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ORIGINAL ARTICLE

Normalization of Insulin Resistance, Glucose Intolerance, and Lipid Profile by *Swietenia Mahagoni* (L.) Jacq. Leaf Extract in Fructose-Induced Diabetic Rats

Naveen Y. Puttaswamy, Asna Urooj

Department of Studies in Food Science and Nutrition, University of Mysore, Mysore, Karnataka, India

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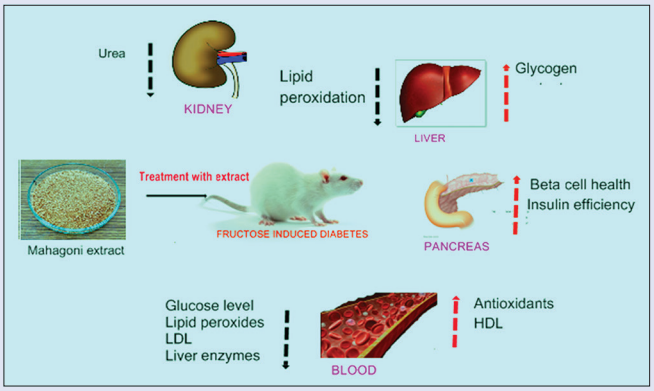
ABSTRACT

Ethanopharmacological Relevance: *Swietenia mahagoni (L.) Jacq.* is a medicinal plant used in various medicinal practices for the treatment of various human ailments. **Materials and Methods:** The present study evaluates the antidiabetic potential of *Swietenia mahagoni aueous* extract (MAE) in fructose-induced diabetic rats with insulin resistance. MAE was analyzed using high-performance liquid chromatography (HPLC) and liquid chromatography mass spectrometry (LCMS) for the identification of constituent bio-actives present. **Results:** MAE treatment improved glucose levels, reduced the insulin resistance, improved glucose tolerance, improved pancreatic β-cell health, decreased glycated hemoglobin content, reduced lipid peroxides, and improved glycogen content and the activity of enzymes involved in the synthesis of glycogen in the liver. There was also overall improvement in the antioxidant status of the treated groups when compared with that of the diabetic control. The treatment also improved the lipid profile of diabetic rats. The HPLC analysis indicated the presence of gallic acid, scopoletin, coumarin, epicatechin, and ellagic acid, and their presence was confirmed by LCMS analysis. **Conclusion:** Results supported the antidiabetic potential of MAE in animal model where it can act on multitargets and ameliorate the overall diabetic condition rather targeting to control hyperglycemia. Thus, the study supports the scope of *Swietenia mahagoni* as an adjuvant in type 2 diabetic patients.

Key words: Antidiabetic, fructose model, high-performance liquid chromatography, insulin resistance, lipid profile, *Swietenia mahagoni* (L.) Jacq

SUMMARY

- The present research work is a preclinical evaluation of antidiabetic potential of *Swietenia mahagoni* leaf extract (MAE) in fructose-induced diabetic rat model, which simulates the pathophysiological condition present in type II diabetic patients. The physical observations, biochemical results, and histopathological results indicated the MAE. The extract along with improving the glycemic status also ameliorated the insulin resistance, improved the health of beta-cells of pancreas, reduced oxidative stress, and improved lipid profile. These observations were also supported by improved body weight, polyphagia, polydipsia, and overall wellness of the animals. With the support of the results of the current investigation, we strongly support the MAE and its usage as an adjuvant in the management of diabetes mellitus.



Abbreviations used: ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; TBARS: Thiobarbituric acid-reactive substance; MET: Metformin; OGTT: Oral Glucose Tolerance Test; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; HOMA-β: Homeostatic Model Assessment for Beta-Cell Health; HPLC: High-performance liquid chromatography; LCMS: Liquid chromatography–mass spectrometry; M/Z: Mass/charge ratio; HbA1c: Hemoglobin A1c; CQS: Chloqine resistant; HIV: Human immunodeficiency virus; BW: Body weight.

Correspondence:

Dr. Asna Urooj,
Department of Studies in Food Science and Nutrition, University of Mysore,
Manasagangothri, Mysore, Karnataka, India.
E-mail: asnaurooj@foodsci.uni.mysore.ac.in
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INTRODUCTION

Diabetes is a chronic metabolic disease characterized by relative or absolute lack of insulin, resulting in hyperglycemia. Prolonged hyperglycemia will lead to a variety of secondary complications such as retinopathy, nephropathy, neuropathy, and increased risk of cardiovascular disease. Recent data on worldwide prevalence of diabetes show that 9.2% of female and 9.8% of male population are diabetic, with approximately 347 million people suffering from the disease worldwide in 2008.[1]

Several effective antidiabetic drugs are available for the management of diabetes; however, few drugs have been developed which have no side effect(s). Since the existing drugs for the treatment of diabetes do not satisfy our need completely, the search for new drugs continues. In recent years, herbal remedies for the unsolved medical problems have been gaining immense importance in the field of pharmacology. Traditional

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knowledge with its holistic and systematic approach supported by scientific documentation can serve as an innovative and powerful discovery engine for newer, safer, and affordable medicines.[2] Evaluation of plant products to treat diabetes mellitus is of growing interest as plants contain many bioactive substances with therapeutic potential and possibly works on multitargets to ameliorate the disorder

Animal models play a key role in the development of antidiabetic drug; various diabetic animal models are employed in the evaluation of compounds as an antidiabetic drug. Models such as streptozotocin-induced diabetes, alloxan-induced diabetic rats, and fructose-induced diabetic models are widely used in the preclinical evaluation of antidiabetic drugs.[3] *Swietenia mahagoni* has been extensively studied in our laboratory for its phytochemical composition and antidiabetic activity using various established methods. As an extension of *in vitro* antidiabetic assays, the plant has been evaluated for antidiabetic potential in streptozotocin-induced diabetic model.[4] Although the streptozotocin-induced model simulates most of the physiological conditions in the diabetic patients, the model has limitation as insulin resistance cannot be studied using the model. Since insulin resistance and its consequences play a very crucial role in type-II diabetic patients, the study was planned to assess the antidiabetic potential of the plant extract using fructose-induced diabetic model.

MATERIALS AND METHODS

Procurement of animals

Thirty albino rats of Wistar strain weighing 180–240 g were procured from the Animal House University of Mysore. The obtained rats were kept in polyacrylic cages (four rats in each cage of dimension 43.2 cm × 34.0 cm × 19.8 cm) in the room provided by the Central Animal House Facility of the University maintained at standard conditions (25°C ± 2°C, 45%–60% relative humidity, and 12 h photo period) and acclimatized for about 15 days.

Induction of diabetes

After 2 weeks of acclimatization, the rats were divided into five groups using random block design. The segregated groups were named Groups I–IV. Group I–Group IV were taken as normal control (CON), diabetic control (DC), metformin (MET) treated (50 mg/kg), sample treated (250 mg/kg BW), and sample treated (500 mg/kg BW), respectively [Table 1]. After 2 days of segregation, all the groups, except the CON group, received 20% W/V fructose in water along with normal chow for the induction period of 8 weeks. The fructose solution was given in the normal water bottles used to feed water to the rats. After the induction period, rats were fasted overnight, and the blood sample was collected in the tail vein to analyze the glucose and insulin levels.[5]

Collection of blood samples

To evaluate the blood glucose level at different intervals of treatment in various groups, the blood was collected from the tail vein by using an artificial rat restrainer. About 100 µl of blood was collected at each sampling time.

Table 1: Treatment groups

Groups	Treatment	Dosage
I	CON	-
II	DC	-
III	MET	50 mg/kg BW
IV	MAE-I	250 mg/kg BW
V	MAE-II	500 mg/kg BW

CON-Control; DC-Diabetic control (only fructose trested); BW: Body weight; MAE: Mahagoni aqueous extract; MAE-I-Mahagoni extract at a dose of 250 mg/kg BW; MAE-II-Mahagoni extract at a dose of 500 mg/kg BW; MET: Metformin

After treatment for 45 days, all the animals were decapitated. Blood was collected through cardiac puncture into the tubes coated with a precoagulant. The coagulated blood was centrifuged to separate serum. The separated serum was used for various biochemical analyses.

Oral Glucose Tolerance Test in diabetic rats

The experiment was performed in DC and antidiabetic potential of *Swietenia mahagoni* leaf extract (MAE)-treated groups at the end of the treatment (45th day). The two groups were fasted overnight, and oral glucose load (2 g/kg) was administered. The blood glucose level in both the groups was analyzed at 0, 30, 60, 120, and 160 min after the administration of glucose.

Histological section preparation of the target organs

For histological examinations, small sections of kidney and pancreas were fixed in Bouin's solution for 24 h, dehydrated through graded concentration of ethanol, embedded in paraffin wax, sectioned at 5-µm thicknesses, and stained with Mayer's hematoxylin and eosin and photographed in a complex microscope with a camera.

Biochemical analysis

Liver marker enzymes (alkaline phosphatase [ALP], alanine transaminase [ALT], and aspartate transaminase [AST]), total protein, albumin, urea, bilirubin, total cholesterol, and triglycerides were analyzed using respective standard kits from Agappe diagnostics as described in the previous section. Insulin levels were analyzed using DRG Rat insulin Elisa Kit. Glycated hemoglobin was analyzed using hemoglobin A1c (HbA1c) kit.

Antioxidant status

Superoxide dismutase

The procedure adopted was that of Beauchamp and Fridovich[6] with minor modifications. The principle of superoxide dismutase (SOD) activity assay was based on the inhibition of nitroblue tetrazolium (NBT) reduction. Illumination of riboflavin in the presence of O^2 and electron donor such as methionine generates superoxide anions, and this has been used as the basis of assay of SOD. The reduction of NBT by superoxide radicals to blue-colored formazan was followed at 560 nm. One unit of SOD activity is defined as the amount of enzyme required to inhibit the reduction of NBT by 50% under the specified conditions.

Catalase

The enzymatic activity of catalase was assayed by the method of Goth.[7] The principle was based on the colored stable complex formation of hydrogen peroxide with ammonium molybdate. In brief, 0.2 mL of serum was incubated with 1.0 mL of substrate (65 µmol/ml hydrogen peroxide in 60 m mmol/L sodium potassium phosphate buffer, pH 7.4) at 37°C for 60 s. The enzyme reaction was stopped by the addition of 1 mL ammonium molybdate (32.4 mmol/l), and the yellow-colored complex was measured at 405 nm against blank 3.

$$\text{Serum catalase activity } \left(\frac{KU}{L}\right) = \frac{A\left(\text{Sample}\right) - A\left(\text{Blank}\,1\right)}{A\left(\text{Blank}\,2\right) - A\left(\text{Blank}\,3\right)} \times 271$$

Glutathione

Reduced glutathione (GSH) was determined by the method of Moron *et al.*[8] Reduced GSH on reaction with 5,5'-dithiobis nitro benzoic acid (DTNB) produces a yellow-colored product that absorbs light

at 412 nm. The serum (0.1 mL) was made up to 1.0 mL with 0.2 M sodium phosphate buffer (pH 8.0). Two mL of freshly prepared DTNB solution was added, and the intensity of the yellow color developed was measured in a spectrophotometer at 412 nm after 10 min. Standard GSH corresponding to concentrations ranging between 2 and 10 nmol (?) were also prepared. The values are expressed as nmoles GSH/g sample.

Estimation of liver glycogen content

1 g of the liver was weighed and transferred to 10 mL test tube. 3 mL of potassium hydroxide mount (30%) was added, and the contents were boiled on a water bath for 20 min to digest the liver and to release glycogen. The contents were cooled to room temperature, and 0.2 mL of Na_2SO_4 was added. Then, the glycogen was precipitated by adding 95% ethanol. The contents were centrifuged to completely sediment the glycogen. The precipitate was dissolved in distilled water, and the solution was made up to 10 ml. 1 mL of the glycogen solution was taken into 10-mL test tube and 1 mL of HCl (1.2 N) was added and heated on a boiling water bath for 2 h. The contents were neutralized using the NaOH of the similar strength. Then, the glucose content in the sample was determined by anthrone method. The concentration of glucose obtained was deduced to glycogen levels (mg of glycogen per 100 g of tissue) using the following relation:[9]

$$\text{Glycogen} = \frac{\text{DU}}{\text{DS}} \times 0.1 \times \frac{\text{Volume of extract}}{\text{Gram of tissue}} \times 100 \times 0.9$$

Where DU = optical density of the unknown, DS = optical density of the standard, 0.1 = mg of glucose in 2 mL of standard solution, and 0.9 = factor for converting glucose value to glycogen value.

Insulin resistance index calculation: Fasting Insulin Resistance Index (FIRI) was calculated according to the following formula:

$$\text{FIRI} = \frac{\text{Fasting insulin (mU/mL)} \times \text{Fasting glucose (mg/dl)}}{25}$$

Homeostatic Model Assessment β-cell Functionality Index (HOMA-β) calculation: The assessment was done according to calculations of Livy, 1998

$$\text{HOMA-}\beta = \frac{(360 \times \text{insulin})}{\text{Glucose-63}} \%$$

Isolation and partial characterization of the bio-active components

High-performance liquid chromatography analysis

The HPLC system (Waters) consisting of photodiode array detector (W2998), dual pump system (515 Waters), temperature control module II (TC2 Waters), pump control module (PC2 Waters), system controller (EMOAA01712), and a reverse-phase HPLC analytical Waters Symmetry column (C18, 5-µm particle size, 4.6 mm × 250 mm). The mobile phase was 1% acetic acid in water (solvent A) and acetonitrile: methanol (solvent B, ratio 1:1), and the separation was performed by the following linear gradient: 70:30 (solvent A: B) for 10 min, 60:40 (solvent A: B) for another 10 min, and the flow rate was adjusted to 0.6 mL/min. Injection volume was 10 µL. The chromatogram of water extract was compared with the individual chromatogram of standards scopoletin, bergenin, caffiec acid, cinnamic acid, and kaempferol (Sigma Chemical Co., St. Louis, USA). Data analysis was done using Empower software.

Liquid chromatography–mass spectrometry analysis

Liquid chromatography–mass spectrometry (LCMS) analysis was done using Water's system with C18 acuity UPLC® column (1.7 µm particle size, 1.0 mm × 50 mm). For the LC, the mobile phase was 0.1% formic acid in water (solvent A) and acetonitrile (solvent B), and the separation was performed by the following linear gradient: 70:30 (solvent A: B) for 5 min and 60:40 (solvent A: B) for another 5 min. The separated components were ionized and analyzed in an integrated mass detection system.

RESULTS

Bodyweight

Body weights (BWs) of various groups are presented in Figure 1. BW of DC group increased steadily during the study period when compared to the CON group. Treatment with MET has decreased the weight gain when compared to DC. In MAE-treated groups, the decrease in BW was dependent on dosage, and the group with 500 mg/kg BW has shown weight comparably prominent than 250 mg/kg BW.

Water intake

Water intake pattern of experimental groups during the study period is presented in Figure 2. Water consumption was highest and showed gradual increase in DC group when compared to that of CON group and all the other treated groups. Treatment with MAE showed gradual reduction in the amount of water consumption. It was noted that, at the end of the study period, the amount of water consumption in MAE-treated group was comparable to that of CON group.

Food intake

Food intake pattern of experimental groups is presented in Figure 3. It was noted that food consumption increased during the study period in DC and CON groups. The increase was higher in DC when compared to CNT. All the treated groups (MET, MAE-I, and MAE II) had similar food consumption at the beginning of the study period, and then gradually decreased with the treatment duration. Decrease in the quantity of food consumed was more prominent in MAE-II group. At the end of the study period, all the treated groups had food consumption similar to that of CNT group, whereas in DC group, consumption was much higher than that of all experimental groups.

Glycemic control

Serum glucose level of experimental groups during the study period is presented in Figure 4. It was noted that, at the beginning of the experiment and after induction of diabetes, serum glucose level was much higher in diabetic groups when compared to normal CON group. In diabetic groups, treatment with MAE showed reduction in the serum

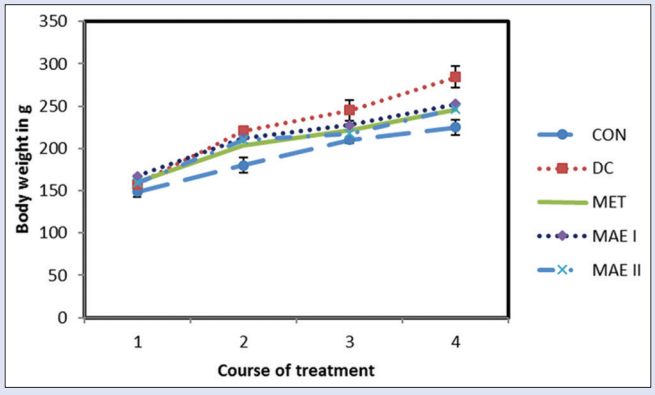

Figure 1: Bodyweights of experimental groups in various intervals of study period. CON: Normal control; DC: Diabetic control; MET: Metformin (50 mg/kg); MAE I: 250 mg/kg MAE treated; MAE II: 500 mg/kg MAE treated

glucose level from the initial levels, and the reduction was comparable to standard drug MET. Whereas in DC group, there was no improvement in the serum glucose levels and serum glucose level increased during the study period.

Serum biochemical parameters

Various serum biochemical parameters are presented in Table 2. There was a significant increase in the liver marker enzymes, namely, ALT, AST, and ALP, in the DC group when compared to CON and all the other treated groups. The reduction in liver marker enzymes in MAE-II group was greater compared to that of all the treated groups. In DC group, there was reduction in innate antioxidant components and enzymes, namely SOD, catalase and GSH, when compared to CON group. Treatment with MET and MAE has improved the antioxidant status, and the effect was more prominent in MAE-II group. Thiobarbituric acid-reactive substance content was highest in DC group, whereas in treated groups (MET and MAE), reduction in the level was noted, and the levels were comparable to CON group and reduction was more prominent in MAE-II group. There was no significant difference in albumin level between the experimental groups. Cholesterol levels were higher in DC group when compared to all the other experimental groups and were lowest in CON group. The group treated with MAE has shown significant reduction in total cholesterol level, which was lower than CON group.

Triglyceride levels were elevated in DC group compared to that of the CON group, the treated grouped showed reduction in the triglyceride levels, and reduction was more prominent in MAE-II group and was comparable to CON group.

Glycogen content in liver

Liver glycogen content in all the experimental groups is presented in Figure 5. There was depletion in glycogen content in DC group when compared to that of the CON group. The treatment with MET improved the glycogen content when compared to all the other experimental groups. MAE treatment also has improved the glycogen content, which was comparable to MET group.

Glycated hemoglobin

Glycated hemoglobin content of experimental groups is presented in Figure 6. Glycated hemoglobin levels were higher in DC group compared

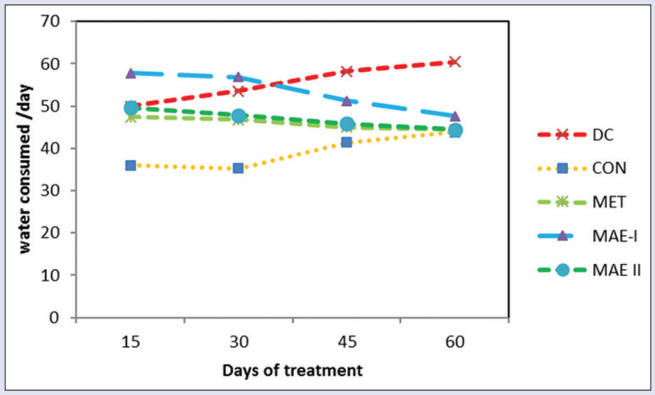

Figure 2: Water intake in experimental groups at various intervals of the study. CON: Normal control; DC: Diabetic control; MET: Metformin (50 mg/kg); MAE I: 250 mg/kg MAE treated; MAE II: 500 mg/kg MAE treated

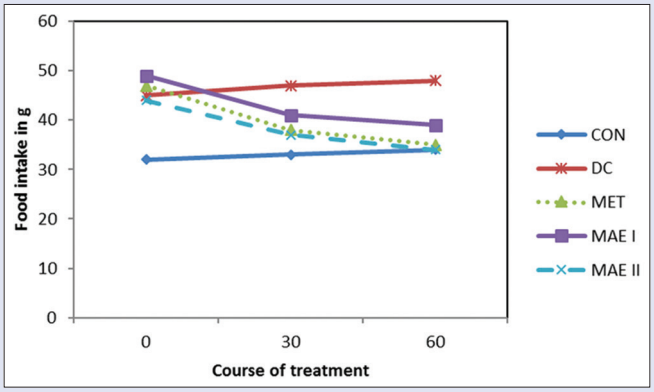

Figure 3: Food intake in experimental groups in various intervals of the study period. CON: Normal control; DC: Diabetic control; MF: Metformin (50 mg/kg); MAE I: 250 mg/kg MAE treated; MAE II: 500 mg/kg MAE treated

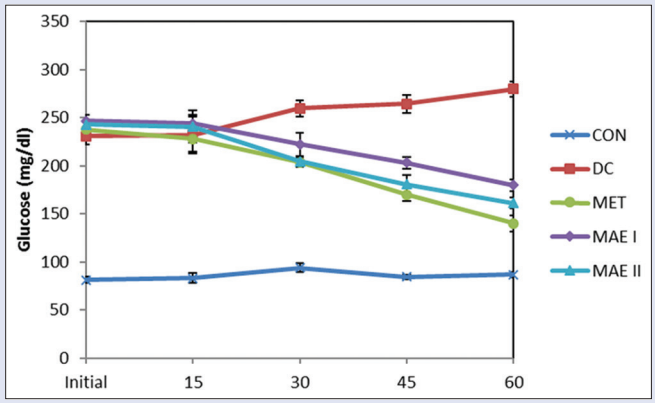

Figure 4: Effect of treatment on serum glucose level. CON: Normal control; DC: Diabetic control; Met: Metformin (50 mg/kg): MAE I: 250 mg/kg MAE treated; MAE II: 500 mg/kg MAE treated. All the values are mean of each group of rats ($n = 6$) with standard deviation. Initial refers to sample taken at the end of diabetic induction and at the start of the treatment. 15, 30, 45, and 60 days refer to sampling at various intervals of the treatment period

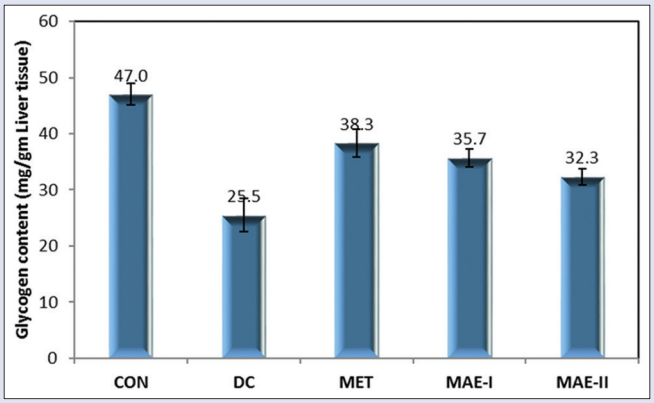

Figure 5: Glycogen content in liver. CON: Normal control; DC: Diabetic control; Met: Metformin (50 mg/kg); MAE I: 250 mg/kg MAE treated; MAE II: 500 mg/kg MAE treated. All the values are mean for each group (n = 6) with standard deviation. The rats were overnight fasted (16 h), and glycogen content in the excised liver was done on the same day of the excision of liver. **Bars carrying different superscripts letters a, b, c… differ significantly ($P \leq 0.05$)

Table 2: Serum biochemical parameters of various groups

	ALP (U/L)	ALT (U/L)	AST (U/L)	Pro (g/dl)	Alb (g/dl)	Urea (mg/Dl)	Bilirubin (mg/dl)	TBARS (ng/mg Pro)	GSH (µM/mg Pro)	Cholesterol (mg/dl)	TGL (mg/dl)	SOD (IU/mg Pro)	CAT (KU/L)
CON	126.5^{a}±113.4	12.9^{a}±1.36	48.5^{a}±1.89	7.4^{a}±0.52	2.1^{a}±0.24	46.8^{a}±2.09	0.2^{a}±0.01	20.4^{a}±1.03	0.8^{c}±0.03	70.2^{b}±5.44	99.1^{a}±2.75	15.8^{e}±1.19	291^{c}±14
DC	206.75^{d}±12.1	25.0^{b}±0.96	65.0^{c}±1.77	7.7^{ab}±0.26	2.1^{a}±0.19	51.9^{b}±2.55	0.3^{a}±0.02	28.6^{b}±2.51	0.4^{a}±0.12	80.0^{c}±3.37	161.7^{d}±9.97	4.1^{a}±0.47	237^{a}±21
MET	181.5^{c}±15.6	21.7^{b}±1.7	58.3^{bc}±3	8.0^{b}±0.5	2.5^{a}±0.2	37.8^{a}±0.8	0.3^{a}±0.01	25.6^{b}±1.2	0.6^{b}±0.1	75.9^{b}±2.6	121.7^{c}±10.2	5.7^{b}±0.4	228^{a}±11
MAE-I	192^{c}±9.1	22.9^{b}±1.6	52.8^{b}±2	7.5^{a}±0.3	2.2^{a}±0.3	41.7^{a}±3	0.2^{a}±0.01	23.8^{a}±2.1	0.6^{b}±0.1	64.7^{a}±2.7	111.0^{b}±11.8	7.6^{c}±0.6	261^{b}±12.8
MAE-II	160^{b}±13.1	20.5^{b}±1.7	52.5^{b}±3	7.2^{a}±0.5	2.0^{a}±0.2	42.6^{a}±1.9	0.2^{a}±0.01	21.0^{a}±1.2	0.7^{bc}±0.1	63.0^{a}±2.6	112.3^{b}±10.4	9.6^{d}±0.4	264^{b}±8.7

**Values carrying different superscripts letters a, b, c…in columns differ significantly (P≤0.05). All the values are mean of each group of rats (n=6) with SD. CON: Normal control; DC: Diabetic control; MET: Metformin (50 mg/kg); MAE: Mahagoni aqueous extract; MAE I: 250 mg/kg MAE treated; MAE II: 500 mg/kg MAE treated; ALP: Alkaline phosphatase; ALT: Alanine transaminase; AST: Aspartate transaminase; Pro: Protein; Alb: Albumin; TBARS: Thiobarbituric acid reactive substance; GSH: Glutathione; TGL: Triglycerides; SOD: Superoxide dismutase; CAT: Catalase; SD: Standard deviation

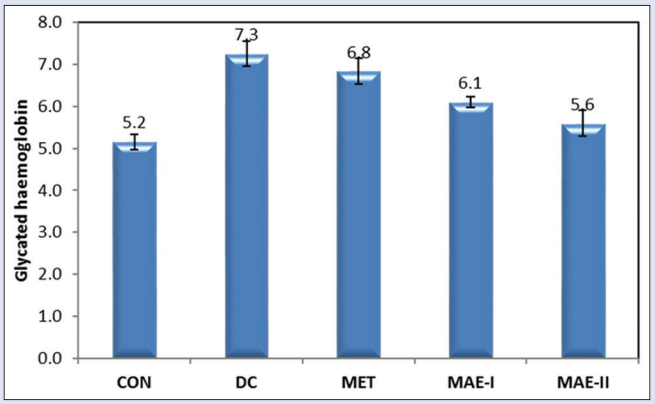

Figure 6: Glycated hemoglobin levels. Cont: Normal control; DC: Diabetic control; Met: Metformin (50 mg/kg); MAE I: 250 mg/kg MAE treated; MAE II: 500 mg/kg MAE treated. All the values are mean for each group (n = 6) with standard deviation. **Bars carrying different superscripts letters a, b, c… differ significantly ($P \leq 0.05$)

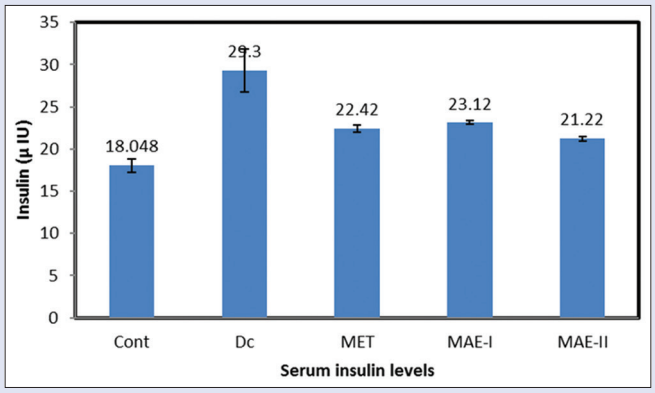

Figure 7: Serum insulin levels. Cont: Normal control; DC: Diabetic control; Met: Metformin (50 mg/kg): MAE I: 250 mg/kg MAE treated; MAE II: 500 mg/kg MAE treated. All the values are mean for each group (n = 6) with standard deviation. **Bars carrying different superscripts letters a, b, c… differ significantly ($P \leq 0.05$)

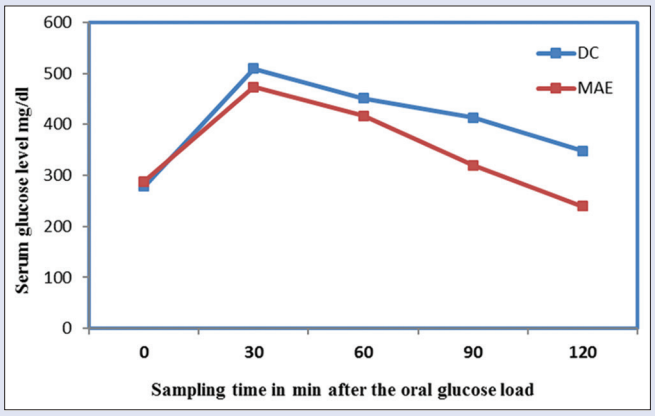

Figure 8: Glucose levels at various intervals during Oral Glucose Tolerance Test**.** DC: Diabetic control group; MAE: 500 mg/kg MAE-treated group. The experiment was conducted at the end of the treatment period before the dissection day. 0th min indicated the blood sample taken just after oral glucose load was given. Blood samples were taken from the tail vein

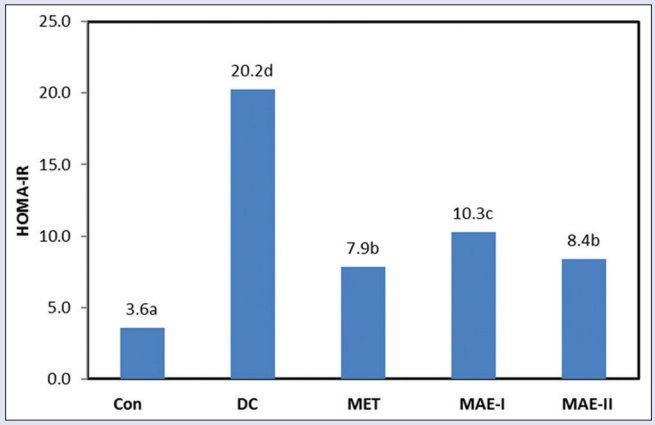

Figure 9: Homeostatic Model Assessment for Insulin Resistance values. Con: Normal control; DC: Diabetic control; MET: Metformin (50 mg/kg); MAE I: 250 mg/kg MAE treated; MAE II: 500 mg/kg MAE treated. All the values are mean of each group of rats (n = 6). HOMA-IR: Homeostatic Model Assessment-Insulin Resistance Index. **Bars carrying different superscripts letters a, b, c… differ significantly ($P \leq 0.05$)

to that of all the other experimental groups, and CON group had the lowest level. MET and MAE groups showed significant reduction in HbA1c compared to DC group. The reduction was more significant in MAE-II group ($P \leq 0.05$).

Serum insulin level

Insulin level of all the experimental groups is presented in Figure 7. Insulin levels were highest in DC group when compared to that of all the other groups and lowest in CON group. Treatment with MET and MAE

has reduced the levels of insulin in the serum of respective groups. The reduction was more significant in MAE-II group.

Oral Glucose Tolerance Test

This test was performed in the animals 3 days prior to their sacrifice to study the impact of MAE treatment on oral glucose tolerance and compared with the DC group. Figure 8 shows the blood glucose levels in MAE-treated and DC groups after a glucose load (10 g/kg BW). It was observed that the blood glucose levels at all time intervals were significantly low in MAE-treated (500 mg/kg BW) group, thus the area under the glucose curve was also significantly low compared to that of the untreated DC group.

Homeostatic Model Assessment of Insulin Resistance

Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) values for all the experimental groups are presented in Figure 9. IR index was highest in DC group compared to all the other experimental groups. There was a significant reduction in IR index in MET group. Reduction in IR index values was also noted in MAE-treated groups; however, the reduction was more significant in MET-treated group compared to that of all the other treated groups.

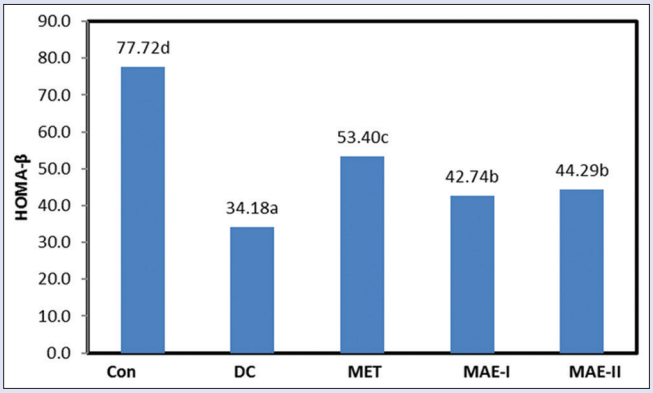

Figure 10: HOMA-β index. Con: Normal control; DC: Diabetic control; MET: Metformin (50 mg/kg); MAE I: 250 mg/kg MAE treated; MAE II: 500 mg/kg MAE treated. All the values are mean of each group of rats ($n = 6$). HOMA-β: Homeostatic Model Assessment β-cell Functionality Index. **Bars carrying different superscripts letters a, b, c… differ significantly ($P \leq 0.05$)

Homeostatic Model Assessment of β-cell Health

HOMA-β values for all the experimental groups are presented in Figure 10. Health index was lowest in DC group compared to that of all the other groups and highest in CON group. Treatment with MET and MAE improved the health index value compared to DC group, and improvement is more prominent in MET-treated group.

Histopathology

The histopathology sections of pancreas of all the groups are presented in Figure 11. A reduction in mass of Islets of Langerhans was noted in all the diabetic groups. The highest mass reduction was observed in DC group. Treatment with MAE has improved the islet mass.

Kidney sections

Histopathology sections of kidney of all the groups are presented in Figure 12. Intact glomeruli, basement membrane, and tubules were observed in the CON kidney section, whereas in DC group, distortions in glomeruli structure, basement membrane, and compressed tubular structures were noted. In the groups MAE-I and MAE-II, restoration toward the basal structural integrity can be observed.

High-performance liquid chromatography analysis

The extract was subjected to HPLC to achieve the separation of constituent phytochemicals which were present in the extract and to identify them by comparing with the purified standard compounds. A method was optimized based on the previous reports on the HPLC separation and also by varying the basic program in the given software of the instrument used. The chromatogram obtained is shown in Figure 13. The purified standards were also run using the same method and compared with the separation pattern of the aqueous extract. The separated peaks of the extracts were identified by matching with the retention time of the peaks of purified standards. To further confirm the identified component in the aqueous extract, the extract was analyzed using spiking technique, where the purified standard was added to the extract and observed for spiking of the identified peak. In the same way, some of the peaks were identified and ascertained to previously known compounds.

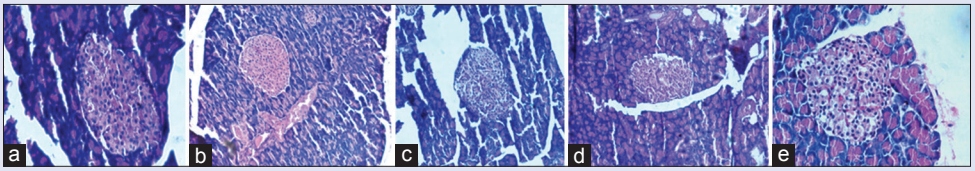

Figure 11: Histopathology of pancreas. A – Normal control, B – Diabetic control (DC), C – Metformin-treated group (Met), D-MAE 250 mg/kg-treated group (MH-I), E-MAE 500 mg/kg-treated group (MH-II). Photographs shown are taken at ×20 resolution

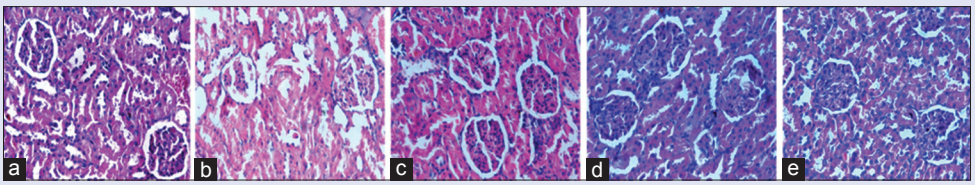

Figure 12: Histopathology of kidney. A – Normal control, B – Diabetic control (DC), C – Metformin-treated group (Met), D-MAE 250 mg/kg-treated group (MAE-I), E-MAE 500 mg/kg-treated group (MAE-II). Photographs shown are taken at ×20 resolution

The peaks were identified as gallic acid, scopoletin, coumarin, epicatechin, and ellagic acid. The identified compounds were further confirmed using the molecular weight analysis of individual peaks by subjecting to LCMS analysis and then by comparing with the reported molecular weight of the respective phytochemical compounds.

Liquid chromatography mass spectrometry analysis

LCMS analysis was done in two modes, namely positive mode and negative mode, so that most of the phytochemicals get ionized into either positive ions or negative ions, and the molecular weights are determined. LC profile and the corresponding molecular ion pattern in MS are depicted in Figures 14 and 15, respectively. From the molecular weight comparison, the presence of the phytochemicals which were identified in the aqueous extract by HPLC analysis was authenticated. The molecular ion (M/Z) peak of 145.0412 corresponded to coumarin (146.14), (M/Z) peak 169.1121 corresponded to gallic acid (170.12), (M/Z) peak 289.1021 corresponded to epicatechin (290.260), (M/Z) peak 301.23 corresponded to ellagic acid (302.197), and (M/Z) peak 191.06 corresponded to scopoletin (192.16). Values in the parenthesis are the molecular weights of un-ionized molecules; as the components are subjected to negative ionization, they tend to lose proton and thus will show molecular weight of 1 less than the corresponding un-ionized form of the respective molecules.

DISCUSSION

Type II diabetes is frequently associated with a number of pathologies which include obesity, dislipidemia, impaired glucose tolerance, and insulin resistance. Thus, to study the antidiabetic potential of any natural/synthetic drug, a diabetic model with induced mentioned pathologies will be more appropriate. *Swietenia mahagoni* has been extensively studied for its antidiabetic potential in our laboratory. The plant has been screened for *in vitro* antidiabetic potential. Based on the results of the *in vitro* studies, it was selected for the *in vivo* models, namely streptozotocin-induced diabetic model. However, streptozotocin-induced model cannot completely simulate the pathological conditions that prevail in type II diabetic patients. Hence, it was planned to study the anti-diabetic potential of the plant in fructose-induced diabetic model. Fructose is a natural sweetener used in beverages and found mostly in all the fruits. Studies on human subjects and experimental animals have shown that fructose induces lipogenicity leading to hyper-triglyceridemia, insulin resistance, metabolic imbalance, and development of a pre-diabetic or diabetic state.[10] High fructose content has also shown accumulation of hepatic triglycerides, impaired glucose tolerance, altered lipid metabolism, and increased risk for cardiovascular disease.[11] In the present study, feeding of rats with high content of fructose led to increased triglyceride levels in serum, impaired glucose tolerance, insulin resistance (as indicated by HOMA-IR), and decreased pancreatic β-cell health (as indicated by HOMA-β). The resulting altered conditions in the rats were very similar to pathophysiological conditions that prevail in diabetic patients.[12] Thus, the diabetic condition created by feeding the rats with the fructose is a suitable model to study the antidiabetic potential of MAE.

IR leads to increased blood glucose levels even in the presence of sufficient insulin. The condition leads to excess production of insulin and increases hepatic glucose production, further increasing the blood glucose levels. Treatment with MAE has reduced the insulin levels and also consistently reduced the fasting blood glucose, indicating the insulin-potentiating ability of the MAE. The effect may be mediated through a combination of inhibition of gluconeogenesis in liver and improving the insulin response by the peripheral tissues. HOMA-IR index depicts the level of IR in experimental animals. In DC group, HOMA-IR was higher, indicating severe IR; MAE treatment has reduced the index, indicating the overall effect of MAE on the insulin. The treatment also has improved the islet histology, thus the positive effect of MAE on glycemia may be mediated through improving the glucose response, improving the insulin action, improving β-cell health, and by inhibiting the hepatic glucose production. The *in vitro* assays on glucose-6-phosphatase inhibition, glucose uptake in the rat hemidiaphragum, and glucose production from the liver slices strongly support the insulin-potentiating and antigluconeogenesis potential, and thus may be responsible for the decrease in insulin and fasting glucose levels in serum of diabetic rats (unpublished results).

Glycogen levels are depleted in diabetic rats due to activation of the glycogenolytic pathways in the liver. MAE treatment has improved the glycogen levels, indicating the inhibitory effect on gluconeogenesis and promoting effect on glycogen synthetic pathway. Severe hyperglycemia leads to glycation of proteins and decreases the innate antioxidant potential. The resulting condition leads to chronic oxidative stress, which leads to several comorbidities associated with the diabetic condition.[13] Along with improving the glycemia, MAE treatment has reduced the level of HbA1c and improved the level of antioxidant enzymes and antioxidant components. Thus, MAE treatment acts on multitargets to control not only hyperglycemia, but also chronic inflammation caused due to prolonged oxidative stress. The major phytochemicals present in the extract such as scopoletin, epicatechin, ellagic acid, gallic acid, and coumarin have shown antioxidant potential in *in vitro* assays,[14,15] thus these components may be responsible for the improvement of oxidative stress in the diabetic rats.

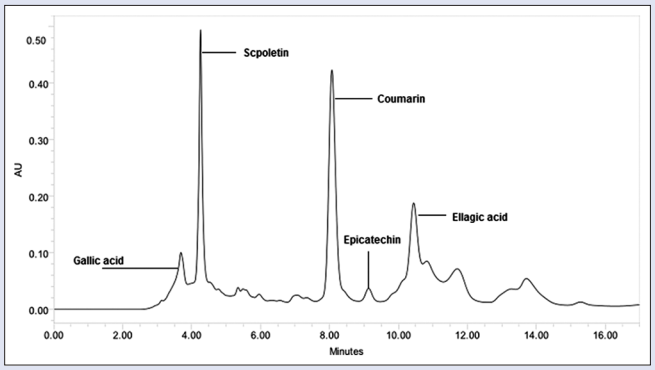

Figure 13: High-performance liquid chromatogram of the antidiabetic potential of *Swietenia mahagoni* leaf extract

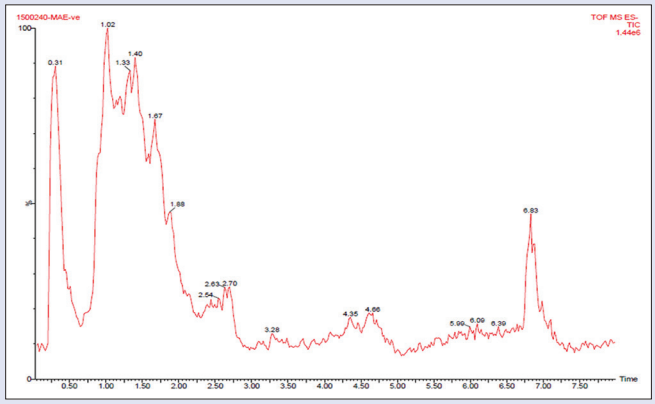

Figure 14: Liquid chromatogram of the antidiabetic potential of *Swietenia mahagoni* leaf extract

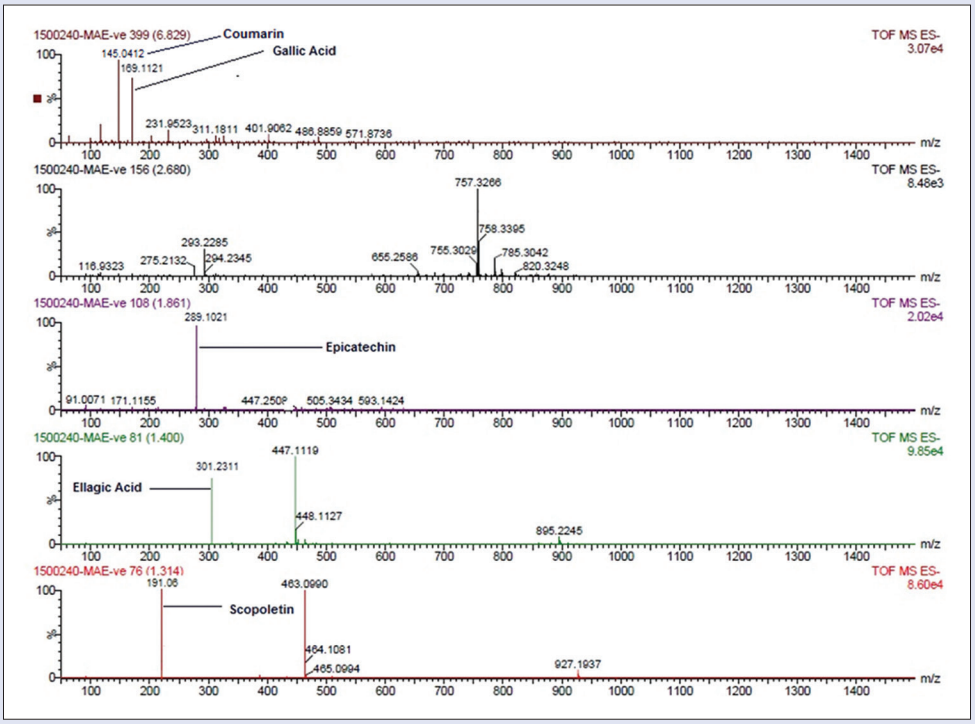

Figure 15: Molecular weight of the antidiabetic potential of *Swietenia mahagoni* leaf extract constituent phytochemicals

IR also leads to severe dyslipidemia characterized by an elevated level of triglycerides, total cholesterol, decreased high-density lipoprotein levels and increased low-density lipoprotein levels.[16] There was an elevated level of triglycerides and total cholesterol in the serum of DC group. MAE-treated group showed normalization of triglyceride and cholesterol levels.

HPLC and LCMS are the analytical and quantitative techniques extensively used by the scientific fraternity to characterize the chemical structure of synthetic or natural compounds. Pharmacologists also have identified the constituent phytochemicals in the active extracts using HPLC and LCMS techniques. The present study identifies the presence of scopoletin, epicatechin, gallic acid, ellagic acid, and coumarin as major phytochemicals which were present along with many un-identified phytochemicals in the extract using the HPLC separation and LCMS methods. Scopoletin (6-methoxy-7-hydroxycoumarin) is a class of coumarins and has been first isolated and characterized from the plants *Castanea crenata* and *Crossostephium chinensis*. The scopoletin has shown to posses various biological activities such as antihyperglycemic, anticholinesterasic, antithyroid, antioxidant, hypouricemic, antitumoral, and anti-inflammatory activities.[17,18] Epicatechin is a group of flavanoids found abundantly in green tea. The phytochemical has shown various pharmacological activities acting as a wide-spectrum agent. It has been showed to inhibit the proliferation of two *Plasmodium falciparum* strains, 3D7 drug sensitive (chloqine resistant) and FCR-1/FVO resistant to chloroquine and thus has showed its potential as an antimalarial agent. Epicatechin has a strong inhibitory effect on human immunodeficiency virus replication in cultured peripheral blood cells.[8] It has shown to have potential antioxidant effect by effectively scavenging free radicals such as reactive oxygen species, reactive nitrogen species, and superoxide.[19] Ellagic acid is a phenolic compound found in many medicinal plants and in fruits such as strawberries, walnuts, grapes, and pomegranates. It has shown to exhibit various physiological beneficial effects, namely anti-allergic, antioxidant, anti-inflammatory, and anticancer activities. Even the plant extracts having rich content of ellagic acid have shown to possess many pharmacological properties.[20-23]

CONCLUSION

The presence of the above bio-active beneficial phytochemicals may be responsible for hypoglycemic, insulin-sensitizing, insulin-potentiating, antioxidant, and hypo-cholesterolemic activities shown by the aqueous extract in animal models. Even the data on pharmacodynamic properties show the absorbability of these components into the blood system. Thus, MAE forms a beneficial adjuvant where it can act on multitargets and affects the overall diabetic condition, rather targeting to control hyperglycemia.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, *et al*. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: Systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2·7 million participants. Lancet 2011;378:31-40.
2. Azaizeh H, Fulder S, Khalil K, Said O. Ethnobotanical knowledge of local Arab practitioners in the Middle Eastern region. Fitoterapia 2003;74:98-108.
3. King AJ. The use of animal models in diabetes research. Br J Pharmacol 2012;166:877-94.

4. Naveen YP, Urooj A. Amelioration of diabetes by *Swietenia mahagoni* (L.) Jacq. in streptozotocin induced diabetic rats. Int J Pharm Sci Res 2015;6:3892.
5. Divi SM, Bellamkonda Dasireddy SK. Evaluation of antidiabetic and antihyperlipedemic potential of aqueous extract of *Moringa oleifera* (Make it italic) in fructose fed insulin resistant and STZ induced diabetic wistar rats: A comparative study. Asian J Pharm Clin Res 2012;5:67-72.
6. Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal Biochem 1971;44:276-87.
7. Góth L. A simple method for determination of serum catalase activity and revision of reference range. Clin Chim Acta 1991;196:143-51.
8. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta 1979;582:67-78.
9. Carroll NV, Longley RW, Roe JH. The determination of glycogen in liver and muscle by use of anthrone reagent. J Biol Chem 1956;220:583-93.
10. Ginsberg HN, Huang LS. The insulin resistance syndrome: Impact on lipoprotein metabolism and atherothrombosis. J Cardiovasc Risk 2000;7:325-31.
11. Shulman GI. Cellular mechanisms of insulin resistance. J Clin Invest 2000;106:171-6.
12. Zarfeshani A, Mutalib MS, Khaza'ai H. Evaluating of high fructose diet to induce hyperglycemia and its inflammatory complications in rats. Pak J Nutr 2012;11:21-6.
13. Mogana R, Teng-Jin K, Wiart C. Anti-inflammatory, anticholinesterase, and antioxidant potential of scopoletin isolated from *Canarium patentinervium* miq. (Burseraceae kunth). Evid Based Complement Alternat Med 2013;2013:734824.
14. Kadhum AA, Al-Amiery AA, Musa AY, Mohamad AB. The antioxidant activity of new coumarin derivatives. Int J Mol Sci 2011;12:5747-61.
15. Garg A. Insulin resistance in the pathogenesis of dyslipidemia. Diabetes Care 1996;19:387-9.
16. Shaw CY, Chen CH, Hsu CC, Chen CC, Tsai YC. Antioxidant properties of scopoletin isolated from *Sinomonium acutum*. Phytother Res 2003;17:823-5.
17. Chang TN, Deng JS, Chang YC, Lee CY, Jung-Chun L, Lee MM, *et al*. Ameliorative effects of scopoletin from *Crossostephium chinensis* against inflammation pain and its mechanisms in mice. Evid Based Complement Alternat Med 2012;2012:595603.
18. Capra JC, Cunha MP, Machado DG, Zomkowski AD, Mendes BG, Santos AR, *et al*. Antidepressant-like effect of scopoletin, a coumarin isolated from *Polygala sabulosa* (Polygalaceae) in mice: Evidence for the involvement of monoaminergic systems. Eur J Pharmacol 2010;643:232-8.
19. Calderón AI, Wright BJ, Hurst WJ, van Breemen RB. Screening antioxidants using LC-MS: Case study with cocoa. J Agric Food Chem 2009;57:5693-9.
20. Bárta I, Smerák P, Polívková Z, Sestáková H, Langová M, Turek B, *et al*. Current trends and perspectives in nutrition and cancer prevention. Neoplasma 2006;53:19-25.
21. Rogerio AP, Fontanari C, Melo MC, Ambrosio SR, de Souza GE, Pereira PS, *et al*. Anti-inflammatory, analgesic and anti-oedematous effects of *Lafoensia pacari* extract and ellagic acid. J Pharm Pharmacol 2006;58:1265-73.
22. Murugan V, Mukherjee K, Maiti K, Mukherjee PK. Enhanced oral bioavailability and antioxidant profile of ellagic acid by phospholipids. J Agric Food Chem 2009;57:4559-65.
23. Bell C, Hawthorne S. Ellagic acid, pomegranate and prostate cancer – A mini review. J Pharm Pharmacol 2008;60:139-44.