

Effect of Mahogany (*Swietenia mahagoni* Jacq.) Extract on the Islet Cells' Number and Blood Glucose Levels of Alloxan-induced Diabetic Rat

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RESEARCH ARTICLE

Effect of Mahogany (*Swietenia mahagoni* Jacq.) Extract on the Islet Cells' Number and Blood Glucose Levels of Alloxan-induced Diabetic Rat

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ABSTRACT

Effect of Mahogany (*Swietenia mahagoni* Jacq.) extract on the Islet Cells' Number and Blood Glucose Levels of Alloxan-Induced Diabetic Rat. This study was to know the effect of ethanol extract of mahogany (*S. mahagoni* Jacq.) seeds on the number of islet cells in the pancreatic Langerhans and blood glucose levels of alloxan-induced diabetic white rat (*Rattus norvegicus*). Twenty-five male rats were divided into five groups consists of: (C-) 1-mL/day aquadest without alloxan induction, (C+) 120 mg/kgBW alloxan and 1-mL/day aquadest, (T1, T2, T3) 120 mg/kgBW alloxan and 250, 500, 1000 mg/kgBW of ethanol extract of mahogany seeds respectively. Alloxan were injected intraperitoneal with single dose and ethanol extract of mahogany seeds administered via intragastric gavage for 14 days. At the end of the treatment period, the number of islet cells in the pancreatic Langerhans was counted and blood glucose levels were measured. This research showed that the ethanol extract of mahogany seeds have antidiabetic effect that can repair damaged islet cells in the pancreatic Langerhans at effective dose of 500 mg/kgBW with a mean number of 151.83 ± 11.07 that significantly higher ($p < 0.05$) than treatment group 1 (T1), treatment group 3 (T3) and positive control group (C+) and also decreased the blood glucose levels from >200 mg/dL to normal range (50–135 mg/dL). This is due to the antioxidant property of mahogany seeds extract which is beneficial in repairing pancreas damage due to oxidative stress.

Keywords: Blood glucose levels, Health care, Islet cells in the pancreatic Langerhans, Mahogany seeds extract, Rats.

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INTRODUCTION

Diabetes nowadays is considered a major health problem that has reached alarming levels and creates huge concerns among scientists regarding public health. In 2019, the World Health Organization (WHO) estimated that 463 million people will have diabetes. This number is expected to reach 578 million by 2030 and 700 million by 2045. In animals, diabetes also could occur, especially to domestic and pet animals, including cats and dogs. Although Diabetes mellitus (DM) could affect animals of all ages, animals, especially pets are stated to be more susceptible to diabetes if they were in older ages, male pets who were castrated, obese, and lack exercise, as well as the genetic factors.

Diabetes is a group of metabolic diseases characterized by hyperglycemia. Generally, initial hyperglycemia develops when blood glucose ≥ 200 mg/dL in rats² and > 180 mg/dL in human.³

It is caused by defects in insulin secretion, insulin action, both caused by genetic and environmental factors. Insulin is one of the hormones that regulate Blood glucose levels (BGL).⁴ Hyperglycemia that caused DM can develop Reactive oxygen species (ROS) and oxidative stress which caused several cellular changes.⁵ ROS is a reactive compound consists of the free radical group and non-radical. High ROS concentration will increase oxidative stress, leads to damage in β -cells of the pancreas, and decrease insulin concentration in blood. Free radicals that caused oxidative stress leads to protein modification, oxidative modification of lipids, DNA damage, and also glycation of some products⁶ that can make an imbalance in cellular oxidation and reduction, as seen in pancreas tissue. Oxidative stress as a result of hyperglycemia can be treated by antioxidants by scavenging free radicals. Antioxidants provide electrons to free radicals without reducing their stability. These antioxidants can be found in plants as a part of herbal medicine.

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Scientific reports and laboratory studies show that plants contain a large variety of substances that have antioxidant activity. Ethnopharmacological surveys also found more than 1200 plants for their affiliation in hypoglycemic activity.⁷ The plants are not only hypoglycemic or insulin mimetic, but also prevent further complications; which no synthetic drug provides both properties. In this aspect, extract of *Swietenia mahagoni* Jacq. seeds have been used to treat DM because it contains flavonoids as antioxidants and also proven to have hypoglycemic activity. Mahogany seeds also reported contain other bioactive compound such as tannin, saponin, and triterpenoid which also have an antidiabetic effect.⁸ According to the previous description, this study investigates the antidiabetic and antioxidant properties of *S. mahagoni* Jacq. in various doses and observe its effect on the number of islet cells in the pancreatic Langerhans and blood glucose levels of alloxan-induced diabetic rat.

MATERIALS AND METHODS

Ethical approval

This type of research is explanatory research with pure experiments (true experiments) carried out in the experimental animal laboratory. This research has obtained ethical eligibility with Ethical certificate number: 1.KE.032.03.2021.

Experimental Animals

Research design in this study is a laboratory experiments research using true experiments post-test completely randomized design. Twenty-five white male rats (*Rattus norvegicus*) with 200 g body weight were divided into five groups and were kept at the Laboratory of Experimental Animals in the Faculty of Veterinary Medicine, Universitas Airlangga.

Mahogany Seeds Extract Preparation

Approximately 500 g mahogany seeds weighed, cleaned, and dried by aerating without direct sunlight. After dried, the mahogany seeds blended to form a powder. The mahogany seeds powder was filtered with a sieve and then extracted using a maceration method. The maceration method was carried out by putting the seeds powder into the macerator. Maceration was carried out with 96% ethanol for 24 hours. The residue and filtrate are separated using filter paper. The residue is then macerated with new ethanol solvent and then evaporated with a rotary evaporator at 50°C until thick but still can be poured to remove the solvent. The results of the fraction process obtained 50 g of mahogany seeds extracted and stored at -20°C. The thick extract of mahogany seeds was made in three doses (250, 500, 1000 mg/kgBW).

Alloxan Preparation

Diabetic conditions in rats were made by inducing alloxan monohydrate intraperitoneally which was dissolved using the normal saline solution at a single dose of 120 mg/kg BW.⁹ The rats were fasted 12 hours before induced with alloxan. After 4 days, the blood glucose was measured to ensure the hyperglycemic rat. Then, the rats were administered with ethanol extract of mahogany seeds for 14 days. The treatment

groups were: (C-) aquadest only, (C+) induced with 120 mg/kgBW alloxan, (T1) induced 120 mg/kgBW alloxan+250 mg/kgBW ethanol extract of mahogany seeds, (T2) induced 120 mg/kgBW alloxan+500 mg/kgBW ethanol extract of mahogany seeds, (T3) induced 120 mg/kgBW alloxan+1000 mg/kgBW ethanol extract of mahogany seeds.

Pancreas Histology Sample Preparation

After 14 days of treatment, all the rats were sacrificed by cervical dislocation. Then, the rats' abdominal was dissected with surgery equipment to collect the pancreas then they were stored in a pot that was filled with 10% Buffer Neutral Formalin (BNF) for 24 hours.

Blood Glucose Levels Measurement

Rats were measured for their blood glucose levels for three times (pre-treatment measurement at day 7, after alloxan induction measurement at day 12, and post-treatment measurement at day 26), which were obtained from the blood at the tip of the tail (lateral vein). Then the tail moistened with warm water for vasodilation of the blood vessels, after which blood was drawn using a blood lancet or making minor cuts using a sterile surgical scissor. Measurements of blood glucose use a glucometer.

Examination and Data Analysis

The examination was carried out on the comparison of negative control with the positive control, and three treatments using different doses. Microscopic examination carried out by making histopathological slides with Hematoxylin and eosin (H and E) staining and microscope at 400x magnification on three different islets of Langerhans, if the sample or slide found contains more than three islets of Langerhans, then the three islets of Langerhans were selected subjectively based on their area, each the widest, medium and narrowest. On each islet of Langerhans that have been selected then counted the entire number of cells. The data of the number of islet cells of the pancreatic Langerhans and the blood glucose levels obtained arranged in table form then the average was calculated; a parametric test analyzed using Analysis of Variant (ANOVA) and the significant difference results between the treatment groups ($p < 0.05$) was proceed with the Tukey Honestly significant difference (HSD) test.¹⁰

RESULTS

Number of Cells in Langerhans Islets

The calculation results in Table 1 showed that the highest mean of the number of islet cells of pancreatic Langerhans is in group T2 and the lowest is in group C+. The treatment of C+ was significantly different from the other entire group; C-, T1, T2 and T3 ($p < 0.05$). Through further testing using the Tukey HSD test the results showed that T1, T2, and T3 did not show any significant difference ($p > 0.05$), so in order to determine the most efficient dose in the use of ethanol extract of mahogany seeds, it was necessary to compare the three treatments with negative control (C-). However, the statistical

Table 1: The mean number of islet cells in the pancreatic Langerhans and the average blood glucose levels in five groups

Groups	Islet cells Mean \pm SD	Average blood glucose levels day 7 /Pre-treatment (Mean \pm SD)	Average blood glucose levels day 26/Post-Treatment (Mean \pm SD)
C-	141.97 ^{bc} \pm 20.82	88.50 \pm 17.61	86.00 ^a \pm 11.75
C+	82.10 ^a \pm 12.44	90.25 \pm 15.63	270.25 ^b \pm 30.01
T1	113.87 ^b \pm 14.54	91.25 \pm 5.44	83.00 ^a \pm 19.80
T2	151.83 ^c \pm 11.07	90.00 \pm 16.12	85.50 ^a \pm 17.82
T3	136.43 ^b \pm 9.01	97.00 \pm 8.45	113.00 ^a \pm 11.16

Note: Different superscripts in the same column represent the significant difference ($p < 0.05$). (C-) aquadest (C-), induced with 120 mg/kgBW alloxan (C+), (T1) induced 120 mg/kgBW alloxan+250 mg/kgBW (T1), 500 mg/kgBW (T2), and 1000 mg/kgBW ethanol extract of mahogany sedes (T3).

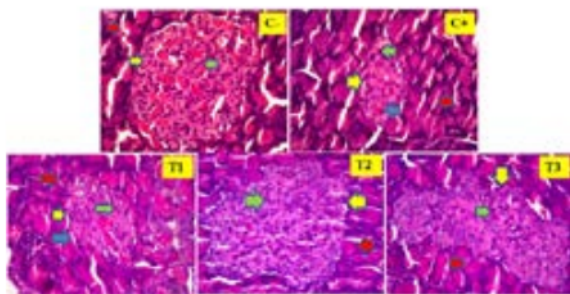


Figure 1: Histopathological imaging of Langerhans islets on white rats with 400x magnification and HE staining on group treatment C-, C+, T1, T2, and T3.



analysis showed that whether from T1, T2, or T3 treatments, all of them showed that there's no significant difference from C- ($p > 0.05$). Based on the data from Table 1, it can be seen that C+ as the positive control has the lowest number of Langerhans islet cells if compared to T1, T2, and T3. On treatment T1, T2, and T3, the mean results closest to C- was from T2 because it has a higher mean number of cells compared to T1 and T3, even from the C- itself.

It can be seen in Figure 1, the histopathological imaging of the pancreatic islets of Langerhans from each treatment group. In the C- group, the islets of Langerhans are clearly demarcated with dense cell populations and clear nuclei. Meanwhile, the C+ group showed the islets of Langerhans were smaller in size than the other treatment groups, the cell population was not as dense as that of the C- group, indicating cell rupture, and necrotic cells were also seen at the edge of the islets. In T1 group, the size of the islet is seen larger than the C+ group, but the boundaries of the islets are still unclear, there are also necrotic cells on the edges of the islet. In the T2 group, Langerhans islet are clearly demarcated but not perfectly circular in shape, the size looks bigger than other groups, the population also looks denser. In the T3 group the size of the islets was larger than the C+ group but the shape was irregular, the cell population in the islets was not too dense.

Blood Glucose Levels

The results in the pre-treatments up (day 7) show homogeneous results or did not show significant differences ($p > 0.05$) between the treatment groups. This is because the group is in normal conditions without any treatment. On day 26, the blood glucose levels were measured and the results show that the C+ group has the highest blood glucose levels with > 200 mg/dL. This is because the C+ was induced by alloxan and not given the extract mahogany seed therapy.

Blood glucose levels of rats after treatment with mahogany seed extract in groups T1, T2, and T3, respectively were decreased from > 200 mg/dL to 85.50 ± 17.82 mg/dL; 83.00 ± 19.80 mg/dL; and 113.00 ± 11.16 mg/dL. This data when analyzed statistically with the average of the positive control group shows that there is a significant difference ($p < 0.05$). The average blood glucose levels of rats in each group.

DISCUSSION

The results of the histopathological examination on the number of islets cells of the pancreatic Langerhans showed that the positive control group (C+) has obtained the lowest mean compared to number cell mean from other treatment groups. On the blood glucose levels result, this group showed high blood glucose levels (> 200 mg/dL) meaning that hyperglycemia still occurred. This is because in this group white rats were induced by alloxan 120 mg/kgBW that could cause hyperglycemia.

The diabetogenic effect of alloxan is due to excess in production of ROS. Excess ROS leads to toxicity in pancreatic cells resulting in reduced synthesis and release of insulin.¹¹ The toxicity of ROS in cells leads to cells swelling and becoming necrosis. Therefore, the population of pancreatic cells including beta cells would decrease and affect the insulin secretion. The decrease of insulin levels in the circulation would triggered the blood glucose levels to rise above normal.¹² Decreased viability and dysfunction of pancreatic β -cells leads to the development of diabetes.¹³ Therefore, the number of cells in the islets of Langerhans and blood glucose levels BGL are presented as indicators of repair or improvement of the pancreas organ in diabetic rat.

On another note, it needs to be emphasized that if entire Langerhans islets were destroyed, a decrease in other cells such as the alpha and delta cells of the pancreas should be expected.

The alpha cells that co-occupy the islets in association with beta cells have been long recognized as the source of glucagon, a hyperglycemia-producing and diabetogenic hormone. However, little is known about the effect of alloxan on α -cells. The fact that both alpha and beta cells originate from pancreatic ductular epithelium provides an ontogenetic basis for the notion that if the diabetic beta cell is genetically defective (in this case caused by the induction of alloxan), the diabetic alpha cell may also be defective.¹⁴

Based on the research result, administration of mahogany seed extract, especially at a dose of 500 mg/kgBW in group T2, was able to stop the process of pancreatic cell damage caused by alloxan. It showed better improvement than the other doses. This can be seen from the mean number of islet cells of Langerhans and blood glucose levels between T2 which are not significantly different from C-. The results of the T2 treatment group with mahogany seed extract at 500 mg/kgBW showed an effective dose of mahogany seed extract that could protect pancreatic endocrine cells. This process of improvement could occur because mahogany seed extract contains compounds and active ingredients that have antioxidant activity.

Damage to pancreatic cells caused by oxidative stress in diabetes will increase the formation of ROS in the mitochondria and exacerbate oxidative damage to pancreatic cells. A good way of handling to reduce damage due to oxidative stress on pancreatic cells is to use synthetic and artificial antioxidant compounds.¹⁵ Antioxidant activity is thought to be able to capture free radicals that cause pancreatic cell damage and inhibit pancreatic cell damage so that the remaining cells still function. When there is an improvement in pancreatic β cells which are insulin-producing, then there is an increase in the amount of insulin in the body and is able to facilitate the entry of blood glucose into the cells, therefore also resulting in a decrease in blood glucose levels in the body.¹⁶

According to Halliwell and Gutteridge¹⁷ mechanisms of antioxidant action can include suppressing ROS formation either by inhibition of enzymes or chelating trace elements involved in free radical production, scavenging ROS, and upregulating or protecting antioxidant defenses. Phytochemical compounds possessed by mahogany seed extract as an antioxidant, inter alia, flavonoid, swietenine, saponin, and tannin compound, which have been shown to have antioxidant,¹⁸ hypoglycemic,¹⁹ and antidiabetic activity.²⁰

Flavonoids were proposed as potential antidiabetic agents before because they exert multiple actions on β -cells. In this respect, the functions of flavonoids can be divided into protection against β -cell damage, increased proliferation of β -cells, and preservation of insulin signaling by increased insulin secretion. The protective effects of flavonoids in diabetes as antioxidants are due to their capacity to scavenge free radicals and to activate antioxidant enzymes.²¹ Flavonoids also inhibit the enzymes responsible for superoxide anion production, such as xanthine oxidase and protein kinase C which all involved in ROS generation.

Another compound that is thought to play a role in the antidiabetic activity of mahogany seeds is swietenine.

Swietenine is a class of tetranortriterpenoid compounds which is a natural Peroxisome proliferator activated receptor (PPAR γ) agonist. It has a working mechanism of activating insulin responsive genes that can stimulate insulin to form and translocate Glucose transporter (GLUT) to cell membranes in peripheral organs so that the absorption and use of peripheral glucose increases.^{22,23}

Compared with T1 and T3, the mean cell number of Langerhans islets of the white rats in T2 group in this study was more increased. The mean cell number of the T2 treatment group was also higher than the C- group. This is because a Langerhans islet with such a large size and containing the most cells were found in the histopathological preparation of the T2 group, which resulted in the mean number of cells being higher than the C- group. During the therapy with mahogany seed extract, proliferation of islet β -cells increasing due to the flavonoid property of mahogany seed extract could happen so well that resulted in this finding. However, statistical calculations using ANOVA test and Tukey HSD test showed that there was no significant difference between the averages of the T2 and C- groups ($p > 0.05$).

The experimental animals in this study were believed to have suffered from diabetes mellitus because of the alloxan, a chemical diabetogenic agent at the dose of 120 mg/kgBW and the results of glucose measurements on the fourth day after alloxan administration was more than 200 mg/dL.

Panda *et al.*²⁴ on his study stated that tannins, the main preliminary phytochemical analysis of mahogany seeds is one type of polyphenols which are well-known natural antioxidants due to their electron-donating properties, either scavenging principal propagating radicals or halting the radical chain, thus being potential drugs for the treatment of non-insulin dependent diabetes mellitus.

Another component of mahogany seeds was saponin which is able to reduce elevated plasma blood glucose, making saponin an excellent alternative in the treatment of diabetes mellitus. Several ways of hypoglycemic action by saponin for example are through restoration of insulin response therefore could create improvement in insulin signaling, increase plasma insulin levels and induction of insulin release from the pancreas, inhibition of disaccharide activity, activation of glycogen synthesis, inhibition of gluconeogenesis, inhibition of α -glucosidase activity, inhibition of mRNA expression of glycogen phosphorylase and glucose 6 phosphatase, and also increase the expression of Glut4.²⁵

Glucose levels in the blood are influenced by insulin secreted by pancreatic β cells, so that when pancreatic β cells are decreased or damaged, the insulin produced will decrease and glucose levels in the blood increase. According to research and statement by Suryani²⁶ that in mahogany seed extract therapy, the decrease in blood glucose levels occurs due to the improvement of pancreatic tissue, so that it can increase insulin secretion, therefore glucose in the blood can be absorbed into cells and can be converted into energy or stored in the form of glycogen in the liver and muscles. This is also in accordance

with the correlation analysis between blood glucose levels and insulin, which has a significant negative correlation: increasing plasma insulin levels can reduce blood glucose levels.

In this study, the increase²⁹ in the number of pancreatic Langerhans islet cells and decrease in blood glucose levels of alloxan-induced diabetic white rats were proved by the statistical analysis from the number of cells and blood glucose levels in this treatment which were not significantly different ($p > 0.05$) from the negative control treatment group³⁴ that was in normal condition and significantly different ($p < 0.05$) with the positive control treatment group which induced by diabetogenic agent alloxan. In the treatment group doses of 250 mg/kgBW and 1000 mg/kgBW were not as effective when used as a therapeutic dose for diabetes mellitus, but these doses still could reduce damage to endocrine cells in the islets of Langerhans and lower the blood glucose levels.

CONCLUSIONS

Ethanol extract of mahogany (*S. mahagoni* Jacq.) seed have effect on improving the number of islet cells in the pancreatic Langerhans and decreasing the blood glucose levels of alloxan-induced diabetic white rat (*Rattus norvegicus*) with the effective dose was at 500 mg/kgBW extract mahogany seeds therapy.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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PAGE 1

PAGE 2

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PAGE 5
