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The effects of the ethanolic extract of mahogany seeds (*Swietenia macrophylla* King) on the renal function of streptozotocin-induced diabetic rats

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ABSRACT

Diabetes-associated oxidative stress causes glomerular hypertrophy, decrease of glomerular filtration rate and inhibits cell proliferation that lead to the decrease of renal function as indiated by the increase of serum creatinine level and the presence of protein in urine. Mahogany seed (*Swietenia macrophylla* King) has been proven to have antidiabetic activity. This study was conducted to evaluate the effect of the ethanolic extract of mahogany seeds on the renal function of streptozotocin-induced diabetic rats. Six normal rats as control (Group I) and 24 diabetic rats were used in this study. The diabetic rats were randomized allocated into four groups with six rats in each group. Group II was considered as diabetic rats control and received aquadest. Group III-V were considered as extract administered diabetic group and received ethanolic extract of *S. macrophylla* seed for 21 days at a dose of 50, 100 and 200 mg/kg BW, respectively. Serum malondialdehyde (MDA), serum creatinine, and urine protein levels were monitored, before and after the ethanolic extract of *S. macrophylla* seed administration. Serum MDA, serum creatinine and urine protein levels of all rats after STZ induction (Group II-V) were significantly higher than without STZ induction ($p < 0.05$). A significant decrease in the serum MDA and serum creatinine as well as urine protein levels were observed after the treatment with ethanolic extract of *S. macrophylla* seed compared to before treatment ($p < 0.05$). In conclusion, the ethanolic extract of *S. macrophylla* seed is able to correct renal dysfunction of streptozotocin-induced diabetic rats.

ABSTRAK

Stres oksidatif akibat diabetes dapat menyebabkan hipertropi glomerulus, penurunan kecepatan filtrasi glomerulus dan penghambatan proliferasi sel yang berakibat terjadinya penurunan fungsi ginjal sebagaimana ditunjukkan dengan penurunan kreatinin serum dan adanya urin protein. Biji mahoni (*Swietenia macrophylla* King) terbukti mempunyai aktivitas antidiabetes. Penelitian ini dilakuan untuk mengkaji efek ekstrak etanol biji mahoni pada fungsi ginjal tikus diabetes yang diinduksi streptozotosin. Enam tikus normal sebagai kontrol (Kelompok I) dan 24 tikus diabetes digunakan dalam penelitian. Tikus diabetes dibagi menjadi 4 kelompok secara random dengan 6 ekor tikus masing-masing kelompok. Kelompok II sebagai kontrol tikus diabetes menerima air suling. Kelompok III-V sebagai

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kelompok perlakuan menerima ekstrak etanol biji mahoni (S. macrophylla King) selama 21 hari dengan dosis berturut-turut 50, 100 dan 200 mg/kg BB. Kadar malodehaldehid (MDA) serum, kreatinin serum dan protein urin diukur sebelum dan sesudah pemberian ekstrak etanol biji mahoni. Kadar MDA serum, kreatinin serum dan protein urin pada semua tikus setelah induksi STZ (Group II-V) lebih tinggi secara bermakna dibandingkan tanpa induksi STZ ($p < 0,05$). Penurunan secara nyata terhadap kadar MDA serum, kreatinin serum dan protein urin dijumpai setelah pemberian ekstrak etanol *S. macrophylla* King dibandingkan sebelum pemberian ($p < 0,05$). Kesimpulan, ekstrak etanol biji mahoni dapat memperbaiki disfungsi ginjal tikus diabetes yang diinduksi streptozotosin.

Key words : hyperglycemia, *Swietenia macrophylla* King, serum creatinine, MDA, urine protein

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia due to a decrease in insulin secretion, insulin action, or both.[1] Complications of diabetes due to hyperglycemia includes macrovascular (coronary artery disease, peripheral arterial disease, and stroke) and microvascular (diabetic nephropathy, neuropathy, and retinopathy) dysfunctions.[2] Oxidative stress caused by hyperglycemia plays an important role in those macrovascular and microvascular complications of DM.[2,3] Oxidative stress occurs as result of the increase of peroxidation product as a mediator of tissue damage and leads to the activation of pathogenesis complication pathways of DM.[3]

Malondialdehyde (MDA) is a product of lipid peroxidation due to the increase of reactive oxygen species (ROS) level on the oxidative stress conditions. Plasma MDA is frequently used as biomarker for oxidative stress. In diabetics condition, plasma MDA level increases due to the decrease of antioxidant level and leads the early systemic complications of diabetes as expressed by the increased AGEs, protein, lipid serum, lymphocytes, and urine of diabetic patients.[4,5] Moreover, oxidative stress causes glomerular hypertrophy, decrease of glomerular filtration rate and inhibits cell proliferation that lead to the decrease of renal function as indiated by the increase of serum creatinine level and the presence of protein in urine.[5-7]

Previous studies showed the benefits and potential of some medicinal plants in dealing with oxidative stress in both the treatment of diabetes and its complication.[8] Mahogany (*Swietenia macrophylla* King) was proven to have antidiabetic activity. The aqueous-methanolic extract of the mahogany seed induces the pancreatic β cells regeneration and increases the insulin production due to its antioxidant activity through glucose auto-oxidant and protein glycation pathways.[9] In addition, the alcoholic mahogany seed extract stimulates the glucokinase enzyme, a major catalyst in the posporilation of glucose, in lowering blood glucose levels.[10]

Streptozotocin (STZ) is widely used to induce experimental both insulin-dependent and non-insulin-dependent diabetes mellitus in animals, respectively. The mechanism of STZ action in β cells has been intensively investigated and is well understood.[11] Streptozotocin entering the B cell *via* a glucose transporter (GLUT2) causes DNA damage due to alkylation of DNA. The DNA damage induces activation of poly ADP-ribosylation leads to depletion of cellular NAD^+ and ATP

resulting in the formation of ROS. Moreover, STZ liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage. As a result of the STZ action, β cells undergo the destruction by necrosis.[12,13]

The present study was designed to evaluate the effects of the ethanolic extract of mahogany seeds (*S. macrophylla* King) on the renal function of streptozotocin-induced diabetic rats. The renal function was expressed by serum creatinine and urinary protein levels. Furthermore, the plasma MDA level after the ethanolic administration was also evaluated.

MATERIALS AND METHODS

Ethanolic extract preparation

The *S. macrophylla* King seeds were collected in The Bogor Botanical Gardens, Indonesia and identified by a botanist in The Faculty of Pharmacy, Universitas Gadjah Mada Yogyakarta. The seeds were separated from the fruits, dried under open air conditions and powdered using a blender. The powdered seeds were extracted with ethanol by the ethanol method for three days. The extract was filtered through Whatman filter and the filtrate was collected and concentrated in a rotary evaporator at 40 ^{o}C. The concentrated extract was dried under open air and stored under refrigeration until further use.

Induction of diabetes by streptozotocin

Wistar strain male albino rats, 6-8 weeks of age, weighing from 180 to 200 g were selected for this study. All rats were acclimated for a period of 7 days in our laboratory condition prior to the study. Rats were housed at room temperature under 12 hours cycles of dark and light and were fed standard food and water *ad libitum.* Induction of diabetes in the rats were performed by a single intraperitoneal injection of STZ at dose of 60 mg/kg BW in 0.1 mL of freshly citrate buffer (0.01 M and pH = 4.5). The day of STZ injection was designated as day 0 (D-0). Development of diabetes was confirmed by measuring blood glucose level four days after STZ injection (D-4). Rats with the blood glucose level higher than 200 mg/dL were considered to be diabetic. The diabetic rats were then selected for the study. This study has been approved by Medical and Health Research Ethics Committee, Faculty of Medecine, Universitas Gadjah Mada, Yogyakarta.

Animal treatment

Six normal rats and 24 diabetic rats were used in this study. The normal rats were considered as non diabetic rats control and received aquadest (Group I). The diabetic rats were randomized allocated into four groups with six rats in each group. Group II was considered as diabetic rats control and received aquadest. Group III-V were considered as extract administered diabetic group and received ethanolic extract of *S. macrophylla* seed at a dose of 50, 100, and 200 mg/kg BW, respectively. The ethanolic extract was administered one time per day at 08.00 AM started from day 4 and continued for next 21 days. Blood glucose level was measured and monitored on day 0 (D-0), 4 (D-4), 18 (D-18) and 25 (D-25), respectively. In addition, serum creatinine, serum MDA and urine protein levels were also monitored, however only on day 4 (D-4) and 25 (D-25), respectively.

Biochemical analysis

A volume of 2 mL orbital sinus blood sample was collected from each study rat and assayed by standard methods for estimation of blood glucose, serum creatinine and MDA levels. The blood glucose level was measured

by glucose oxidase peroxidase (GOP-PAP) method.14 The serum creatinine level was measured by Jaffe method[15], MDA level was measured by TBARS metod[16] and urine protein level was measured by colorimetric test pyrogallon red metod.[17] All spectrophotometric measurements were carried out in a DR6000 UV-Visible spectrophotometer (Hach-US).

Statistical analysis

All the results were expressed as mean ± standard deviation (SD) for six animals in each group. Statistical comparisons were performed using one way analysis of variance (ANOVA) followed by *Post Hoc Tests Least Significant Difference* (LSD). p-values of less than 0.05 were considered to indicate statistical significance.

RESULTS

The effect of ethanolic extract of mahogany seeds on blood glucose level

Blood glucose level of all rats in all groups before STZ induction were in normal level (TABLE 1). A significant increase in the level of blood glucose level was observed four days after STZ induction (D-4) when compared to before STZ induction ($p < 0.05$). All rats on Group II-V had the blood glucose level higher than 200 mg/dL ($p > 0.05$). Furthermore, a significant decrease in the level of blood glucose level was observed 14 (D-18) as well as 21 (D-25) days after the treatment with ethanolic extract of *S. macrophylla* seed (Group III-V) ($p < 0.05$). The blood glucose levels in diabetic rats of the Group III-V were in normal level (<200 mg/mL) on D-18 and D-25. In contrast, the blood glucose level in diabetic rats of Group II (without treatment with the ethanolic extract) remained higher on D-18 and D-25 ($p > 0.05$) (TABLE 1).

The effect of ethanolic extract of mahogany seeds on serum MDA level

Serum MDA level of all rats four days after STZ induction (Group II-V) were significantly higher than without STZ induction ($p < 0.05$) (TABLE 2). A significant decrease in the serum MDA level was observed 21 (D-25) days after the treatment with ethanolic extract of *S. macrophylla* seed (Group III-V) compared to before treatment (D-4) ($p < 0.05$). No significant difference in the serum MDA level was observed on D-4 compared to on D-25 in Group II ($p > 0.05$) (TABLE 2).

TABLE 1. Effect of ethanolic extract of *S. macrophylla* seed on blood glucose levels (mg/dL) in diabetic rats

Group	Treatment	Mean ± Standard Deviation			
		D-0	D-4	D-18	D-25
I	Aquadest	82.42 ± 5.24	77.75 ± 3.09	82.73 ± 5.04	84.90 ± 6.75
II	STZ + aquadest	74.83 ± 1.81	230.10 ± 2.99	233.48 ± 6.06	234.15 ± 3.23
III	STZ + extract 50 mg	74.12 ± 2.55	227.66 ± 8.69	183.28 ± 13.06	145.43 ± 5.83
IV	STZ + extract 100 mg	75.76 ± 2.45	230.45 ± 3.74	146.07 ± 14.81	124.57 ± 2.06
V	STZ + extract 200 mg	75.31 ± 3.28	230.86 ± 4.30	127.01 ± 23.55	102.92 ± 4.42

D-0: day on STZ induction; D-4: 4 days after STZ induction; D-18: 14 days after administration of ethaolic extract; D-25: 21 days after administration of ethanolic extract.

TABLE 2. Effect of ethanolic extract of *S. macrophylla* seed on serum MDA level (mg/dL) in diabetic rats

Group	Treatment	Mean ± Deviation Standard	
		D-4	D-25
I	Aquadest	2.27 ± 0.62	1.31 ± 0.13
II	STZ + aquadest	8.72 ± 0.64	8.56 ± 0.44
III	STZ + extract 50 mg	8.31 ± 0.77	5.23 ± 0.53
IV	STZ + extract 100 mg	8.08 ± 0.47	3.71 ± 0.42
V	STZ + extract 200 mg	8.22 ± 0.37	2.56 ± 0.35

D-4: 4 days after STZ induction; D-25: 21 days after administration of ethanolic extract.

The effect of ethanolic extract of mahogany seeds on serum creatinine level

Serum creatinine level of all rats four days after STZ induction (Group II-V) were significantly higher than without STZ induction ($p < 0.05$) (TABLE 3). A significant decrease in the serum creatinine level was observed 21 (D-25) days after the treatment with ethanolic extract of *S. macrophylla* seed (Group III-V) compared to before treatment (D-4) ($p < 0.05$). No significant difference in the serum creatinine level was observed on D-4 compared to on D-25 in Group II ($p > 0.05$) (TABLE 3).

TABLE 3. Effect of ethanolic extract of *S. macrophylla* seed on serum creatinine level (mg/dL) in diabetic rats

Group	Treatment	Mean ± Deviation Standard	
		D-4	D-25
I	Aquadest	1.26 ± 0.33	0.67 ± 0.04
II	STZ + aquadest	3.49 ± 0.15	3.90 ± 0.23
III	STZ + extract 50 mg	3.50 ± 0.26	2.12 ± 0.22
IV	STZ + extract 100 mg	3.62 ± 0.18	1.37 ± 0.34
V	STZ + extract 200 mg	3.56 ± 0.14	0.68 ± 0.09

D-4: 4 days after STZ induction; D-25: 21 days after administration of ethanolic extract.

Urine protein level of all rats four days after STZ induction (Group II-V) were significantly higher than without STZ induction ($p < 0.05$) (TABLE 3). A significant decrease in the urine protein level was observed 21 (D-25) days after the treatment with ethanolic extract of *S. macrophylla* seed (Group III-V) compared to before treatment (D-4) ($p < 0.05$). No significant difference in the urine protein level was observed on D-4 compared to on D-25 in Group II ($p > 0.05$) (TABLE 4).

TABLE 4. Effect of ethanolic extract of *S. macrophylla* seed on urine protein level (mg/dL) on diabetic rats

Group	Treatment	Mean ± Deviation Standard	
		D-4	D-25
I	Aquadest	2.27 ± 0.62	1.31 ± 0.13
II	STZ + aquadest	8.72 ± 0.64	8.56 ± 0.44
III	STZ + extract 50 mg	8.31 ± 0.77	5.23 ± 0.53
IV	STZ + extract 100 mg	8.08 ± 0.47	3.71 ± 0.42
V	STZ + extract 200 mg	8.22 ± 0.37	2.56 ± 0.35

D-4: 4 days after STZ induction; D-25: 21 days after administration of ethanolic extract.

DISCUSSION

In this study, STZ induction resulted in a significant increase in blood glucose level (Group II-V) compared to the control group (Group I). All rats on Group II-V had the blood glucose level higher than 200 mg/dL indicating the rats became diabetes. Diabetic Streptozotocin-induced diabetes has been described a useful experimental model to study the activity of antidiabetic agents.[18] Streptozoocin destroys β-cells of the pancreas and induces hyperglycemia.[11-13,19]

After administration of ethanolic extract of *S. macrophylla* seed to the diabetic rats on Group III-V for 21 days, a significant decrease in blood glucose level was observed which was close to the normal level (< 200 mg/dL). In addition, the antidiabetic effect of the ethanolic extract of *S. macrophylla* seed was in a dose-dependent manner (TABLE 1). The antidiabetic effect of *S. macropylla* seed extract has been previously reported by some authors.[9,10,17] Its mechanism of action as antidiabetic has been also investigated. Kalaivanan *et al*.[10] reported that ethanolic extract of mahogany seed can increase insulin production and stimulate the regeneration of β-cells of the pancreas. *Swietenia macropylla* seed extract was also reported can activate the enzyme glucokinase in lowering blood glucose levels.[9] Moreover, Maiti *et al.*[20] and Dewanjee *et al*.[21] have successfully isolated swietenine, a tetranortriterpenoid, from the *S. macrophylla* seeds and have proven its hypoglycemic activity in type 2 diabetic rats.

Serum MDA level of rats four days after STZ induction significantly higher than without STZ induction indicating that oxidative stress developed in diabetic rats after STZ induction. After administration of ethanolic extract of *S. macrophylla* seed to the diabetic rats on Group III-V for 21 days, a significant decrease in serum MDA level was observed (TABLE 2). It indicates that the ethanolic extract of *S. macrophylla* seed is able to correct the diabetes-induced oxidative stress. The ethanol extract of *S. macrophylla* seeds was proven to have antioxidant activity in the streptozotocin-induced diabetic rats.[17] Furthermore, the antioxidaive compounds from *S. macropylla* have been isolated such as swietemacrophyllanin, catechin, and epicatechin.[22]

Serum creatinine and urine protein level of rats four days after STZ induction significantly higher than without STZ induction indicating the decrease of renal function. Diabetes-associated oxidative stress causes glomerular hypertrophy, decrease of glomerular filtration rate and inhibits cell

proliferation that lead to the decrease of renal function.[5-7] After administration of ethanolic extract of *S. macrophylla* seed to the diabetic rats on Group III-V for 21 days, a significant decrease in serum creatinine and urine protein levels were observed (TABLE 3 and 4). It clearly demonstrates that the ethanolic extract of *S. macrophylla* seed is able to protect the decrease of renal function due to the diabetes-induced oxidative stress. As explained by Debasis *et al.*[9], the renal protective effect of the extract of *S. macrophylla* seed may be explained by two ways. One way may be due to the insulinotrophic effect of this extract that results correction in blood glucose level that prevents the ROS generation by preventing glucose autooxidant and by glication. Another way may be the presence of antioxidative compounds in this extract that lowered the levels of end products of free radicals like MDA.

CONCLUSION

In conclusion, the ethanolic extract of mahogany seed (*S. macropylla* King) is able to correct renal dysfunction of streptozotocin-induced diabetic rats as indicated by the decrease of serum creatinine and urine protein after administration of this extract. The renal protective effect of the ethanolic extract of mahogany seed may be due to its antidiabetic and antioxidant effects.

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