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Hypoglycemic Activity of *Gymnema sylvestre* Extracts on Oxidative Stress and Antioxidant Status in Diabetic Rats

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ABSTRACT: Diabetes mellitus, which is associated with oxidative damage, has a significant impact on health, quality of life, and life expectancy. An ethanol extract of *Gymnema sylvestre* leaf was examined in vitro and in vivo to investigate the role of antioxidants in diabetic rats. The extract exhibited strong antioxidant activity in the assays, including TBA (56%), SOD-like (92%), and ABTS (54%). Blood glucose levels in the diabetic rats fed *G. sylvestre* extract decreased to normal levels. The presence of the antihyperglycemic compounds gymnemagenin and gymnemic acids in *G. sylvestre* extract was detected by LC/MS analysis. Lipid peroxidation levels were decreased by 31.7% in serum, 9.9% in liver, and 9.1% in kidney in the diabetic rats fed the extract. Feeding *G. sylvestre* extract to the diabetic rats decreased the activity of glutathione peroxidase in cytosolic liver and glutamate pyruvate transaminase in serum to normal levels.

KEYWORDS: *antidiabetes, antioxidants, blood glucose level, ethanol extract of Gymnema sylvestre leaf*

INTRODUCTION

Diabetes mellitus is characterized as a consequence of a genetically based disorder and dietary indiscretion also known as diabetes type 2.[1] It is a chronic metabolic disease that has a significant impact on health, quality of life, and life expectancy.[2] Recently, an increase in patients with adult diseases, including diabetes, has become a serious problem in advanced countries. The prevalence of diabetes has been estimated to be 25.8 million in children and adults in the United States, which was about 8.3% of the population in the year 2010.[3]

It has been assumed that oxidative stress in the body is one of the most serious contributors to the occurrence of diabetes.[4] Even though our bodies possess some defense systems against oxidative damage such as the presence of superoxide dismutase (SOD), oxidative damage, which includes alteration in antioxidant enzymes,[5] impairment of glutathione metabolism, and decreased antioxidant levels, reportedly causes diabetes.[6] Therefore, antioxidants must play an important role in the prevention of diabetes.

Among 800 known Asian medicinal plants, several medicinal herbs have been known to be effective in reducing the incidence of diabetes.[7] *Gymnema sylvestre* R. Br., an herb cultivated in southern Asia and the East Indies, has been used frequently for the treatment of hyperglycemic patients.[8] The leaves of *G. sylvestre* are reported to lower blood sugar, to stimulate the heart, uterus, and circulatory systems, and to exhibit antisweet and hepatoprotective activities.[9] Recently, this leaf extract also demonstrated a potential hypoglycemic and subsequent blood cholesterol lowering property in streptozotocin-induced diabetic rats.[10]

In the present study, the antioxidant activity of *G. sylvestre* leaf was examined to investigate the role of antioxidants in diabetic rats. *G. sylvestre* leaf extract was also analyzed by LC/MS to identify possible components with antioxidant and antidiabetic activity.

MATERIALS AND METHODS

Chemicals and Reagents. All chemicals (analytical grade) including streptozotocin (STZ) and reagents used in the present study were obtained from Sigma Chemical Co. (St. Louis, MO, USA) except 2-thiobarbituric acid (TBA), which was purchased from MP Biomedical Co., Inc. (Denver, CO, USA). *G. sylvestre* fresh leaves were obtained from a local market in New Delhi, India, in 2008. The fresh leaves were air-dried in shade at room temperature for one week and then ground into fine powders with an electric blender (Tefal BL210 Blender, Tefal UK Ltd., Slough, Berkshire, U.K.). The *G. sylvestre* leaf powders were kept in an airtight container and stored in a refrigerator at 4 °C until used.

Preparation of Plant Extracts. *G. sylvestre* leaf extracts were prepared for in vitro chemical studies and in vivo animal studies by two different processes.

Extracts for Chemical Studies. The dried powders (50 g) were soaked in 2 L each of various ethanol/water solutions (0:100, 20:80, 40:60, 60:40, 80:20, and 100:0) overnight. An extract was filtered, and the residual materials were dissolved in 2 L of 100% ethanol, allowed to stand for 24 h, and then filtered. The two filtrates were combined, and the solvents were removed by a rotary evaporator at 40 °C under reduced pressure (95 mmHg) and then lyophilized by a freeze-dryer

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(IlShinBioBase, Kyungki, Korea). The greenish-black powder obtained (10−15 g) was stored at −4 °C until used.

Extract for Animal Studies. The dried powders (50 g) were soaked in 2 L of 100% ethanol overnight. The ethanol extract was filtered, and the residue was dissolved in 2 L of 100% ethanol again, allowed to stand for 24 h, and then filtered. The two filtrates were combined and the solvents removed by a rotary evaporator at 40 °C under reduced pressure (95 mmHg) and then freeze-dried to be lyophilized. The resultant greenish-black powder (10−15 g) was stored at −4 °C until used.

Determination of Total Phenolic Content. The total phenolic content of the extracts prepared for the chemical studies was calorimetrically determined according to the Folin−Denis procedure[11] with slight modifications. A solution of each extract (0.1 mL) and 2 mL of Na_2CO_3 (2%) was mixed and allowed to stand for 2 min at room temperature, after which 50% Folin−Ciocalteu (2N) reagent (2 mL) was added. After the reaction mixture had been allowed to stand for 30 min at room temperature, UV absorbance was measured at 750 nm using a spectrophotometer (Pharmacia Biotech, Ultraspec 3000, Cambridge, U.K.). The experiment was repeated three times.

Determination of in Vitro Antioxidant Activity of *G. sylvestre* Leaf Extracts. The antioxidant activity of the extracts was determined by various antioxidant assays using a spectrophotometer to measure the UV absorbance of the samples. The antioxidant activity was calculated using the following equation:

$$\text{antioxidant activity (\%)} = \frac{\text{absorbance of control} - \text{absorbance of testing sample}}{\text{absorbance of control}}$$

All assays were repeated three times.

Thiobarbituric Acid (TBA) Assay. The TBA assays were performed according to a previously reported method[12] with slight modifications, using egg yolk lecithin or 2-deoxyribose. Various concentrations of testing samples (0.2 mL) were added to an aqueous solution (2 mL) containing 200 μL of Tris buffer (pH 7.4), 300 μL of 1 M KCl, 400 μL of 1% sodium dodecyl sulfate (SDS), egg yolk lecithin (0.1 mg), 40 μL of $FeCl_2$ (1.0 μM), and 20 μL of H_2O_2 (0.5 μM) in a brown non-transparent vial (10 mL) to avoid any oxidation caused by UV irradiation. Samples were incubated for 30 min at 37 °C while shaking, and then oxidation was terminated by adding 50 μL of 4% BHT solution in ethanol solution. Two milliliters of the TBA reagent solution (0.67% TBA, 0.67% trichloroacetic acid, 1% SDS, 5 N HCl) was added, and the sample solutions were heated at 100 °C for 45 min and then cooled in an ice bath for 10 min. A blank sample was prepared following the same procedure without a test sample. The TBA−MA adduct formed was measured by a spectrophotometer at 532 nm.

TBA assays were also performed using 2-deoxyribose as the oxidized agent according to a previously reported method.[13] Briefly, the solution containing 200 μL of 10 mM $FeSO_4 \cdot 7H_2O$, 10 mM EDTA, and 10 mM 2-deoxyribose was mixed in a 1.8 mL phosphate buffer solution (0.1 M, pH 7.4), and then a 200 μL H_2O_2 solution (10 mM) was added. The solution was incubated at 37 °C for 2 h. After incubation, 2.8% trichloroacetic acid (1 mL) and 1.0% TBA (1 mL) were added to the reaction mixture. After the reaction mixture had been boiled for 10 min and cooled, its absorbance was measured at 532 nm.

Superoxide Dismutase (SOD)-like Activity Assay. SOD-like activity was assayed according to the previously reported method.[14] The reaction solution was prepared by mixing 0.2 mL of the sample solution, 3 mL of the Tris-HCl buffer (50 mM + 10 mM EDTA, pH 8.5), and 0.2 mL of 7.2 mM pyrogallol. The solution was allowed to stand for 10 min at 25 °C. The pyrogallol oxidized was measured at 420 nm using a spectrophotometer after the reaction was terminated by adding 0.1 mL of 1.0 N HCl solution.

2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Assay. ABTS assays were performed according to the previously reported method.[12] Each test sample (0.1 mg) dissolved in methanol (1 mL) was mixed with 100 μL of 0.1 M phosphate buffer solution (pH 5.0) and 20 μL of H_2O_2 solution (100 μM) in a test tube (10 mL). After the solution had been incubated at 37 °C for 5 min, 30 μL of ABTS (1.25 mM) and 30 μL of peroxidase (1 unit/mL) were added. Again, the solution was incubated at 37 °C for 10 min. The absorbance was read with an ELISA plate reader at 405 nm.

LC/MS Analysis of *G. sylvestre* Components. Identification of chemicals in *G. sylvestre* leaf extracts was conducted by an AQAmax series LC/MS single-quadrupole mass spectrometer (Thermoquest Co., Manchester, U.K.) with electrospray ionization (ESI). Chromatographic separation was accomplished with a 100 × 4.6 mm Varian Polaris RP column (Varian, Walnut Creek, CA, USA) with a 3 μm particle size at 30 °C. The HPLC mobile phase consisted of 0.1% methanol/formic acid (9/1, v/v) set at a flow rate of 0.1 mL/min. The ESI source was set at the positive ionization mode.

Treatment of Experimental Animals for in Vivo Studies. Sprague−Dawley male rats (180−200 g body wt) (Samtaco Co., Osan, Korea) were housed individually at 24 ± 1 °C in metabolic cages, permitting separate collection of urine and feces, with a 12 h light/12 h dark cycle. All rats were allowed free access to a diet (Super Feed Clear Corp., Korea) and water for 1 week for adaptation to the new environment. The food intake was equal in both groups. The Laboratory Animal Care Advisory Committee of Hoseo University, Korea, approved all animal protocols.

Diabetes was induced in overnight-fasted rats by intravenous injection of 200 mg/kg body weight streptozotocin (STZ, Sigma Chemical Co., St. Louis, MO, USA) using a 5% solution of freshly prepared streptozotocin in 0.1 M citrate buffer (pH 4.5). Rats in the normal group were injected with a 0.1 M citrate buffer (pH 4.5) solution alone.

A total of 40 rats (20 STZ-treated diabetic rats, 20 normal rats) were fed a basal diet[15] for 4 weeks. The rats were divided into four groups (10 rats/group): group 1 (control), rats were fed the basal diet alone; group 2 (*G. sylvestre*-fed), rats were fed the basal diet plus *G. sylvestre* extract (100 mg/kg/bw/day); group 3 (STZ-treated), STZ-treated rats were fed the basal diet alone; group 4 (STZ-treated−*G. sylvestre*-fed), STZ-treated rats were fed the basal diet plus *G. sylvestre* extract (100 mg/kg/bw/day).

Sample Preparations for Analysis of Substrates from Experimental Animals. Blood, liver, and kidney samples were obtained from the rats treated according to the method described above. The blood was obtained from a tail vein once a week 2 h after fasting to monitor the glucose levels. For serum, liver, and kidney samples the rats were fasted for 12 h and then killed by anesthetization with diethyl ether. Blood was taken from the abdominal aorta and then allowed to clot at room temperature for 30 min. The clot was removed by centrifuging at 2000*g* for 10 min in a refrigerated centrifuge. The resulting serum was separated and stored at −80 °C until used.

After the liver and kidney were removed and washed with a cold phosphate-buffered saline solution (PBS, pH 7.4), a portion of each liver and kidney was homogenized in a cold PBS solution using a homogenizer (T 10 basic Ultra-Turrax, IKA Works Inc., Wilmington, NC, USA). Samples were stored at −80 °C.

The preparation of the cytosolic liver for glutathione analysis and determination of enzyme activity were conducted as follows: The liver tissue (1 g) was homogenized with 9 mL of 0.1 mM PBS buffer. The homogenate was centrifuged at 2000*g* for 10 min, and then the supernatant was further centrifuged at 38000*g* for 60 min. After centrifuging, the supernatant was removed and stored at −80 °C until used. The protein level was measured in the supernatant according to a previously reported method.[16]

Measurement of Chemicals in the Substrates from Experimental Animals. Measurement of glucose levels in whole blood and levels of insulin, total triglycerides, cholesterol (high-density lipoprotein (HDL), low-density lipoprotein (LDL), and total cholesterol) was conducted to investigate the role of *G. sylvestre* leaf extract in diabetes. Glutathione content in cytosolic liver supernatant was also measured. All experiments were repeated three times.

Glucose in Whole Blood. The glucose levels were determined using a whole blood glucose analyzer (Super Glucocard TMII, Dongbang Co., Incheon, Korea).

Plasma Insulin in Serum. Serum insulin was determined with an enzyme-linked immunosorbent assay (ELISA) using a Boehringer-Mannheim (Mannheim, Germany) kit with a Boehringer Analyzer ES300 according to a previously reported method.[17]

Lipids in Serum. Total triglycerides and cholesterols in the serum were determined by enzymatic colorimetric assay (Hitachi 917, Roche Diagnostics, Mannheim, Germany). The HDL-cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate−magnesium. LDL-cholesterol was calculated using the Friedewald formula.

Glutathione in Cytosolic Liver. The amount of glutathione was determined according to a previously reported method.[18] Briefly, 1 mL of supernatant prepared as described above was mixed with 0.5 mL of Ellman's reagent (19.8 mg of 5,5-dithiobis(nitrobenzoic acid) in 100 mL of 0.1% sodium nitrate) and 3 mL of PBS (0.2 M, pH 8.0) at 37 °C for 30 min. The absorbance was read at 412 nm.

TBA Assay on Samples from the Liver and Kidney. Aliquots of each homogenate (1 mL) were incubated at 37 °C for 2 h in PBS (10 mmol/L, pH 7.4) containing ferrous sulfate (15 μmol/L). To measure 2-thiobarbituric acid reactive substances (TBARS), 1 mL of each mixture was mixed with 2 mL of trichloroacetic acid (0.46 mol/L) in a test tube. After centrifugation at 700*g* for 10 min, 2 mL of each supernatant was added to 1 mL of TBA (49 μmol/L) solution. The reaction mixture was boiled for 10 min and allowed to cool, and the absorbance at 532 nm was measured. TBARS were calculated as malonaldehyde (MA) equivalents.[12] The assay was repeated three times.

Measurement of Enzyme Activities. The effect of *G. sylvestre* extract toward activities of enzymes was investigated to examine the role of *G. sylvestre* extract in diabetes. The enzymes tested were glutathione-*S*-transferase (GST), glutathione peroxidase (GSH-Px), catalase in cytosolic liver, and glutamate oxaloacetate transaminase (GOP) and glutamate pyruvate transaminase (GPT) in serum. The experiments were repeated three times.

Catalase Activity. Catalase was measured using a previously reported method.[19] Reaction mixtures (1.5 mL) containing 1 mL of PBS (0.01 M, pH 7.0), 0.1 mol of a tissue homogenate, and 0.4 mL of 2 M H_2O_2 solution were incubated at 37 °C for 15 min. The reaction was stopped by the addition of 2.0 mL of dichromate−acetic acid reagent (5% potassium dichromate/glacial acetic acid, 1:3). Catalase activity was monitored at 620 nm using a calorimeter (Pharmacia Biotech, Ultraspec 3000, Cambridge, U.K.) and expressed as moles of H_2O_2 consumed per minute per milligram of protein.

GSH-Px Activity. GSH-Px activity was measured according to a previously reported method.[20] Briefly, a PBS solution (0.8 mL) containing 1 mM EDTA, 1 mM $NaHCO_3$, 0.2 mM NADPH, 1 unit/mL glutathione reductase, 1 mM glutathione, and 100 mM KH_2PO_4 (pH 7.0) was mixed with the 0.2 mL of cytosolic liver supernatant and incubated at 37 °C for 10 min. After 0.2 mM H_2O_2 (0.2 mL) had been added, $NADP^+$ was monitored at 340 nm. GSH-Px activity was determined by monitoring the formation of $NADP^+$ from NADPH in the catalytic cycle.[21] GSH-Px activity was expressed as glutathione consumed in units per milligram of protein per minute and reduced glutathione as micrograms per milligram of protein.

GST Activity. GST activity was determined spectrophotometrically according to a previously reported method. An aqueous solution (3.0 mL) containing a homogenate (0.1 mL), 0.3 M PBS (1.0 mL, pH 6.5), and 0.1 mL of 1-chloro-2,4-dinitrobenzene solution (30 mM) was incubated at 37 °C for 15 min. After 0.1 mL of glutathione had been added, the change in optical density (OD) was read at 340 nm for 3 min at 30 s intervals. A reaction mixture without the enzyme was used as a blank. The GST activity was expressed as units per milligram of protein per minute.

GOP and GPT. The activities of GOP and GPT were determined using a previously reported method.[22] The serum (0.2 mL) was incubated at 37 °C for 1 h with the assay mixture containing 1 mL of *o*-dianisidine solution (10 mg/mL) and 1 mL of 0.1 M PBS (pH 7.0) in a test tube (5 mL). The reaction was stopped by the addition of 1 mL of 2,4-dinitrophenyl hydrazine (DNPH). After the reaction solution had been allowed to stand at room temperature for 30 min, 5 mL of 0.4 N NaOH solution was added, and the color that developed was monitored at 540 nm. The activity was expressed as micromoles of pyruvate liberated per milligram of protein per hour.[23]

Statistical Processing. The results of the present study were averaged, and the comparison between experimental groups was drawn through an ANOVA based on the SAS system. After the ANOVA, the level of significance was computed using Duncan's multiple-range test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Antioxidant Activities of Extracts from *G. sylvestre* Leaf. It is recommended that at least two different assays be performed to determine the antioxidant activity of target samples. Therefore, three commonly used assays were used in the present study. One was the TBA assay, which is a type of assay associated with lipid peroxidation. The other two were the ABTS assay and the SOD-like assay, which are types that are involved in electron or radical scavenging.[12]

Results of the TBA Assays. Figure 1 shows the results of antioxidant activity of the ethanol extracts. In the assay with egg yolk, all of the samples exhibited moderate antioxidant activities. The extract from 80% ethanol showed the strongest antioxidant activity (55.56 ± 3.56%), which was higher than that of α-tocopherol (47.38 ± 0.86%), followed by 100% extract (50.67 ± 5.16%), 20% extract (47.38 ± 1.04%), and water extract (46.49 ± 5.18%). Because the known antioxidant α-tocopherol exhibited only moderate activity, this TBA assay system may not respond well to antioxidants.

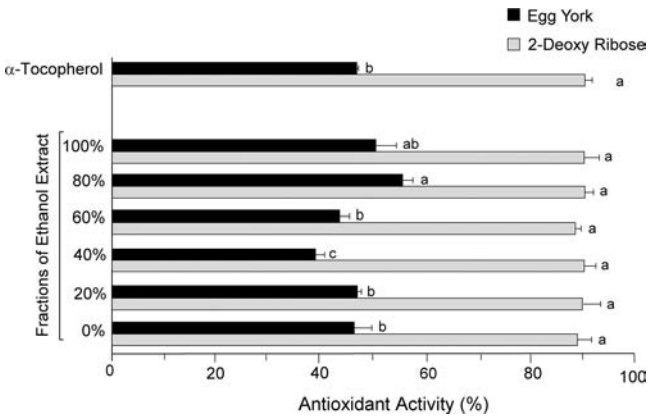

Figure 1. Antioxidant activity of the ethanol extracts from *G. sylvestre* leaf tested by TBA assays. Values are the mean ± SD ($n = 3$). Letters indicate significant levels computed by Duncan's multiple-range test at $\alpha = 0.05$ after the ANOVA.

On the other hand, all extracts showed strong antioxidant activity in the TBA assay with 2-deoxyribose ranging from 90.80 ± 2.36% (80% ethanol extract) to 88.93 ± 4.07% (water extract). These activities were comparable to that of α-tocopherol (90.80 ± 2.44%).

Results of SOD-like and ABTS Assays. Figure 2 shows the results of antioxidant activity of the ethanol extracts tested with two different assays. All extracts exhibited strong antioxidant activities ranging from 91.67 ± 0.45% (80% ethanol extract) to 74.01 ± 2.26% (water extract) in the SOD-like assay. Their activities were comparable to those of α-tocopherol (99.13 ± 0.10%). The antioxidant activities of ethanol extracts tested by the ABTS assay were relatively lower than those of the SOD-like assay. The values ranged from 54.02 ± 0.27% (80% ethanol extract) to 27.60 ± 0.82% (60% ethanol extract). α-Tocopherol

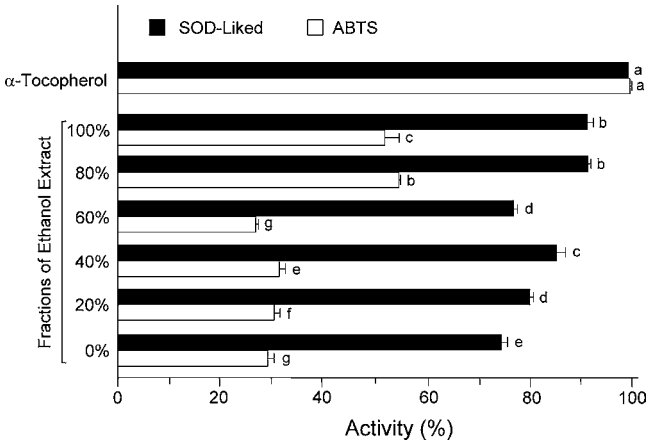

Figure 2. Antioxidant activity of the ethanol extracts from *G. sylvestre* leaf tested by SOD-like and ABTS assays. Values are the mean ± SD (n = 3). Letters indicate significant levels computed by Duncan's multiple-range test at α = 0.05 after the ANOVA.

exhibited potent antioxidant activity (99.61 ± 0.68%) in the ABTS assay. The ABTS assay, which is also called the ABTS radical assay, has been widely used to evaluate antioxidant activities of components in foods and beverages due to its applicability in aqueous and lipid phases.[12]

The results obtained from antioxidant assays in the present study indicate that *G. sylvestre* leaf contains potent antioxidants. The phenolic compounds found in natural plants are known to have a number of beneficial health effects associated with natural antioxidants,[24] such as suppressing LDL-cholesterol oxidation[25] and reducing the risk of heart disease.[26] Therefore, the total amount of phenolic compounds in the extracts from *G. sylvestre* leaf was determined. The results showed that all extracts contained appreciable levels of phenolic compounds ranging from 47.84 ± 0.37 μg/mL (80% ethanol extract) to 22.94 ± 0.19 μg/mL, suggesting that the antioxidant activity of the extracts was at least partially achieved by phenolic components. The SOD present in the body is known to inhibit the reaction of converting active oxygen into hydrogen peroxide in cells. In addition, the intake of food with high antioxidant contents has been recommended to prevent the diseases caused by oxidation.[27]

LC/MS Analysis of 80% Ethanol Extract of *G. sylvestre* Leaf. Figure 3 shows the LC/MS chromatogram of 80% ethanol extract from *G. sylvestre* leaf. Peaks at 4.93, 5.20, and 5.35 min were tentatively identified as gymnemoside-c, gymnemoside-d, and gymnemoside-e, respectively. The quasi-molecular ion peak of gymnemosides was observed at m/z 829 $[M + H]^+$ from gymnemoside-c, at m/z 947 $[M - H]^-$ from gymnemoside-d, and at m/z 1255 $[M - H]^-$ from gymnemoside-e. These triterpene glycosides have been isolated and identified in the leaves of *G. sylvestre* previously, and the structures of these triterpene glycosides have also been well elucidated with their MS and NMR.[28] Comparison of these published MS data with the MS of the peaks in Figure 3 allowed us to tentatively identify the above three chemicals.

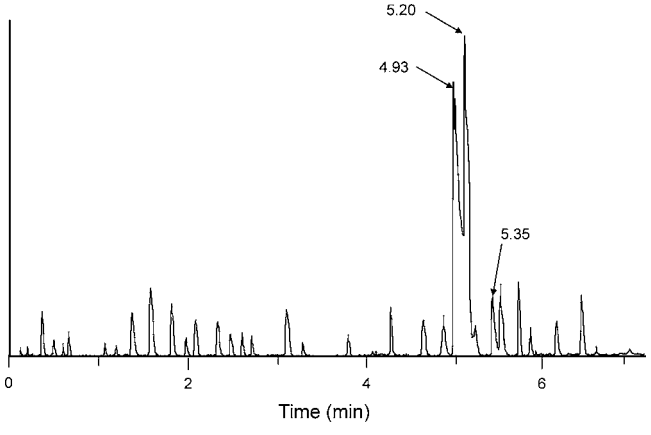

Figure 3. Typical LC/MS chromatogram of 80% ethanol extract from *G. sylvestre* leaf.

Results of in Vivo Studies on *G. sylvestre* Extract. The body weights of the experimental rats are shown in Table 1. The initial weight of the rats ranged from 190 to 195 g, indicating that the average initial body weight was not significantly different across the four groups. The body weight of the control group increased the most, by 52.7%, after 4 weeks, whereas the STZ-treated group lost weight (by 3.6%). Among the rats with diabetes, feeding the *G. sylvestre* extract increased body weight slightly, by 12.5%.

Table 1 also shows the weight of the organs collected from the experimental rats after 4 weeks. Feeding *G. sylvestre* extract increased liver weight slightly, whereas weight change was not observed in the case of the kidney. However, livers and kidneys from the diabetic rats were significantly heavier than those from the normal rats, suggesting that diabetes increases the weight of the liver and kidney.

Blood Glucose Levels of Experimental Rats. Figure 4 shows the results of blood glucose analysis in the four groups of experimental rats. One week after the injection of STZ was set as zero week because generally diabetes is induced within one week after STZ injection. The glucose levels of the two normal groups (control and *G. sylvestre*-fed groups) were maintained at normal levels (near 100/dL) over 7 weeks. The glucose levels of the diabetic groups (STZ-treated and STZ-treated–*G. sylvestre*-fed) increased to 482.2 ± 89.3 and 435.8 ± 129.4 mg/dL, respectively, by the second week. The glucose levels of the STZ-treated group continued to increase to the sixth week (569.8 ± 48.7 mg/dL), whereas that of the group fed *G. sylvestre* leveled off after the second week and decreased slightly until the animals were sacrificed in the seventh week. These results were consistent with previous papers.[29] The present study showed that *G. sylvestre* decreased the level of glucose in diabetic rats. The effect of *G. sylvestre* extract in decreasing the elevated blood glucose of the rats to normal levels is an essential trigger if the liver is to return to its normal homeostasis during experimental diabetes. *G. sylvestre* is reported to be rich in gymnemagenin and gymnemic acids, the presence of which in *G. sylvestre* extrzact was recognized by LC/MS analysis in the present study, that are responsible for the antihyperglycemic effect.[30]

Amounts of Serum Insulin, Triglycerides, Total Cholesterol, HDL, and LDL in Experimental Rats. Table 2 shows the results of the serum analysis of the experimental rats. Feeding *G. sylvestre* extract increased insulin levels slightly in the control rats (8.4% increase). On the other hand, feeding *G. sylvestre* extract increased the insulin levels significantly in diabetic rats (48% increase), suggesting that *G. sylvestre* can be used to treat diabetes. A previous study showed that *G. sylvestre* enhanced the production of endogenous insulin.[31] It is proposed that a part of the antihyperglycemic activity of this plant is due to its effect in enhancing release of insulin from the pancreas. Earlier

Table 1. Body Weights of Experimental Rats and Their Liver and Kidney Weights after 4 Weeks[a]

	body weight (g)			weight of organs (mg/100 g bw)	
	initial	final	gain	liver	kidney
control	192.0 ± 0.4 NS	293.3 ± 17.2 a	101.1 ± 17.1 a	30.3 ± 4.0 b	7.0 ± 2.0 b
G. sylvestre-fed	190.0 ± 0.6 NS	183.3 ± 36.8 c	−6.9 ± 36.7 c	40.0 ± 8.0 a	11.0 ± 1.0 a
STZ-treated	191.0 ± 0.4 NS	281.3 ± 18.9 a	90.0 ± 18.9 a	34.0 ± 2.0 b	7.0 ± 4.0 b
STZ-treated−*G. sylvestre*-fed	194.9 ± 0.3 NS	219.2 ± 46.1 b	24.3 ± 46.2 b	43.0 ± 10.0 a	11.0 ± 2.0 a

[a]Values are the mean ± SD (n = 10). NS, not significant. Letters indicate significant levels computed by Duncan's multiple-range test at α = 0.05 after the ANOVA.

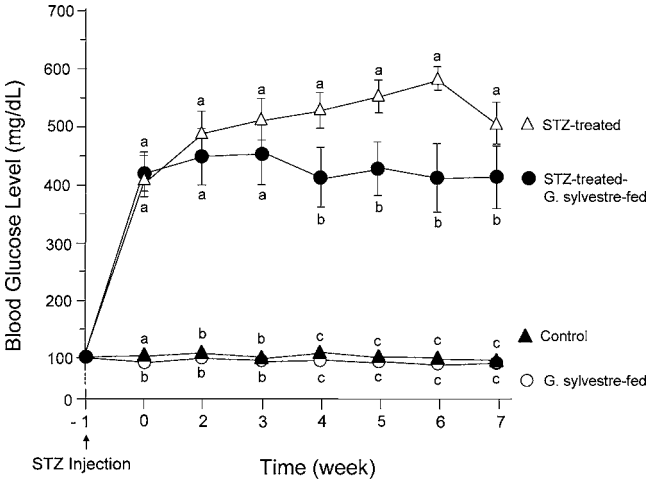

Figure 4. Results of blood glucose analysis in the four groups of experimental rats. Values are the mean ± SD (n = 10). Letters indicate significant levels computed by Duncan's multiple-range test at α = 0.05 after the ANOVA.

studies have reported that the extract of *G. sylvestre* reduced blood glucose and increased plasma insulin levels in diabetic rats.[32] The results of the present study are consistent with those of earlier studies.

Feeding *G. sylvestre* extract decreased the levels of serum triglyceride in control and STZ-treated rats by 6.7 and 22.3%, respectively. Oral administration of *G. sylvestre* extract increased the levels of HDL slightly, whereas it decreased LDL levels considerably (27.4% decrease) in the diabetic group (STZ-treated−*G. sylvestre*-fed). However, feeding *G. sylvestre* extract decreased the total cholesterol levels significantly in both control (15.9% decrease) and diabetic rats (17.7% decrease). The results of the present study clearly show that *G. sylvestre* extract has a transitory lowering activity on lipid levels in serum. There is substantial evidence that lowering the serum lipid level, particularly the LDL level, would lead to a reduction in the incidence of coronary heart disease.[33] As there is a close relationship between elevated serum triglyceride levels and the occurrence of atherosclerosis,[34] the efficacy of the extract in selective reduction of triglycerides through the reduction of LDL components could be beneficial in preventing atherosclerotic conditions, thereby reducing the possibility of coronary heart disease in general.

Effect of G. sylvestre Extract toward in Vivo Lipid Peroxidation. One previous study reported that antioxidants inhibited lipid peroxidation in STZ-induced diabetic rat tissues.[35] It is well-known that lipid peroxidation produces many so-called secondary oxidation products, some of which have been used to determine the occurrence of lipid peroxidation. Among the secondary oxidation products, malonaldehyde (MA) has been widely used to study the last stage of lipid peroxidation in the TBA assay.[12] Therefore, the TBA assay was used to investigate the in vivo antioxidant activity of *G. sylvestre* extract. The results of the TBA assay are shown in Figure 5. It is

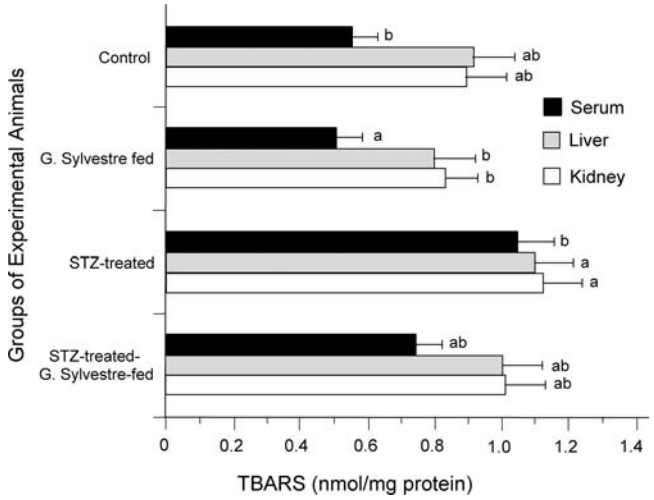

Figure 5. Results of in vivo antioxidant activity of *G. sylvestre* extract tested by the TBA assay. Values are the mean ± SD (n = 10). Letters indicate significant levels computed by Duncan's multiple-range test at α = 0.05 after the ANOVA.

obvious that the levels of MA in the samples from the rats fed *G. sylvestre* were lower than those in the samples from rats not fed *G. sylvestre*. The diabetic rats (STZ-treated) exhibited the highest levels of MA (1.04 ± 0.22 nmol/g protein from serum,

Table 2. Amounts of Serum Insulin, Triglycerides, Total Cholesterol, HDL, and LDL in Experimental Rats[a]

			cholesterol (mg/dL)		
	insulin (μIU/mL)	triglycerides (mg/dL)	HDL	LDL	total
control	15.30 ± 1.60 a	68.44 ± 6.08 c	26.10 ± 4.48 a	14.50 ± 1.77 c	48.90 ± 21.51 c
G. sylvestre-fed	16.70 ± 2.46 a	63.85 ± 5.45 c	29.80 ± 5.43 a	12.50 ± 1.77 c	41.11 ± 17.72 c
STZ-treated	5.70 ± 1.95 c	101.75 ± 8.11 a	16.12 ± 3.44 b	27.87 ± 2.41 a	86.62 ± 10.15 a
STZ-treated−*G. sylvestre*-fed	10.90 ± 1.50 b	79.11 ± 5.81 b	20.55 ± 6.76 b	20.22 ± 3.80 b	71.33 ± 9.12 b

[a]Values are the mean ± SD (n = 10). Letters indicate significant levels computed by Duncan's multiple-range test at α = 0.05 after the ANOVA.

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Table 3. Glutathione Content and Enzyme Activity of Glutathione Peroxidase (GSH-Px), Glutathione-*S*-transferase (GST), and Catalase in Rat Cytosolic Liver[a]

	glutathione content (μg/mL)	enzyme activity		
		GSH-Px (nmol/NADPH oxidized/min)	GST (units/mg protein/min)	catalase (mU/mg protein)
control	29.46 ± 0.29 a	6.06 ± 0.29 a	0.18 ± 0.0 a	36.13 ± 0.82 a
G. sylvestre-fed	30.33 ± 0.79 a	6.17 ± 0.29 a	0.18 ± 0.01 a	37.11 ± 1.22 a
STZ-treated	20.54 ± 1.14 c	2.61 ± 0.36 c	0.13 ± 0.01 c	16.07 ± 1.54 c
STZ-treated−*G. sylvestre*- fed	27.29 ± 0.86 b	5.02 ± 0.90 b	0.16 ± 0.01 b	25.50 ± 1.03 b

[a]Values are the mean ± SD (n = 10). Letters indicate significant levels computed by Duncan's multiple-range test at α = 0.05 after the ANOVA.

1.11 ± 0.20 nmol/mg protein from liver, and 1.10 ± 0.4 nmol/mg protein from kidney), but those levels were decreased by 31.7% in serum, 9.9% in liver, and 9.1% in kidney in the rats fed *G. sylvestre* (STZ-treated−*G. sylvestre*-fed). The present study indicates that lipid peroxidation occurring in the plasma of diabetic rats can be reduced by *G. sylvestre* extract, which is consistent with previous papers.[36]

Effect of *G. sylvestre* Extract toward Glutathione Content and Activity of Enzymes Found in Rat Cytosolic Liver. Table 3 shows the results of activity studies on the enzymes glutathione-*S*-transferase (GST), glutathione peroxidase (GSH-Px), and catalase in rat cytosolic liver along with the glutathione content.

Glutathione Content. In the case of normal groups, the GSH concentration did not show significant differences. The amounts of glutathione increased by 24.7% when *G. sylvestre* extract was fed to diabetic rats. The functions of glutathione have been proposed to associate with many cellular processes including the protection of cells against xenobiotics, carcinogens, radiation, and reactive oxygen species (ROS).[37] Glutathione is also known to be the first line of defense against in vivo oxidation.[34] In the present study, *G. sylvestre* significantly increased the glutathione levels. Therefore, these previous studies and the results of the present study suggest that glutathione plays an important role in the prevention or reduction of oxidative stress. An increase in glutathione level may in turn activate the glutathione-dependent enzymes such as glutathione peroxidase and glutathione-*S*-transferase, which will be discussed next.

GSH-Px Activity. In the case of the normal groups (control and *G. sylvestre*-fed), the GSH-Px activity did not show significant differences. However, feeding *G. sylvestre* extract to the diabetic rats (STZ-treated) decreased GSH-Px activity by 78.7%. GSH-Px has been known as one of the major protective enzymes against the accumulation of peroxides,[38] suggesting that GSH-Px was consumed to protect against diabetes and, thus, that GSH-Px depressed its activity in diabetic rats. These reports and the results of the present study suggest that *G. sylvestre* extract prevents in vivo oxidative damage.

The increased GSH-Px content in the liver and kidney tissues of the rats treated with *G. sylvestre* leaf extracts may be one of the factors responsible for regulating levels of antioxidant markers including GST, GSH-Px, and glutathione. Glutathione has also been known to possess antioxidant activity.[39]

GSH-Px might play an important role in peroxidative stress because the depression of GSH-Px activity was observed in the tissues of diabetic rats.[40]

GST Activity. GST activity in the cytosolic livers of the control rats was not changed by *G. sylvestre* extract feeding. In the case of the normal group (control vs *G. sylvestre*-fed), GST activity did not show significant differences, whereas in the case of the diabetic groups (STZ-treated vs STZ-treated−*G. sylvestre*-fed), feeding *G. sylvestre* increased the level of GST activity moderately by 23.1%. These results indicate that increasing GST activity by *G. sylvestre* extract is beneficial to preventing or reducing the severity of diabetes.

Catalase Activity. In the case of the normal group, catalase activity did not show significant differences, whereas in the case of the diabetic group (STZ-treated vs STZ-treated−*G. sylvestre*-fed), feeding *G. sylvestre* extract increased catalase activity by 55.94%. Catalase is a major antioxidant defense enzyme that detoxifies reactive oxygen radicals by catalyzing the decomposition of H_2O_2 to H_2O with GSH-Px.[41] The liver, kidney, and red blood cells possess relatively high levels of catalase. The present results indicate that *G. sylvestre* extract increases catalase activity and consequently increases the antioxidant activity of organs.

Effect of *G. sylvestre* Extract toward Activity of Enzymes Found in Rat Serum. Table 4 shows the results of activity studies on the enzymes, glutamate oxaloacetate transaminase (GOP) and glutamate pyruvate transaminase (GPT), found in rat serum. The levels of GOT and GPT activity increased slightly by feeding *G. sylvestre* leaf extract to the normal group (control vs *G. sylvestre*-fed). However, feeding *G. sylvestre* leaf extract to the STZ-treated rats decreased the levels of serum GOP and GPT activity considerably, by 30.9 and 32.6%, respectively. The previous study reported elevation of transaminase activity (GOT and GPT) in the liver and kidney in addition to the serum from the STZ-treated rats and the role of these transaminases in gluconeogenesis and ketogenesis in diabetes.[42] The restoration of GOT and GPT activities to their respective normal levels after supplementation of *G. sylvestre* extract further strengthened the antidiabetogenic effect of this extract. Moreover, the present study suggests that GOT and GPT levels can also act as indicators of liver function.

Table 4. Effect of *G. sylvestre* Extract on the Enzyme Activity of Glutamate Oxaloacetate Transaminase (GOP) and Glutamate Pyruvate Transaminase (GPT) in Rat Serum[a]

	activity (μmol pyruvate liberated/mg protein/h)	
	GOP	GPT
control	70.50 ± 7.44 c	26.4 ± 3.59 c
G. sylvestre-fed	72.57 ± 5.27 c	27.4 ± 3.97 a
STZ-treated	149.25 ± 15.30 a	48.25 ± 5.33 a
STZ-treated−*G. sylvestre*-fed	103.87 ± 8.25 b	32.5 ± 3.77 b

[a]Values are the mean ± SD (n = 10). Letters indicate significant levels computed by Duncan's multiple-range test at α = 0.05 after the ANOVA.

The use of traditional medicine and medicinal plants in developing countries is becoming popular as a medical alternative in the treatment of various diseases including diabetes. It has been known that oxidative damages are associated with a number of disease processes including diabetes mellitus.[43] In the present study, the extract from *G. sylvestre* leaf exhibited potent antioxidant activities and increased the activity of enzymes beneficial in the prevention of diabetes.

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Notes

The authors declare no competing financial interest.

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