

Hypoglycemic Effect of Mahogany (*Swietenia macrophylla* King) Bark Extracts in Alloxan-induced Diabetic Rats

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Abstract

In this study, *in vivo* hypoglycemic activity of mahogany (*Swietenia macrophylla*) bark extracts was evaluated against alloxan-induced diabetic rats. The hypoglycemic effect was compared to that of standard glibenclamide. Oral administration of hot water and methanol extracts at a dose of 250 mg/kg body weight for thirteen days of daily treatment to diabetic rats was found to possess significant dose dependant hypoglycemic effect in diabetic rats. It less active than that of glibenclamide at dose of 3.22 mg/kg. However, the hot water extract showed significant hypoglycemic activity compared to that standard drug. Phytochemical analysis of hot water and methanol extracts has shown positive test for the presence of alkaloids, flavonoids, tannins, saponins, dan terpenoids. Histopathological studies of pancreas revealed its significant effect of β -cell count. Therefore, the hot water extract could serve as good adjuvant to other oral hypoglycemic agents and seems to be promising for the development of phytomedicines for diabetes mellitus.

Key words: *Swietenia macrophylla*, bark extract, hypoglycemic activity, alloxan-induced diabetic rats.

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Bowman and Russel 2001). Statistical projections mentioned that number of diabetics in the world will increase from 151 million in the year 2000 up to 221 million in the year 2010, and hence it become Indonesia is the fourth in the highest number of diabetics in the world after India, China, and USA (King *et al.* 1998; Boyle *et al.* 2001; Zimmet *et al.* 2001).

Traditional medicinal plants have been employed successfully by the local communities since long time to treat diabetes without adverse effects. Researches in traditional medicine for appropriate hypoglycemic agents have been focused on plants due to traditional medicine gives better treatments than drugs (Rates 2001). Seeds of mahogany (*Swietenia macrophylla*) have been used for treatment of diabetes as a folk medicine in Indonesia (Kadota *et al.* 1990). The seed also have been used for leishmaniasis and abortion medicine by an Amazonian Bolivian ethnic group (Bourdy *et al.* 2000) and for treatment of hypertension and malaria (Kadota *et al.* 1990). However, bioactivities from the bark have not been investigated extensively. The bark of mahogany, collected from Indonesia, contain flavonoids with high antioxidant activity, namely swietemacrophyllanin, catechin, and epichatechin (Falah *et al.* 2008). In this study, the hypoglycemic effect of mahogany bark extracts were evaluated, and phytochemicals compounds of the extracts were examined. The effect of hot water and methanol extracts were evaluated on diabetic rats and its effects were compared

with glibenclamide, a standard hypoglycemic agent.

Materials and Methods

Plant Material

Mahogany bark was collected from Sumedang, Indonesia since March 2009. A dried bark powder of mahogany (500 g) were boiled in 1 liter of water for 4 h to give a hot water extract (29 g). The extract was filtered with filter paper (Whatman, no. 1) and evaporated with rotary evaporator at 60°C, and the crude extract was used in biological assay. Another 3000 g of the dried bark powder was extracted with acetone for 48 h at room temperature to give acetone extract (237 g), and then the residue was extracted again by methanol to yield methanol extract (184 g). The acetone was used for extraction of non polar substances, i.e. fatty acid, wax. The methanol extract was evaporated, and the extract was used in biological assay.

Chemicals and Drugs

The solvents were of analytical grade and purchased from Merck, Germany. Alloxan and glibenclamide were obtained from Sigma Chemical, USA and Daonil Aventis Pharmacy, USA, respectively. All other chemicals were of analytical grade.

Qualitative Phytochemical Analyses (Harborne 1987)

Alkaloid Test. The hot water and methanol extracts of 0.1 g each were added with 3 mL of chloroform and 3 drops of ammonia. The chloroform fraction was separated and acidified with 10 drops of H₂SO₄ 2M. The H₂SO₄ fractions were taken and added separately with Dragendof, Meyer, and Wagner reagents. The alkaloids content was indicated by white precipitant upon addition of Meyer reagent, orange precipitant upon Dragendof reagent, and brown precipitant upon addition of Wagner reagent.

Saponin Test. The extracts of 0.1 g were added with 2 mL of H₂O and heated for 5 min. The mixtures were cooled down, stirred up until foamy appearance can be observed to indicate the presence of saponin.

Flavonoid Test. The extracts of 0.1 g were soaked with 2 mL of 30% methanol and heated. The filtrates were added with 1 drop of concentrated H₂SO₄. The presence of flavonoid was indicated by the formation of red pigment.

Phenolic Hydroquinone Test. The extracts of 0.1 g were soaked with 2 mL of 30% methanol, heated and filtered. The filtrates were added with 1 drop of NaOH 10%(b/v). The presence of phenolic hydroquinone was indicated by the formation of red color.

Triterpenoid Test. The extracts of 0.1 g were added with 2 mL of 30% ethanol, heated and filtered. The filtrates were evaporated and then diethyl ether was added. The Lieberman Burchard reagent (3 drops of acetic acid anhydride and 1 drop of concentrated H₂SO₄) was added to the ether layer. The presence of triterpenoid was indicated by the formation of reddish-violet pigment.

Tannin Test. The extracts of 0.1 g were added with 2 mL of H₂O and heated for several minutes. The mixtures were filtered and the filtrates were added with FeCl₃ 1% (b/v). The presence of tannin was indicated by the formation of dark-blue or greenish-black color.

Animals

Male Sprague-Dawley rats of 3 weeks old were obtained from The National Agency of Drug and Food Control of Indonesia. They were fed with a standard laboratory diet and allowed food and water ad libitum for an acclimatization periods of 2 weeks prior to experiments. The animals were divided into five groups of seven each and housed individually during the experimental period.

Experimental Design

All the rats (*Sprague dawley* albino male rats) were randomly divided into the five groups.

Group A: Normal rats administered NaCl 0.9% by intraperitoneal and orally aquades 1 ml daily for 13 days.

Group B: Diabetic control rats administered alloxan 150 mg/kg by intraperitoneal and orally aquades 1 ml daily for 13 days.

Group C: Diabetic rats administered standard drug glibenclamide (3.22 mg/kg, orally) daily for 13 days.

Group D: Diabetic rats administered hot water extract (250 mg/kg, orally) daily for 13 days.

Group E: Diabetic rats administered methanol extract (250 mg/kg, orally) daily for 13 days.

Alloxan was injected to all rat groups on 1st day. Treatment with the extracts and glibenclamide was started 48 h after alloxan injection. Blood samples were obtained from the tail vein in fasting rats for 18 h and blood glucose levels were measured using an electronic glucometer (Miles Inc, USA). Fasting blood glucose and body weight were measured on 1st, 3rd, and 15th days.

Statistical Analysis

All the values of body weight and fasting blood sugar were expressed as mean \pm standard error of mean (S.E.M) and analyzed for ANOVA and Duncan's *t*-test. Differences between groups were considered significant at $P < 0.05$.

Histopathological Studies

All the animals were sacrificed on 15th day by cervical dislocation. Pancreases were excised, isolated, and were subjected to histopathological studies and microscopical finding were noted. The pancreas tissues were removed immediately and washed with ice-cooled saline, and then fixed in 10% of neutral formalin. The sections stained in haematoxylin and eosin and mounted were observed under microscope.

Results and Discussion

Phytochemicals assay of hot water and methanol extracts of mahogany bark revealed the presence of the flavonoids, tannins, triterpenoids, saponins and alkaloids (Table 1). Phytochemical compounds such as, flavonoids, triterpenoids, alkaloids, and phenolics are known to be bioactive antidiabetic principles (Nagappa *et al.* 2003; Battu *et al.* 2007; Safithri and Fahma 2008).

The effect of treatment on rat body weight on 1st day showed that rats body weight in all groups did not differ significantly ($P < 0.05$) (Table 2). Rats body weight decreased on 3rd day after alloxan (B, D, and E group) induction; the highest degradation occurred at group D (4.4% from body weight of 1st day). However, body weight degradation on 3rd day in B, D, and E group did not different significantly with A and C group ($P < 0.05$). On 15th day, the rats body weight was measured to evaluate the effect of hot water and methanol extracts of mahogany bark which orally administered at the dose of 250 mg/kg body weight (D and E group). During 13 days treatment (from 3rd day till 15th day), hot water and methanol extracts reduced the body weight by 8.54% and 7.36%, respectively. D and E groups did not differ with B and C group ($P < 0.05$). It means that mahogany bark extracts reduced rat body weight the same as B and C group. Body weight reduction was also indicated by aqueous extract of *Terminalia catappa* at the dose of 42 mg/kg for 12 days treatment in alloxan-induced diabetes up to 63.09% (Nagappa *et al.* 2003) and decoction of *Piper crocatum* of 322 mg/kg for 10 days treatment in alloxan-induced diabetes up to 17.28% (Safithri and Fahma 2008). The decrease of body weight in diabetes is due to

continuous excretion of glucose and glycogen synthesis (Defronzo *et al.* 1992).

Measurement of blood glucose level was carried out on the 1st, 3rd, and 15th day to observe the effect of aquades, glibenclamide, and mahogany bark extracts orally administration and induction of NaCl or alloxan. The induction influences blood glucose rats during experiment. On 1st day (before treatment), rats blood glucose in all groups resulted not significant different ($P < 0.05$) (Table 3) and performed at normal range (60~110 mg/dl). However, after NaCl and alloxan induction (on 3rd day), rats blood glucose increased. Induction of alloxan (150 mg/kg) (B, C, D, and E group) increased blood glucose up to 0.5~1.0 folds. Blood glucose levels data showed B group has the highest increasing by 150.9%. Increasing of blood glucose rats on 3rd day after induction of alloxan, showed significantly different ($P < 0.05$) with A group (Table 3). Rats blood sugar was reduced up to 45.73% and 25.80% in thirteen days after treated with hot water and methanol

extracts at a dose of 250 mg/kg. It is less active than that of glibenclamide which reduced blood sugar level by 48.42%. It was indicated that the hot water extract showed significant antihyperglycemic activity as compared to that of standard drug.

Antihyperglycemic activity from decoction of *P. crocatum* at 322 mg/kg body weight reduced blood glucose level up to 10.46% after ten days given to diabetic rat. The extract contained flavonoids, alkaloids, and tannin (Safithri and Fahma 2008). Blood glucose reduction up to 3.68% occurred from alcoholic extract of Chinese squash (*Benincasa hispida*) at 200 mg/kg after 24 h given to diabetic mice. The extract contained alkaloids, flavonoids, saponins, and steroids (Battu *et al.* 2007). The alcohol extract of gopher plant (*Euphorbia leucophyllum*) at 500 mg/kg in diabetic mice showed that it possessed an antihyperglycemic activity to reduce blood glucose up to 21.54% after 24 h the extract was given (Satyanarayana *et al.* 2006).

Table 1. Phytochemical constituents of mahogany bark.

Test	Hot water extract	Methanol extract
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Triterpenoids	+	+
Tannins	+	+

(+) Positive (-) Negative

Table 2. The effect of 13 days treatment with extract of mahogany bark on body weight.

Group No	Body weight (g)		
	Day 1	Day 3	Day 15
A	380.6 ^{ab}	385.6 ^{ab}	389.6 ^a
B	367.6 ^{abc}	357.6 ^{abc}	352.8 ^{abc}
C	367.2 ^{abc}	370.0 ^{abc}	360.8 ^{abc}
D	372.4 ^{ab}	356.0 ^{abc}	325.6 ^c
E	379.6 ^{ab}	369.2 ^{abc}	342.0 ^{bc}

The same letter(s) indicated not significant different on $P < 0.05$. The groups refer to Table 1.

Table 3. The effect of 13 days treatment with extract of mahogany bark on blood glucose level.

Group No	Average blood glucose level (mg/dl)		
	Day 1	Day 3	Day 15
A	71.0 ^a	91.6 ^a	84.6 ^a
B	66.4 ^a	166.6 ^b	103.6 ^{ab}
C	75.4 ^a	177.6 ^b	91.6 ^a
D	81.2 ^a	173.2 ^b	94.0 ^a
E	81.8 ^a	118.6 ^b	88.0 ^a

The same letter(s) indicated not significant different ($P < 0.05$). The groups refer to Table 1.

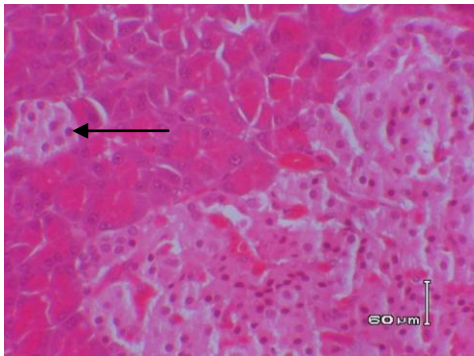


Figure 1. Pancreas of normal health rat, H & E staining (x100). The islet of langerhands is shown by arrow.

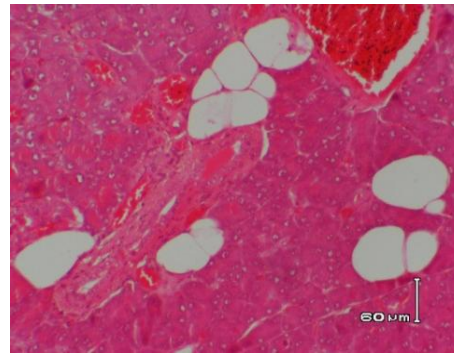


Figure 2. Pancreas of diabetic control (alloxan-induced diabetic) rat, H & E staining (x100). The islet of langerhands couldn't be found.

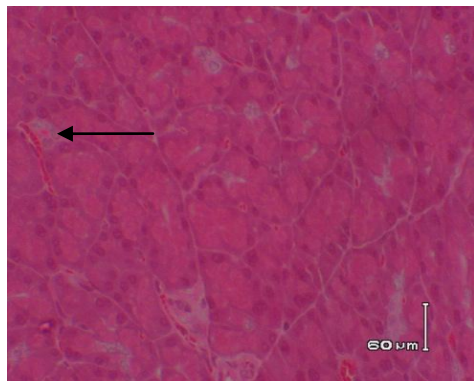


Figure 3. Pancreas of diabetic rat treated with Glibenclamide 0.25 mg/kg body wt, H & E staining (x100). The islet of langerhans is shown by arrow.

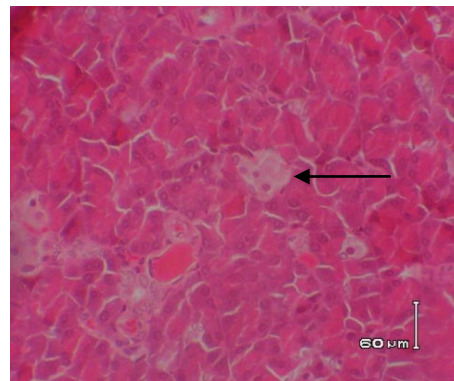


Figure 4. Pancreas of diabetic rat treated with 250 mg/kg body wt hot water extract, H & E staining (x200). The islet of langerhans is shown by arrow.



Figure 5. Pancreas of diabetic rat treated with 250 mg/kg body wt methanol extract, H & E staining (x200). The islet of langerhans is shown by arrow.

The histological sections of the pancreas, tissues were observed to know the effect of extract of mahogany bark in alloxan diabetic rats. The cellular integrity and architecture were intact in the A group. Besides that, in group A there was no specific abnormalities, and easy to find the islets of Langerhans (Figure 1). Pancreatic sections stained with hematoxylin and eosin (H & E) showed that alloxan caused fat necrosis; acinar cell necrosis, and hemorrhage and the

islet of Langerhans cannot be found (Figure 2). However, administration of glibenclamide at the dose of 3.22 mg/kg/day orally in alloxan diabetic rats showed no necrosis. The size and the number of islets of Langerhans is smaller and fewer than normal group, respectively (Figure 3). Meanwhile, administration of hot water extract of mahogany bark (250 mg/kg/day, orally) in alloxan diabetic rats showed fat necrosis, acinar cell necrosis, the number

and size of islets of Langerhans smaller than normal group (Figure 4). Furthermore, administration of methanol extract of mahogany bark (250 mg/kg/day, orally) in alloxan diabetic rats showed necrosis and easy to be found the islets of Langerhans (Figure 5).

In this study, the pancreatic β cells were destroyed with the help of alloxan. Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from streptozotocin. Alloxan has a destructive effect on the β cells of the islets of Langerhans (Szkdeliski 2001). The alloxan produce permanent hyperglycemia by selective destruction of the β cells of the islets of Langerhans are in agreement with those of Singh and Gupta (2007a). The histopathological study of diabetic treated with the extracts indicated the increasing of volume density of islets and percentage of β cells, in the diabetic rats that received the extracts, which may be a sign of regeneration. Signs of regeneration of β cells, potentiation of insulin secretion from surviving β cells of the islets of Langerhans and decrease of blood glucose have been reported following consumption of some plant extracts (Yadav *et al.* 2008; Singh and Gupta 2007b). Hot water and methanol extracts of mahogany bark may have some chemical components that exert regenerative effects on β cells, stimulate these cells to produce more insulin (pancreatotropic action) or may have some insulinlike substances. Induction of regenerative stimulus in diabetic state triggers pancreatic regenerative processes, thereby restoring functional activities of the pancreas (Adewole and Ojewole 2007). A higher dose of the extract has a greater restorative effect on the islet cells of diabetic rats than a lower dose of extract.

Conclusions

The hot water and methanol extracts of mahogany bark contained flavonoids, tannins, triterpenoids, saponins and alkaloids. Oral administration of hot water and methanol extract at a dose of 250mg/kg for thirteen days of daily treatment led to reduce blood sugar level by 45.73% and 25.80%, respectively. It is less active than that of glibenclamide which reduce blood sugar level by 48.42%. However, the hot water extract showed significant antihyperglycemic activity as compared to that of standard drug. Histopathological study indicated the extracts exert regenerative effect on β cells, stimulate to produce more insulin. Further research is needed to explore different mechanisms to reduce blood glucose levels.

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