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Treatment of Songgo Langit Extract (*Tridax procumbens*) Against Blood Glucose Levels and Pancreatic Histopathology of Hyperglycemic Mice

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Abstract

Hyperglycemia is an early symptom of diabetes caused by the pancreas not being able to produce enough insulin. This condition can disrupt blood glucose metabolism, thereby damaging organs in the body such as the pancreas. Hyperglycemia can be treated using traditional plant medicines. Songgo Langit leaves (*Tridax procumbens* L.) are reported to have secondary metabolite compounds that have potential as antidiabetic drugs. This study aims to determine the effect of administering Songgo Langit leaf extract on blood glucose levels and changes in the histopathological structure of the pancreas in hyperglycemic mice induced by alloxan. Using Complete Random Design, mice were divided into 5 groups of 5 mice each. Group K (-) was a negative control (not treated), group K (+) was a positive control (only induced by alloxan), group P1 was induced by alloxan and extract of 0.45 mg/g BW, group P2 was induced by alloxan and extract 0.9 mg /g BW, and group P3 was induced by alloxan and extract 1.8 mg/g BW for 14 days. Glucose level data were analyzed using ANOVA and post hoc test with LSD. Pancreatic histopathological structure data were analyzed using the Kruskal-Wallis test and the Mann-Whitney post hoc test. The results showed that the treatment of ethanol extract of Songgo Langit leaf at doses P1, P2, and P3 were significantly able to reduce blood glucose levels by 61.70%, 65.11%, and 74.79% and was able to repair damage to pancreatic histopathology of mice induced by alloxan.

Keywords: alloxan, hyperglycemia, pancreas, *Tridax procumbens* L.

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INTRODUCTION

Hyperglycemia increases blood glucose levels beyond normal limits [1]. Hyperglycemia can occur because the pancreas cannot produce the hormone insulin or the body cannot use the hormone insulin properly [2]. Disorders of carbohydrate, lipid, and protein metabolism caused by damage to the pancreas in secreting the hormone insulin in the body

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will stimulate hyperglycemia. Hyperglycemia is one of the first symptoms of a person experiencing a metabolic disorder, like diabetes [1].

The way to treat diabetes in Indonesia is through pharmacological therapy, such as oral and injectable drug treatment. Oral and injectable drugs commonly given to people with diabetes mellitus are glibenclamide and insulin. However, the treatment of chemical drugs can cause side effects such as diarrhea, vomiting, hypoglycemia, weight gain, and urinary tract infections [1]. In addition, the continuous use of chemical drugs can trigger organ damage. The use of traditional medicine, especially herbs, in treating Diabetes is safer and has fewer side effects when compared to modern chemical drugs [3].

One of the plants that has potential as a diabetes drug is the Songgo Langit plant (*Tridax procumbens* L.). Previous research on Songgolangit extract showed that this plant has antidiabetic activity and hypoglycemic effects on reducing blood glucose levels in alloxan-induced rats [4]. Traditionally, people in India use leaf powder and other oral herbs to treat Diabetes [5].

Research on the potential of Songgo Langit plants as a medicine in overcoming hyperglycemia conditions has yet to be widely studied in Indonesia. Information related to the function of this plant is still minimal, so it is necessary to conduct this study to determine the effect of giving ethanol extract of Songgo Langit leaves (*T. procumbens* L.) in reducing blood glucose levels and improving the histopathological structure of the pancreas of alloxan-induced hyperglycemia mice.

METHODS

Plant Sample and Extraction

The extract used in this study is the Songgolangit leaf extract. Leaf samples were taken from all the best leaves on the Songgolangit plant. The criteria for good Songgolangit leaves are green, fresh, not hollow, and moldy. The best Songgo Langit leaves were selected, washed with running water, dried for 24 hours, and oven to 40°C for 24 hours. The dried Songgo Langit leaves were then pulverized to a coarse powder and macerated using 96% ethanol solvent for 3 × 24 hours. The macerated Songgolangit leaves were filtered using a Buchner funnel until the filtrate was obtained and then concentrated using a rotary evaporator with a temperature of 50°C until a thick extract was obtained and then put into the oven until the extract was obtained in paste form.

Phytochemical testing aims to determine the secondary metabolite compounds contained in a plant extract. Phytochemical testing procedures are shown in Table 1 [6].

Table 1. The phytochemical testing procedure of Songgo Langit leaf extracts

Type of secondary metabolite assay	Treatment	Indicator
Saponin	0.5 ml sample + 5 ml distilled water, then shaken for 30 seconds	Forms froth or foam
Tannin	1 ml sample + 3 drops of 10% $FeCl_3$ solution	Formed bluish-black solution color
Terpenoid	0.5 ml sample + 0.5 ml glacial acetic acid + 0.5 ml H_2SO_4	Formed red or yellow colored samples
Alkaloid	0.5 ml sample + 5 drops chloroform + 5 drops Mayer reagent (1 g KI dissolved in 20 ml distilled water + 0.271 g $HgCl_2$ to dissolve)	Abrownish-white solution formed
Flavonoid	0.5 ml sample + 0.5 ml g Mg powder + 5 ml concentrated HCl (dropwise)	Red or yellow color solution and foam formed

Steroid	0.5 ml sample + 0.5 ml glacial acetic acid + 0.5 ml H_2SO_4	Formed blue or purple sample color change

This research was conducted from February 2022 to July 2022. Songgolangit (*Tridax procumbens* L.) leaf extract was prepared at the Organic Chemistry Laboratory, Department of Chemistry, University of Lampung. Maintenance of testing animals was carried out in the Experimental Animal Management Unit, Department of Biology, University of Lampung. Induction of alloxan diabetogenic compounds, oral treatment of Songgolangit leaf extract testing materials, blood sampling, and animal necropsy tests were performed at the Zoology Laboratory, Department of Biology, University of Lampung. Preparation of histopathology preparations of pancreatic organs and observations were made at the Laboratory of Anatomical Pathology and Histology, Faculty of Medicine, University of Lampung.

Testing Animals and Experimental Design

Testing animals used in this study were male mice with a body weight of ± 30-40 grams aged 3-4 months. By using a completely randomized design, 25 male mice were divided into 5 groups 5 mice individuals. The first group was coded (K-) as negative control, (K+) as positive control alloxan induced, P1 received extract of 0.45mg/g BW/day, P2 gave extract of 0.9 mg/g BW/day, and P3 treated with extract of 1.8 mg/g BW/day.

Before being treated, mice are acclimatized for seven days to adapt to environmental conditions. Alloxan used to make mice experience hyperglycemia is at a dose of 160 mg/kg bb. Chougale et al. [7] stated that alloxan-induced testing animals at 160 mg/kg BW could maintain rat blood glucose levels at 400 mg / dL for three months. According to Nurfitri et al. [8], inducing alloxan so that mice experience hyperglycemia begins with satisfying male mice for ± 6-8 hours. After that, mice are weighed using a digital scale to measure initial body weight. Blood glucose levels are measured using a glucometer by taking blood through the tail. The results of this measurement were used as the baseline blood glucose level.

Two hours later, after the tail wound had dried, the mice were induced with alloxan subcutaneously at the nape of the neck. The dose of alloxan used in this study was 8mg / 36g BW mice. Alloxan was dissolved using 0.3 ml aqua pro injection. After 24 hours of alloxan induction, mice were given 0.3 ml of 5% sugar solution for one day to prevent the hypoglycemia phase, which will be fatal and usually occurs 4-8 hours after alloxan induction [9]. The second blood glucose level measurement was done after six days of alloxan induction. Mice with blood glucose levels >200 will be used in this study.

Mice that have experienced hyperglycemia are given Songgolangit leaf extract for 14 days at 125 mg/kg bw, 250 mg/kg bw, and 500 mg/kg bw. This dosage refers to Petchi et al.'s [10] research that administering Songgolangit ethanol extract at a dose of 500 mg/kg bw in diabetic Wistar rats can reduce blood glucose levels. Research by Amagbegnon et al. [11] states that the treatment of Songgo Langit extract for 14 days shows antidiabetic potential, as shown by the results of lower blood glucose levels.

Blood Glucose Level Measurement

Measurement of blood glucose levels was conducted four times. Blood glucose levels are measured using a glucometer by taking blood through the tail. The result of this measurement was used as the initial blood glucose level. Two hours later, after the tail wound had dried, the mice were induced with alloxan subcutaneously at the nape of the neck. The dose of alloxan used in this study was 8 mg/ 36 g BW mice. The second blood glucose level measurement was conducted after six days of alloxan induction. Mice with blood glucose levels >200 will be used in this study. After positive hyperglycemia, mice were given treatment for 14 days. The third blood measurement is after seven days of extract treatment, and the fourth is after 14 days.

Necropsy or surgery was performed at the

end of the study. At the time of necropsy, the pancreas organs of mice were taken and washed with ringer's solution so that the blood attached to the organs disappeared. Then, put it into a sample bottle containing a fixative solution. Furthermore, pancreatic organ preparations were made at the Laboratory of Anatomical Pathology and Histology, Faculty of Medicine, University of Lampung.

Pancreatic Histopathology Structure Observation

The observation was carried out to determine differences in the picture of the pancreas structure in each treatment group—examination of pancreatic histopathology using Hematoxylin Eosin staining. Pancreatic histopathology preparations were observed under a microscope with a magnification of 400x and recorded microscopic changes in 5 fields of view. The observation results were scored for the degree of damage with the categories listed in Table 2 [12].

Table 2. Pancreas damage score

Pancreatic Damage Scoring	
Damage Criteria	Score
Normal, there is no change in the islet of Langerhans border, cell number, cell necrosis, and cell shape.	0
Islet of Langerhans boundaries are clear, cell number starts to decrease, cell necrosis is not yet visible, and cell shape is normal	1
Boundaries start to become indistinct, cell numbers decrease, cell necrosis and abnormal cell shape start to occur	2
Indistinct boundaries, reduced cell number, visible cell necrosis, and abnormal shape of many cells	3
Boundaries are very indistinct, cell numbers are much reduced, cells are almost entirely necrotized, and cell shapes are abnormal	4

Data Analysis

Data on blood glucose levels were analyzed using the ANOVA, preceded by normality and homogeneity tests using Kolmogorov-Smirnov (P>0.05), then the LSD test at a 5% absolute level. Normally distributed and homogeneous data were analyzed using one-way ANOVA to test the average difference (mean) of data between groups. The LSD test was conducted at an absolute level of 5% If P<0.05. The SPSS 25 program assisted statistical analysis. A significant value in this study is if the variable analyzed has P < 0.05.

Data from the observation of pancreatic histopathology were collected and analyzed with the Kruskal-Wallis test, followed by the Mann-Whitney test at an absolute level of 5%. The first analysis is the normality test using the Kolmogorov-Smirnov test to determine the normality of distributed data (P>0.05). A non-parametric statistical method, the Kruskal Wallis test, and the Mann-Whitney test, were conducted if the variants are neither normally distributed nor homogeneous. The significant value in this study is if the variable analyzed has P <0.05.

RESULTS AND DISCUSSION

Phytochemical of Ethanol Extract of Songgolangit Leaf

Based on the research on the treatment of ethanol extract of Songgolangit leaves (*Tridax procumbens* L.) to reduce blood glucose levels and histopathological structure of the pancreas of mice (*Mus musculus* L.) hyperglycemia-induced alloxan obtained the following results. The results of phytochemical testing are displayed in Table 3.

Table 3. Phytochemical Test Results of Songgolangit Leaf Extracts

Test type	Results
Saponin	+
Tannin	+
Terpenoid	-
Alkaloid	+
Flavonoid	+
Steroid	+

Note: (-) : No test compound present, (+) : Test compound present

Based on the result of phytochemical testing, the ethanol extract of Songgolangit leaves contains bioactive compounds such as saponins, tannins, alkaloids, flavonoids, and steroids. The primary compound of the extract used in this study is flavonoids. The individual or synergistic activity of flavonoids and other active phytocompounds influences the antihyperglycemic activity of Songgolangit ethanol extract. Flavonoids play a role in lowering blood sugar levels by regulating hypoglycemic activity and increasing the solubility of blood sugar so that it is easily excreted through the urine [13]. In addition, flavonoids inhibit oxidative damage to pancreatic cells. The hypoglycemic activity of flavonoids led to the inactivation of hydroxyl free radicals that attack pancreatic cells so that cells can secrete insulin better [14].

Blood Glucose Levels

The treatment of ethanol extract of Songgo Langit leaves for 14 days to the blood glucose levels of mice induced by alloxan obtained the mean KGD presented in Table 4.

Table 4. Mean blood glucose levels of mice in all treatment groups

Treatment group	Mean blood glucose level of mice (mg/dL) Mean±SD (day-)			
	0	6	14	21
K-	96,00 ± 15,18	108,80 ± 21,24^{b}	125,20 ± 7,19^{c}	124,80 ± 25,34^{bc}
K+	131,00 ± 16,03	322,60 ± 70,54^{a}	306,60 ± 56,14^{a}	276,80 ± 18,15^{a}
P1	108,20 ± 34,47	399,00 ± 110,14^{a}	305,40 ± 69,18^{a}	152,80 ± 36,65^{b}
P2	99,00 ± 7,14	356,60 ± 64,86^{a}	259,00 ± 28,53^{ab}	124,40 ± 12,97^{bc}
P3	111,40 ± 12,70	389,60 ± 81,62^{a}	201,80 ± 68,45^{b}	98,20 ± 22,59^{c}

Notes: Mean ± SD values followed by the same superscript in the same column indicate no significant difference between groups based on the LSD test α 5%

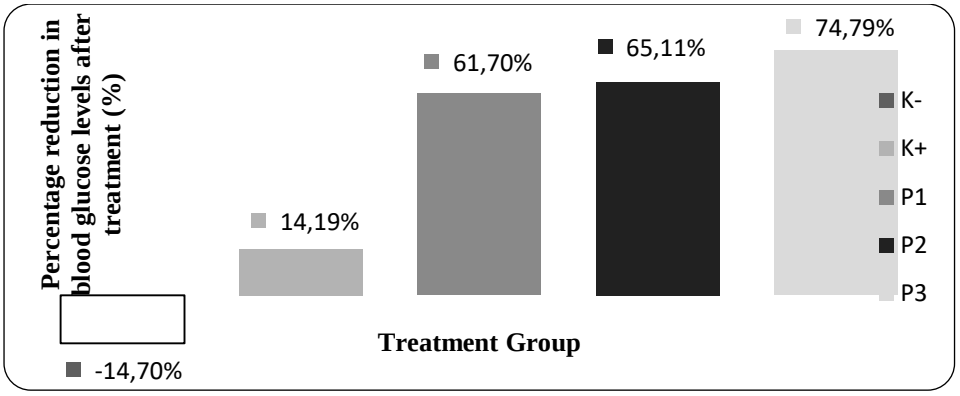

Figure 1. Percentage reduction in blood glucose levels after treatment.
Description :
K- : Not treated
K+ : Alloxan 160 mg/kgbw induced.
P1 : Induced Songgolangit leaf extract dose 0.45 mg/gb/day
P2 : Induced Songgolangit leaf extract dose 0.9 mg/gb/day
P3 : Induced Songgolangit leaf extract dose 1.8 mg/gb/day

In Table 4, mice's average blood glucose levels after alloxan induction by the dose of 160 mg/kg BW show a significant increase. A significant value in this study is if the variable analyzed has p <0.05. Based on the results of statistical analysis of one-way ANOVA on blood glucose levels of mice after treatment of ethanol extract of Songgolangit leaves for 14 days showed a significant difference (p<0.05) between treatment groups on day 6, day 14, and day 21 so that further LSD test was conducted. On day 1, each treatment group did not show a significant difference (normal). On the sixth day, the mice induced by alloxan subcutaneously experienced hyperglycemia. On days 14 and 21, or after being given Songgolangit leaf extract for 14 days orally, showed a gradual decrease in blood glucose levels.

Based on the results of the LSD further test after mice were induced by alloxan on day 6, it shows that the average value of blood glucose levels in K- mice is significantly different from the K+, P1, P2, and P3 groups. On day 14, the K- treatment group significantly differed from the K+, P1, P2, and P3 treatment groups, but there was no difference between the K+ and P3 groups. On the 21st day, the K+ treatment group significantly differed from K-, P1, P2, and P3, while the treatment groups P1, P2, and P3 showed no significant differences. This study shows that the mice induced by alloxan and given ethanol extract of Songgo Langit leaves for 14 days in each treatment group P1, P2, and P3 decreased blood glucose levels to reach normal (Figure 1). The average decrease in blood glucose level in group P1 (group induced alloxan and given extract 4.5 mg/gBW/day) amounted to 61.70%, group P2 (group induced alloxan and given extract 0.9 mg/gBW/day) amounted to 65.11%, and group P3 (group induced alloxan and given extract 1.8 mg/gBW/day) amounted to 74.79%. Group P3 showed the highest percentage reduction in blood glucose levels compared to groups P1 and P2. Secondary metabolite compounds influence the decrease in blood glucose levels in Songgo Langit leaf extract, which acts as antioxidants such as flavonoids, saponins, alkaloids, steroids, and tannins.

Histopathologic Structure of Mouse Pancreas

The treatment of ethanol extract of Songgolangit leaves for 14 days to the histopathological structure of the pancreas of mice induced by alloxan resulted in the damage score presented in Figure 2.

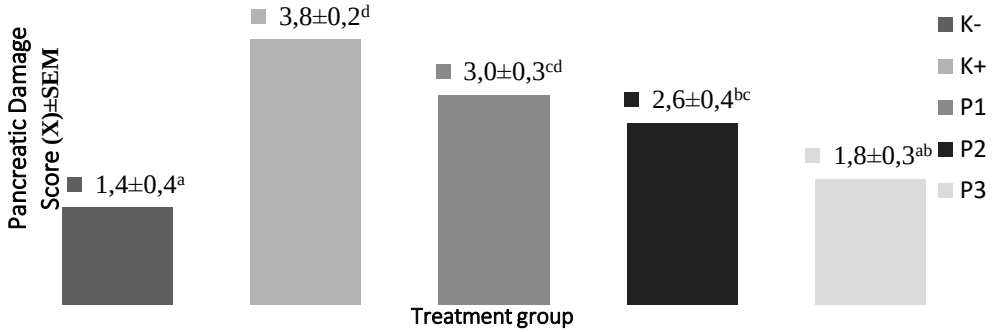

Figure 2. Pancreatic damage score of mice in all treatment groups.

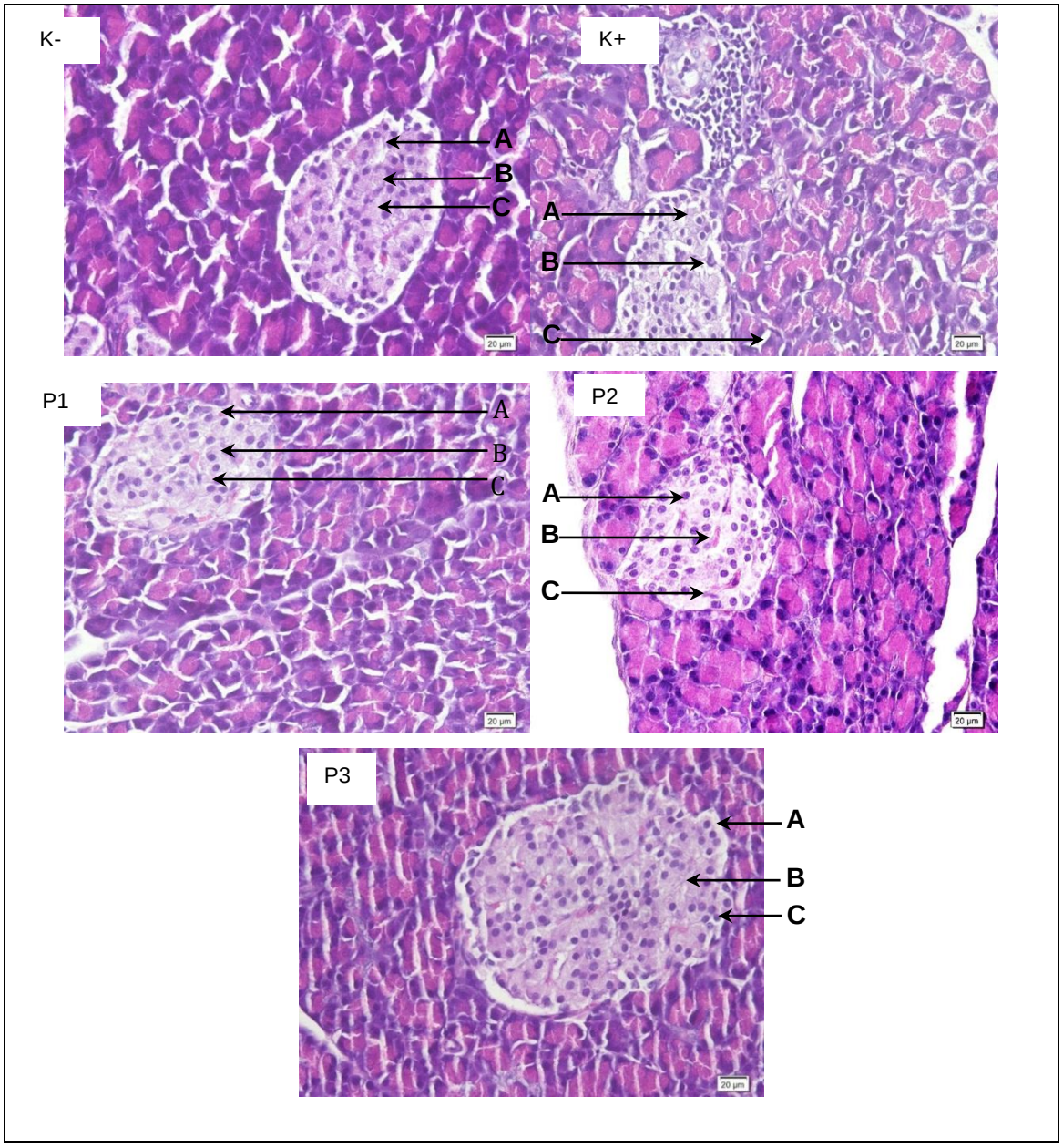

Figure 3. Histopathologic structure of mice pancreas: (K-) Negative Control, (K+) Positive Control, (P1) Dose of 4.5 mg/g BW/day, (P2) Dose of 0.9 mg/g BW/day, (P3) Dose of 1.8 mg/g BW/day
Description: (A) Boundary of Langerhans Island, (B) Number of Beta Cells, (C) Cell Shape

Table 5. Results of Post-Hoc Mann Whitney test of pancreatic islet damage for each treatment.

Testing	Asymp. Sig	Interpretation
K- and K+	0, 006	Significant
K- and P1	0,016	Significant
K- and P2	0,060	Not significant
K- and P3	0,572	Not significant
K+ and P1	0,065	Not significant
K+ and P2	0,041	Significant
K+ and P3	0,009	Significant
P1 and P2	0,371	Not significant
P1 and P3	0,049	Significant
P2 and P3	0,178	Not significant

The results show the level of pancreatic damage in each treatment group given alloxan and Songgolangit leaf extract. The results of the pancreas histopathology image showed that group K- (Figure 2) had the lowest mean damage score of 1.4. Group K- is a normal group uninduced by alloxan and Songgolangit leaf extract. In this group, the borders of the islets of Langerhans are still clearly visible, and there are numbers of beta cells, no beta-cell necrosis, and standard cell shape. It happened because the mice were not given treatment.

The K+ group has the highest average damage score among all treatments, which is 3.8, indicating severe damage. Group K+ induced by alloxan without being given Songgolangit leaf extract. In this group, there was a change from the boundaries of the islets of Langerhans to be very unclear; the decreasing number of beta cells and almost all cells experienced necrosis and abnormal beta cell shape. It shows that the administration of alloxan causes damage to the structure of the pancreas, especially beta cells that undergo necrosis so that insulin secretion into the blood vessels decreases. Alloxan is selectively toxic to pancreatic beta cells that produce insulin due to the accumulation of alloxan, specifically through glucose transporters namely GLUT- 2.

Alloxan in the blood will bind to GLUT-2 (transporters of glucose), facilitating the entry of alloxan into the cytoplasm of pancreatic beta cells. Alloxan causes excessive depolarization of the mitochondria due to the entry of Ca_{2+} ions, followed by excessive energy use, causing energy deficiency in the cell and resulting in pancreatic beta-cell damage [15].

The damage score of the P1 group was 3.0. In this group, there were changes from the boundaries of the islets of Langerhans to be unclear, the decreasing number of beta cells, the cell necrosis was visible, and many cell shapes were abnormal. This group's damage level is smaller than the K+ group. The damage score of group P2 was 2.6. In this group, the boundaries of the islets of Langerhans are unclear. The number of cells decreased, the beginning of the cell necrosis, and the cell shape was abnormal.

The P3 group damage score of 1.8; in this group, there are clear boundaries of Langerhans islands, the number of beta cells began to decrease, cell necrosis was invisible, and the cell shape was standard. The average damage score of the P3 treatment group is close to the K- (Normal) group, which shows a better decrease in the level of damage than the K+, P1, and P2 treatment groups. The P3 dose has more flavonoids and other phytocompounds in the Songgolangit extract than the P1 and P2. The P3 group can regenerate damaged cells with an increase in the number of beta cells of Langerhans islands compared to the P1 and P2 groups.

The results of the Kruskal-Wallis test (P = 0.006) showed a significant difference between treatment groups (P <0.05). Next, the Mann-Whitney test was carried out to determine the difference in effectivity between the doses in repairing pancreatic damage. Based on the Mann-Whitney test (Table 5), the K- (Normal) group was significantly different from the K+ and P1 groups but not significantly different (P>0.05) with P2 and P3. The K+ group was not significantly different from P1 (P>0.05) but significantly different from P2 and P3 (P<0.05). P1 was not significantly different from P2 but significantly different from P3, and the P2 group was not significantly different from the P3 group.

CONCLUSIONS

Oral treatment of ethanol extract from Songgolangit leaves (*Tridax procumbens* L.) for 14 days can reduce blood glucose levels and repair damage to pancreatic histopathology of hyperglycemia mice (*Mus musculus* L.). Treatment of a 1.8 mg/g BW/day showed that mice's blood glucose levels and pancreatic histopathology were not significantly different from the Normal group.

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