Medicine and Health Sciences Faculty of Jambi University (No.B/275/UN21.8/PT.01.04/2019).

RESULTS

Blood glucose data included the average amount of blood glucose in each group before being induced by alloxan (pre-test), three days after alloxan induction, and at the end of the study (post-test). Figure 1 shows a significant difference in blood glucose levels between pre-test blood glucose levels and three days after alloxan induction in the negative control, positive control, P1, P2, and P3 groups.

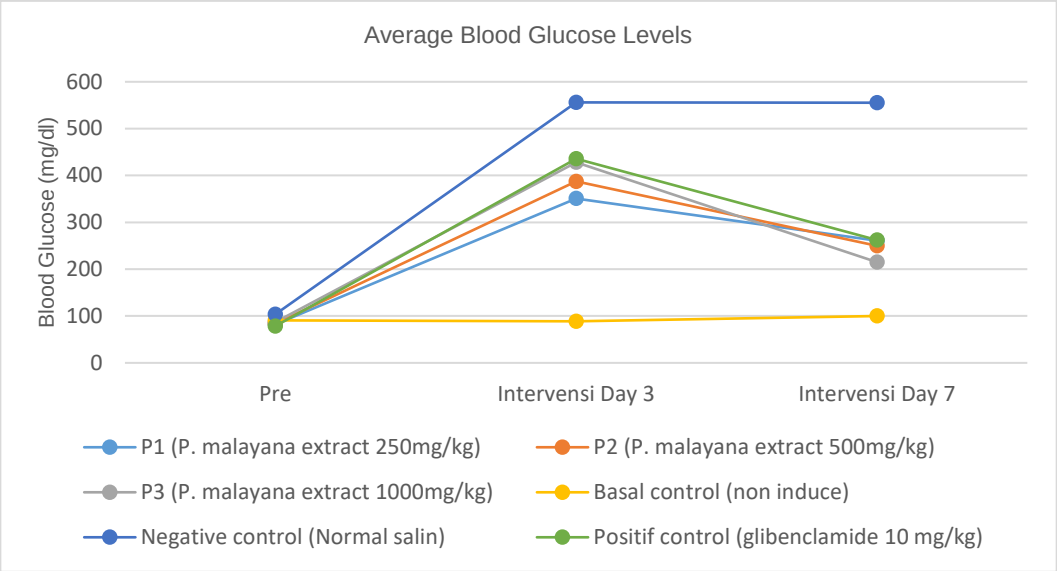

Figure 1. Average blood glucose levels from all test groups

A pre-test was done to determine the initial glucose value and see the increase after alloxan induction. While there was no difference between blood glucose levels before alloxan induction and three days after alloxan induction in the basal control group. There was a decrease in blood glucose levels between three days after alloxan induction and seven days after treatment in the positive control, P2, and P3 groups. While the basal control, negative control, and P1 groups showed no differences between blood glucose levels three days after alloxan induction and seven days after treatment based on the percentage (%) of decreased blood glucose levels of each treatment group compared to the negative group. There was the most significant percentage reduction in blood glucose levels after treatment for seven days in the P3 group (1000 mg /kg BW).

Table 1. One-way ANOVA test results

Glucose	Sum of Squares	Df	Mean Square	F	p-value
Between Groups	0.050	17	0.003	38.063	0.001
Within Groups	0.004	54	0.001		
Total	0.054	71			

Based on the One-way ANOVA test (Table 1). it found that blood glucose levels between each treatment obtained p-value = 0.001; it concluded that there are differences in blood glucose levels between treatment groups. This means that there is an effect of *P. malayana* leaf extract on the reduction of blood glucose levels in white rats (Rattus norvegicus) male Sprague Dawley strain. A further post hoc analysis conducted to show that there were significant differences in group P3.

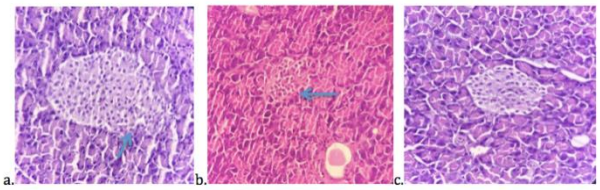

Figure 2. Paraffin section of the rats' pancreas using hematoxylin-eosin staining for (a) the P3 group, (b) the negative control group, and (c) the basal control group (all at 40x magnification).

Analysis of pancreatic islets performed using routine HE staining. The pancreatic Langerhans cell in the basal control group showed a healthy structure (Figure 2c). However, both diabetic and treated rats showed endocrine islet damage (Figures 2a,b). Histopathologically, there are still parts of pancreatic Langerhans cells that are similar to pancreatic Langerhans cells in the negative control group and show a decrease in blood sugar levels even accompanied by Langerhans cell damage.

DISCUSSION

P. malayana leaves contain various active chemicals. The results of the phytochemical analysis indicate of cyclitols, phenolics, sugar acids, free fatty acids, phytosterols, alkaloids, and alcohols. The alkaloid components in these leaves are (-) - Chimonanthus, (+) Chimonanthus, Meso-Chimonanthus, Calychanthine, hodgkinsine, 2-ethyl-6-Mrthilpyrazine, and 3-Methil-1,2,3,4-tetrahydro-gamma-carboline. *P. malayana* has a hodgkinsine level of 52.245%. Alkaloids and flavonoids have reported many pharmacological effects, one of which is antidiabetic. Flavonoids, which are polyphenol compounds, also have antioxidant properties through the reduction of free radicals [8,21].

Various doses of *P. malayana* extract applied in this study. The extract was able to lower the blood glucose level of alloxan-induced type 1 diabetic rats, although not to normal levels (compared with the basal group). In this study, we found that the most significant decrease in blood glucose occurred in the treatment group P3, with extract concentrations of 1000 mg/kg BW, which was a decrease of 49.76%. Research on *P. malayana* extracts found significant results at doses of 1, 2, 3 g/kg in reducing blood glucose levels in diabetic zebrafish models. The study showed that plant extracts 3 g/kg was more effective than other doses of fingerprints based on liquid spectrometry-chromatography [22].

Histopathological examination in this study conducted to evaluate the effects of alloxan in diabetic rats compared with healthy mice. Figure 2 shows that the cells in the pancreatic Langerhans islets from mice injected with alloxan showed a decrease in endocrine islets cellularity [23]. This suggests that alloxan injection affects the rats' pancreatic structure following previous findings [24]. The possible mechanism of the antidiabetic effect of P. malayana extract is due to the increased insulin production in the ß cells, increased peripheral glucose uptake in adipose and muscle tissue, decreased glucose in the liver, and reduced intestinal glucose absorption [22,25]. The first mechanism was not suited in the present study, as this plant extract cannot repair damaged islets of Langerhans, which has been proven by the histological examination (Figure 2).

The result of this study is the following study of the effect of Petroselinum crispum extract in diabetic-induced rats, which reported that P. crispum extract was able to reduce blood glucose levels to normal but did not show effects on pancreatic Langerhans cell [26]. This result corresponds to Benchoula et al., who confirmed the antidiabetic activity of *P. malayana* in diabetic zebrafish [10]. The authors said the mechanism of action that the plant's to decrease blood glucose levels was not through the repair of pancreatic cells, but through stimulating glycolysis and gluconeogenesis inhibition.

CONCLUSIONS AND RECOMMENDATION

Salung leaves (*Psychotria malayana* Jack) extract have an antidiabetic effect by reducing blood glucose levels in a diabetic rat model. The mechanism of this plant in lowering blood glucose levels needs to investigate further.

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