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ANTIHYPERGLYCEMIC ACTIVITY OF *LAGENARIA SICERARIA* AERIAL PARTS ON STREPTOZOTOCIN INDUCED DIABETES IN RATS

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SUMMARY

The present study was carried out to evaluate the antihyperglycemic activity of methanol extract of Lagenaria siceraria *aerial parts (MELS) for its purported use in diabetes. Hyperglycemia was induced by streptozotocin (50 mg/kg, i.p.) in rats. Treatment was done by MELS at doses of 200 and 400 mg/kg, p.o. for 14 days. Glibenclamide (500 µg/kg) was used as a reference drug. Antihyperglycemic potential was assessed by fasting blood glucose (FBG) measurement (on days 0, 4, 8 and 15), biochemical tests (SGPT, SGOT, ALP, total cholesterol, triglycerides), antioxidant assay (lipid peroxide, catalase and glutathione) and histologic study of the liver, kidney and pancreas tissue. Significant reduction (P<0.001) in FBG levels was observed with treatment duration. Antioxidant and biochemical parameters were significantly improved by MELS and glibenclamide treatment. Histologic observations showed good correlations with the results obtained. The study explored the potent antihyperglycemic activity of MELS, which is probably attributable to its rich flavonoid content.*

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by the classical symptom of hyperglycemia, which in turn leads to various acute and chronic complications if left untreated and may cause massive damage to the renal, cardiovascular, retinal and neurologic systems. It occurs as a result of a relative or an absolute lack of insulin, or its action on the target tissue, or both (1,2). Despite the great strides made in understanding and management of diabetes, the incidence of diabetes mellitus is on rise all over the world, especially in Asia and Africa, and is likely to rise to up to 300 million or more by the year 2025 (3,4). Many synthetic hypoglycemic agents are currently available but they are either too expensive or produce undesirable side effects on chronic use (5). Traditionally, many indigenous plants have been used successfully for the management of the disease throughout the world; some of them have been

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evaluated experimentally and their active ingredients have been isolated (6,7). However, a number of such potential plants have remained unexplored.

Lagenaria siceraria (Mol.) Standley, commonly known as bottle-gourd (in English), belongs to the Cucurbitaceae family. The plant is widely available throughout India. It is a climbing or trailing herb, with bottle- or dumb-bell shaped fruits. Both its aerial parts and fruits are commonly consumed as a vegetable. Traditionally, it is used as medicine in India, China, European countries, Brazil, Hawaiian island, etc. for its cardiotonic, general tonic and diuretic properties (8). Further, the antihepatotoxic, analgesic and anti-inflammatory, hypolipidemic, antihyperglycemic, immunomodulatory and antioxidant activities of its fruit extract have been evaluated (9-14). *Lagenaria (L.) siceraria* fruits are a good source of vitamin B complex, ascorbic acid, fibers, proteins, cucurbitacins, saponins, fucosterols and compesterols, polyphenolics and flavone-C-glycoside (9,10,15-17). Methanol extract of its leaves showed the presence of sterols, polyphenolics, flavonoids, saponin, protein and carbohydrates (18). A novel protein, lagenin, has also been isolated from its seeds and it possesses antitumor, immunoprotective and antiproliferative properties (19). Although extensive studies have been carried out on its fruits and seeds, the pharmacology of the aerial parts of *L. siceraria* has not been studied yet. In many countries, this plant has been used traditionally as a single treatment for diabetes mellitus (20). The present investigation was therefore carried out to evaluate the antihyperglycemic potential of the methanol extract of *L. siceraria* aerial part (MELS) on streptozotocin (STZ) induced diabetes in rats.

MATERIALS AND METHODS

Plant material

The aerial parts of *L. siceraria* were collected in November 2008, from Madanpur, West Bengal, India, and identified by the Botanical Survey of India, Howrah, India. A voucher specimen (P/LS/1/08) was retained in our laboratory for further reference.

Preparation of plant extract

The aerial parts were dried under shade and powdered in a mechanical grinder. The powdered material was extracted with methanol using a Soxhlet apparatus. This extract was filtered and concentrated *in vacuo* in a Buchi evaporator, R-114 and kept in a *vacuum* desiccator until use. The yield was 18.13% w/w with respect to dried powder. Aqueous suspension of MELS was prepared using 2% (v/v) Tween-80 and used for oral administration.

Animals

Healthy Wistar albino rats (160±20 g) were used in the present study. They were maintained at standard laboratory conditions and fed commercial pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to laboratory conditions for one week before commencement of experiment. The experiments were performed based on animal ethics guidelines of the University Animals Ethics Committee.

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out following the standard procedures (21).

Acute toxicity study

Healthy Wistar albino rats (160±20 g) of either sex, starved overnight, were divided into five groups (n=4). Group I-IV animals were orally fed MELS in increasing dose levels of 0.5, 1.0, 1.5 and 2.0 g/kg b.w., while group V (untreated) served as control. The animals were observed continuously for the first 2 h for any gross change in behavioral, neurologic and autonomic profiles or any other symptoms of toxicity and mortality if any, and intermittently for the next 6 h and then again at 24 h , 48 h and 72 h for any lethality or death. One-tenth and one-fifth of the maximum safe dose of the extract tested for acute toxicity were selected for the experiment (22).

Study in normoglycemic animals

Healthy rats were divided into two groups (n=6). After overnight fasting with free access to water, fasting blood glucose (FBG) level of each animal was determined at the beginning of the experiment (at 0 h). Animals in control group (group I) received only the vehicle and the test group animals (group II) were treated with a high dose of MELS (400 mg/kg b.w.) orally. Blood sugar levels were determined again at ½ h, 1 h, 2 h and 3 h after oral administration of test samples to assess the effect of test samples on normoglycemic rats.

Induction of diabetes

A freshly prepared solution of STZ (50 mg/kg) in ice-cold citrate buffer (0.1 M, pH 4.5) was injected intraperitoneally to the overnight fasted rats (23). After 72 h of STZ administration, the blood glucose levels were measured and the rats showing blood glucose level >200 mg/dL were considered to be diabetic and were used in the study.

Study in STZ induced diabetic rats

Healthy Wistar albino rats were divided into five groups (n=6). Treatment was done for 14 days. Group I: normal rats received only vehicle; groups II, III, IV and V: STZ induced diabetic rats. Group II received only vehicle and served as STZ control group. Groups III and IV were orally administered with MELS, 200 and 400 mg/kg b.w., respectively, while group V was treated with the reference drug, glibenclamide (0.5 mg/kg, p.o.).

Testing of fasting blood glucose and body weight

FBG level of each animal was monitored on days 0, 4, 8 and 15. Drop of blood was collected from the tip of the tail vein of each rat and FBG level was measured using One Touch Glucometer, Horizon (Lifescan, Johnson and Johnson Company). Initial and final body weights were also recorded.

Estimation of biochemical parameters

On day 15, blood samples were collected from the retro-orbital plexus of the rats and serum was separated for biochemical estimation of serum glutamic pyruvate transaminase (SGPT), serum glutamic oxaloacetate transaminase (SGOT) (24), alkaline phosphatase (ALP) (25), total cholesterol and triglycerides (26). All analyses were performed by using commercially available kit from Span Diagnostics Ltd.

Evaluation of antioxidant properties

After blood collection, all animals were sacrificed by euthanasia. Liver, kidney and pancreas were collected for the estimation of tissue lipid peroxide (LPO) (27), reduced glutathione (GSH) (28) and catalase (CAT) (29) levels for the antioxidant study.

Histologic studies

After sacrificing the rats, parts of the pancreas, liver and kidney tissues were collected for histologic studies. The tissues were washed in normal saline and fixed immediately in 10% formalin for a period of at least 24 h, dehydrated with alcohol, and embedded in paraffin, cut into 4- to 5-μm thick sections and stained with hematoxylin-eosin dye for photomicroscopic observation.

Determination of total phenolic compounds in the extract

The amount of total phenolic compounds in MELS was determined using Folin-Ciocalteu's reagent and sodium carbonate solution, and absorbance was measured at 760 nm (30). A calibration curve of standard pyrocatechol was prepared and the results were expressed as mg of pyrocatechol equivalents/g of dry extract.

Determination of total flavonoid content in the extract

Total flavonoid content of MELS was determined spectrophotometrically (31). Briefly, 0.5 mL of 2% aluminum chloride in ethanol was mixed with the same volume of extract (1.0 mg/mL). Absorption readings at 415 nm were taken after 1 h against a blank (ethanol). Total flavonoid content was determined using a standard curve with quercetin (0-50 mg/L). The mean of three readings was used and expressed as mg of quercetin equivalents/g of dry extract.

Statistical analysis

Values were expressed as mean ± SEM. Data were statistically evaluated by one-way analysis of variance (ANOVA) followed by post hoc Dunnett's test using SPSS software. *P* values less than 0.01 were considered statistically significant.

RESULTS

Preliminary phytochemical screening of MELS revealed the presence of polyphenolics, flavonoids, glycosides, triterpinoids, saponin and carbohydrates.

In the acute toxicity study, MELS did not show any mortality or toxic effect up to the dose of 2 g/kg b.w.; accordingly, 200 and 400 mg/kg b.w. were taken as the low and high dose of MELS for the *in vivo* experiment.

Blood glucose level of normoglycemic study (NG) showed no significant effect of MELS on normoglycemia (results not shown).

The increased FBG level in STZ induced diabetic rats was significantly reduced (*P*<0.001) by MELS treatment and it was found to be lowered up to 65.74% and 68.57% at the dose of 200 and 400 mg/kg b.w., respectively. FBG and change in body weight in the STZ induced diabetic rats in 14-day experiment are

Table 1. **Effect of methanol extract of *Lagenaria siceraria* (MELS) on fasting blood glucose (FBG) level in control and streptozotocin (STZ) diabetic rats**

	Mean serum FBG ± SEM (mg/dL)				
Group	Day 0	Day 4	Day 8	Day 15	% Change
Normal control	82.50±1.09	81.33±2.09	83.17±1.38	15±0.57	0.81
Diabetic control	260.33±9.42[a,#]	315.33±7.25[a,#]	304.33±3.36[a,#]	296.67±2.46[a,#]	13.96
MELS (200 mg/kg)	250.50±5.30	200.33±14.84[b,*]	110.00±6.06[b,*]	85.83±3.73[b,*]	-65.74
MELS (400 mg/kg)	257.17±9.36	128.83±10.83[b,*]	84.33±4.15[b,*]	80.83±1.74[b,*]	-68.57
Glibenclamide (0.5 mg/kg)	260.50±7.42	229.33±4.43[b,*]	169.33±2.91[b,*]	93.00±2.16[b,*]	-64.30

Values are mean ± SEM; (n=6): [a]diabetic control group *vs.* normal control group,
[#]P<0.001; [b]treated groups *vs.* diabetic control group, *P<0.001; the level of significance was assessed by one-way ANOVA followed by post hoc Dunnett's test.

Table 2. **Effect of methanol extract of *Lagenaria siceraria* (MELS) on body weight in control and streptozotocin (STZ) diabetic rats**

	Body weight (g)		
Group	Initial	Final	Change
Normal control	155.00±3.87	156.00±3.54	1.00±0.93
Diabetic control	157.50±3.82	123.33±3.33	-34.17±1.54[a,#]
MELS (200 mg/kg)	161.67±6.15	136.50±6.73	-25.17±1.20[b,*]
MELS (400 mg/kg)	154.17±5.39	135.17±5.91	-19.00±1.39[b,*]
Glibenclamide (0.5 mg/kg)	173.83±2.29	155.83±2.51	-18.00±1.65[b,*]

Values are mean ± SEM; (n=6): [a]diabetic control group *vs.* normal control group,
[#]P<0.001; [b]treated groups *vs.* diabetic control group, *P<0.001; the level of significance was assessed by one-way ANOVA followed by post hoc Dunnett's test.

summarized in Table 1 and Table 2, respectively, indicating MELS as being equipotent to the reference drug, glibenclamide.

After 14-day experiment, the activities of serum enzymes such as SGOT, SGPT and ALP were significantly elevated in diabetic control groups (Fig. 1a), which was found to return to normal level upon supplementation with MELS (200 and 400 mg/kg) and

glibenclamide (0.5 mg/kg). Figure 1b reveals significant reduction in total cholesterol and triglyceride levels in the MELS treated groups as compared to diabetic control group.

As shown in Table 3, lipid peroxide level in the liver, pancreas and kidney tissues increased significantly in STZ induced diabetic rats as compared to normal group, and showed significant reduction ($P<0.001$)

Table 3. **Effect of methanol extract of *Lagenaria siceraria* (MELS) on tissue lipid peroxide (LPO), reduced glutathione (GSH) and catalase (CAT) levels in control and streptozotocin (STZ) diabetic rats**

Group	LPO level (nM/mg wet tissue)			GSH level (µg/mg wet tissue)			CAT level (µM of H_2O_2 decomposed/min/mg wet tissue)		
Normal control	126.86 ± 2.68	220.29 ± 0.59	209.43 ± 0.32	20.88 ± 0.22	69.12 ± 0.70	22.14 ± 0.13	0.54 ± 0.04	3.90± 0.29	1.78 ± 0.21
Diabetic control	302.08 ± 5.07 [a],##	395.40 ± 1.06 [a],##	330.24 ± 0.73 [a],##	8.84 ± 0.20 [a],##	24.93 ± 0.33 [a],##	11.28 ± 0.20 [a],##	0.25 ± 0.02 [a],#	1.25± 0.09[a],#	0.41 ± 0.06 [a],##
MELS (200 mg/kg)	200.94 ± 3.03 [b],**	291.65 ± 2.09 [b],**	235.78 ± 2.05 [b],**	16.03 ± 0.62 [b],**	52.39 ± 1.93 [b],**	20.48 ± 1.22 [b],**	0.39 ± 0.02 [b],*	2.13± 0.04[b],*	1.40 ± 0.03 [b],*
MELS (400 mg/kg)	147.92 ± 2.44 [b],**	232.08 ± 1.54 [b],**	206.47 ± 1.36 [b],**	20.04 ± 1.83 [b],**	59.56 ± 0.53 [b],**	22.51 ± 1.00 [b],**	0.51 ± 0.04 [b],*	3.12± 0.04 [b],*	1.80 ± 0.06 [b],**
Glibenclamide (0.5 mg/kg)	144.32 ± 2.69 [b],**	234.88 ± 0.62 [b],**	212.29 ± 0.46 [b],**	18.53 ± 0.16 [b],**	66.31 ± 0.54 [b],**	20.51 ± 0.33 [b],**	0.53 ± 0.04 [b],*	3.06± 0.31 [b],*	1.43 ± 0.17 [b],*

Values are mean ± SEM; (n=6): [a]diabetic control group *vs.* normal control group,
#$P<0.01$, ##$P<0.001$, [b]treated group *vs.* diabetic control group, *$P<0.01$, **$P<0.001$; the level of significance was assessed by one-way ANOVA followed by post hoc Dunnett's test

Figure 1a. **Effect of methanol extract of *Lagenaria siceraria* (MELS) on some biochemical parameters (serum biomarker enzyme levels) in control and streptozotocin (STZ) diabetic rats. Values are mean ± SEM; n=6 *per* group. Treatment was done for 14 days. [a]Diabetic control group *vs.* normal control group, #$P<0.001$; [b]treated groups *vs.* diabetic control group, *$P<0.001$; the level of significance assessed by one-way ANOVA followed by post hoc Dunnett's test.**
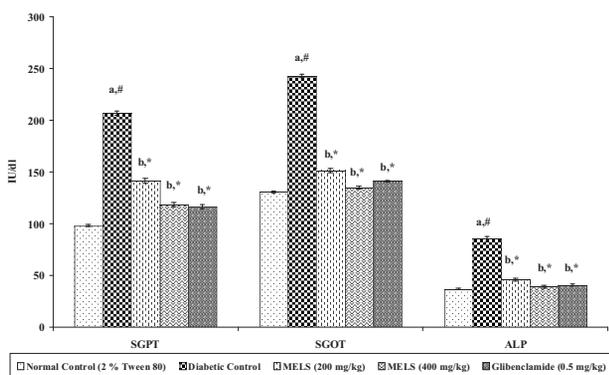

Figure 1b. **Effect of methanol extract of *Lagenaria siceraria* (MELS) on some biochemical parameters (total cholesterol and triglyceride levels) in control and streptozotocin (STZ) diabetic rats. Values are mean ± SEM; n=6 *per* group. Treatment was done for 14 days. [a]Diabetic control group *vs.* normal control group, #$P<0.001$, [b]treated groups *vs.* diabetic control group, *$P<0.001$; the level of significance was assessed by one-way ANOVA followed by post hoc Dunnett's test.**
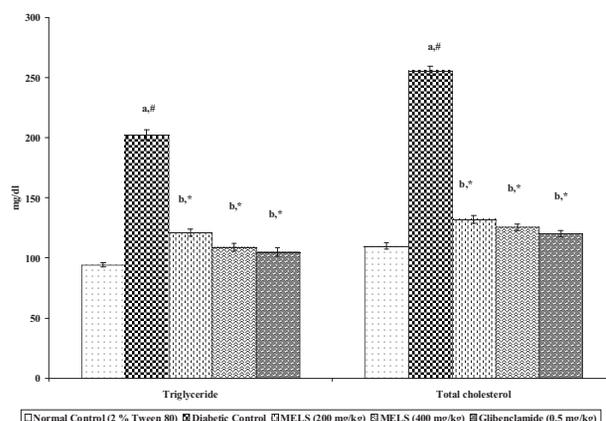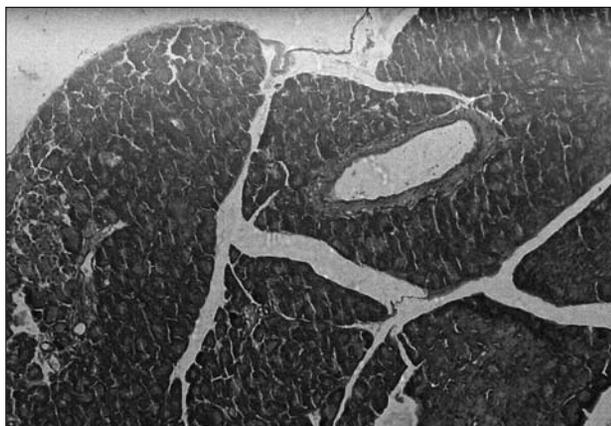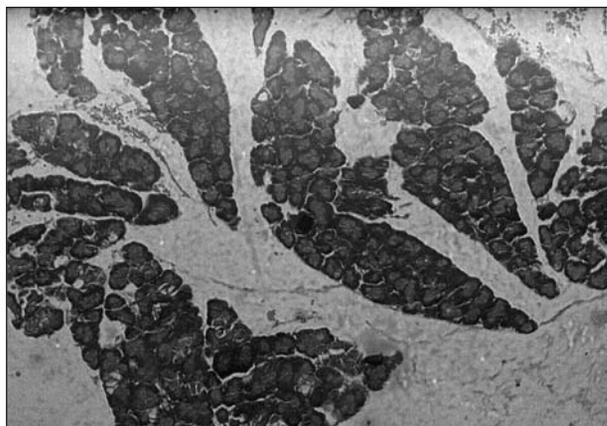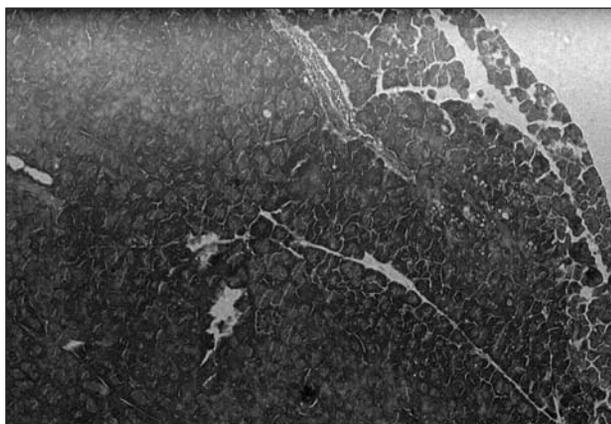
Figure 2. **Histologic examination of 14-day experimental rat pancreas: pancreatic sections of normal rats (a) showed dense Langerhans islets with well preserved cytoplasm and nucleus. Pancreatic sections of streptozotocin (STZ) intoxicated rats (b) showed loss of cell integrity and islet mass, damaged islets, acini degradation and polymorphonuclear leukocyte infiltration. Pancreatic sections of low dose and high dose MELS treated rats (c and d) showed gradual improvement in islet mass and cell integrity. Pancreatic sections of glibenclamide treated animals (e) showed normal tissue architecture with mild damage.**

2a. Pancreas of normal control rats

2b. Pancreas of STZ control rats

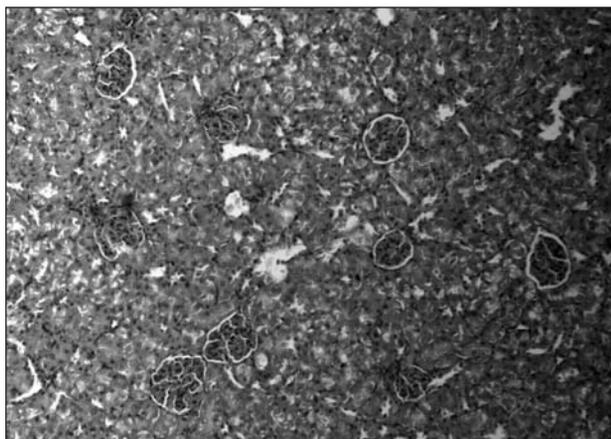**2c. Pancreas of low dose MELS treated rats**
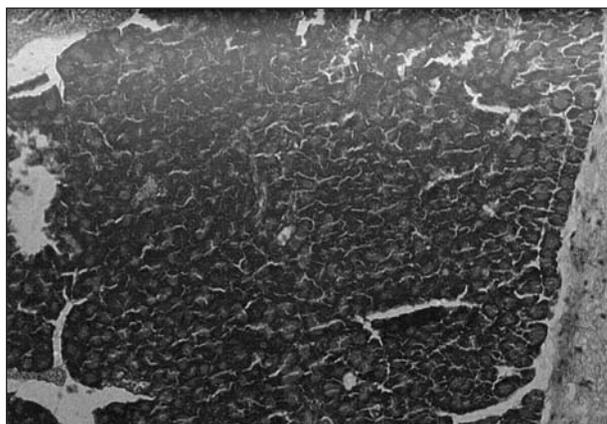
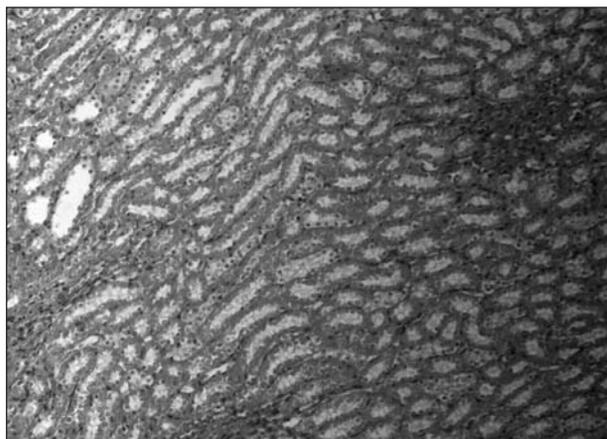**2d. Pancreas of high dose MELS treated rats**
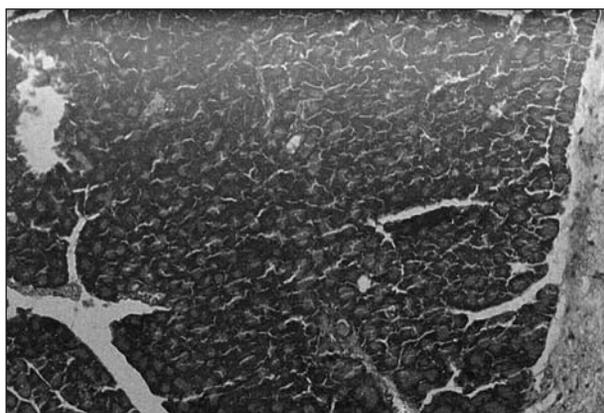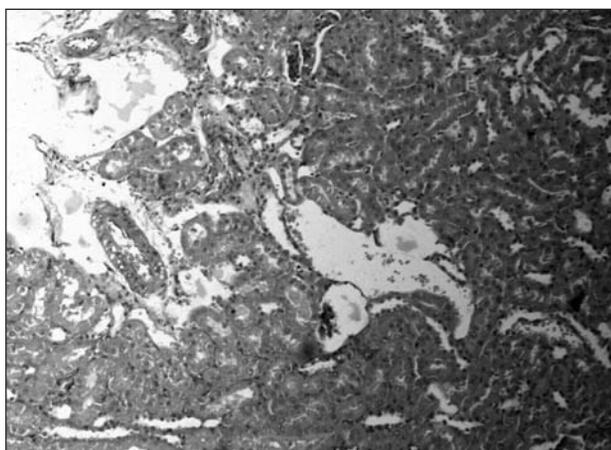

2e. Pancreas of glibenclamide treated reats

Figure 3. **Histologic examination of 14-day experimental rat liver: liver section of normal rats (a) showed well arranged cells and clear large central vein; cytoplasm and nucleus were well preserved. Liver section of diabetic control group (b) showed complete destruction of hepatocytes, degeneration of central vein, fatty degeneration, loss of cell structure and damage in cell membrane. Liver section of low dose MELS treated rats (c) disclosed that MELS (200 mg/kg) treatment was not able to recover completely, however, improvement of damage in the central vein and hepatocyte necrosis was observed to some extent, whereas liver section of the high dose MELS (400 mg/kg) treated rats (d) showed restoration of cell architecture to near normal. Liver section of glibenclamide treated rats (e) showed no damage in the hepatocytes and well arranged cells surrounding the central vein.**
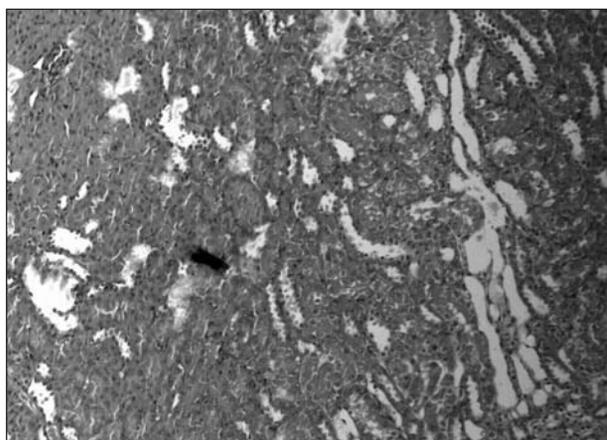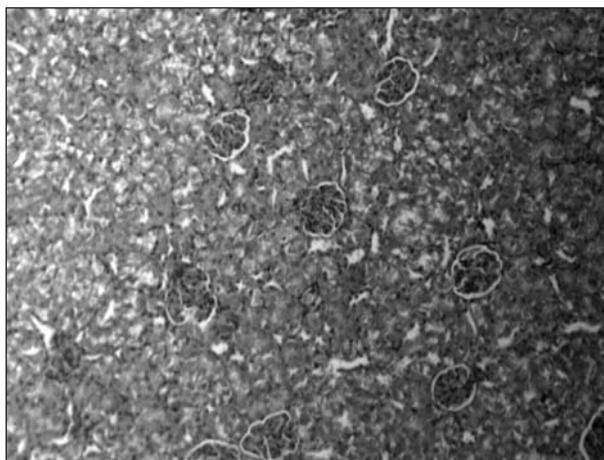

3a. Liver of normal control rats

3b. Liver of STZ control rats

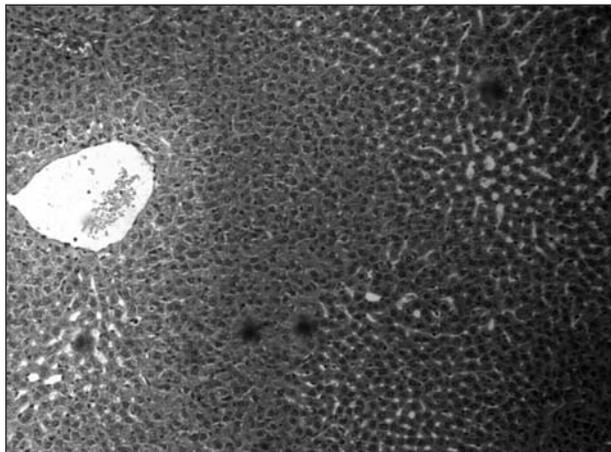**3c. Liver of low dose MELS treated rats**
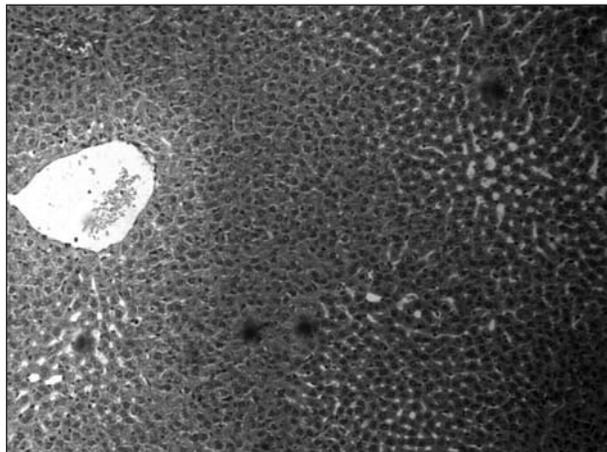

3d. Liver of high dose MELS treated rats

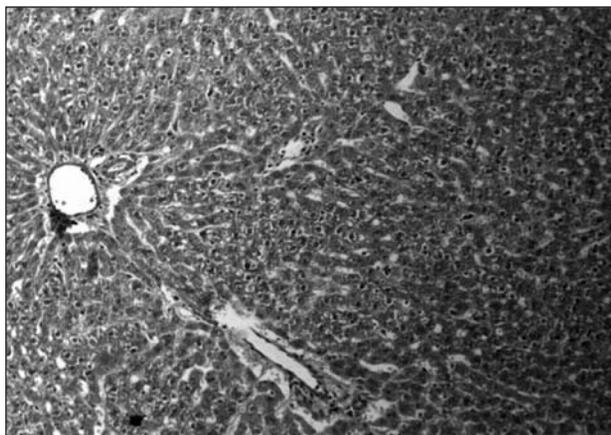**3e. Liver of glibenclamide treated reats**

Figure 4. **Histologic examination of 14-day experimental rat kidney: kidney section of normal rats (a) showed the cortex and medulla portion with lot of well packed glomerular and well arranged tubules. Kidney section of diabetic control group (b) showed damaged cells with hypertrophy and necrosis and derangement of cells with glomerulosclerosis. Kidney section of low dose and high dose MELS treated rats (c) and (d) showed improvement as compared to those of diabetic control group, with slight glomerular hypertrophy in the former group. Kidney section of glibenclamide treated rats (e) showed complete recovery of the damage.**
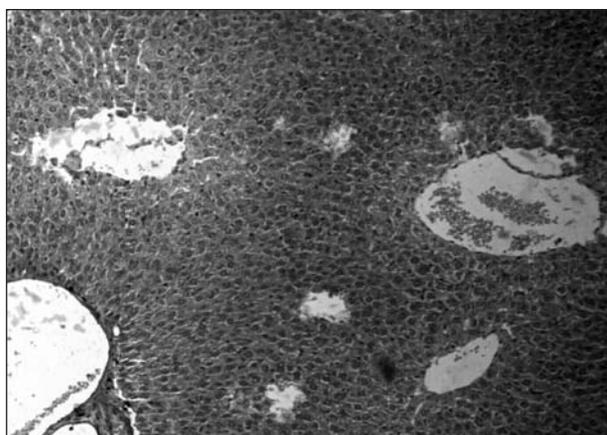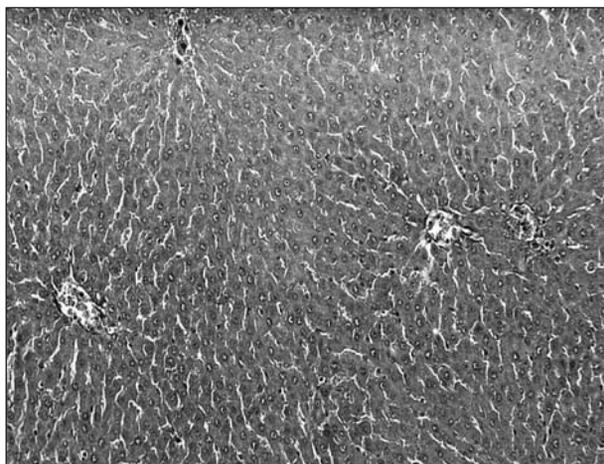

4a. Kidney of normal control rats

4b. Kidney of STZ control rats

4c. Kidney of low dose MELS treated rats

4d. Kidney of high dose MELS treated rats

4e. Kidney of glibenclamide treated reats

upon supplementation with MELS. In the STZ induced diabetic control animals, the GSH content and catalase activity were reduced with respect to normal control animals, and both were improved in the MELS treated group, almost comparable to the standard drug treated animals (Table 3).

Histologic examination revealed degeneration and necrosis of pancreatic islets in the diabetic control group (Fig. 2). The cytoplasm of peri-acinar hepatocytes showed either a single large or multiple small round empty vacuoles that distended the cell cytoplasm and displaced the nucleus to the periphery in histologic liver sections stained with hematoxylin and eosin. Parenchymatous degeneration was observed in peripheral regions. Dissociation of hepatocytes and sinusoidal dilatation occurred due to these changes (Fig. 3). Degenerated cortex and medulla and necrosis of tubules were observed in nephrons of diabetic groups. The glomerulus was emptied and distal tubules were also damaged in diabetic nephrons (Fig. 4). These histopathologic changes were restored to the near normal with MELS treatment.

Total phenolic and flavonoid content of the extract was found to be 65.7±0.46 mg pyrocatechol/g dry extract and 25.32 ±0.80 mg quercetin equivalent/g dry extract.

DISCUSSION

The present study was carried out to evaluate the antidiabetic activity of MELS on streptozotocin (STZ) induced diabetes in rats. STZ-induced hyperglycemia is a useful experimental model for studying antihyperglycemic activity. Because of its structural features, STZ gets selective entry into the β cells of the islets of Langerhans *via* the low affinity glucose transporter GLUT2 in its plasma membrane and causes destruction of β cells, which leads to a reduction in insulin release, which in turn results in a rise in blood glucose concentration, i.e. hyperglycemia (32). Accordingly, significantly high levels ($P<0.001$) of FBG were observed in STZ control group rats and remained high throughout the experimental period. STZ-induced diabetic rats treated with the extract

showed a significant reduction in blood sugar levels compared to STZ control group. This decrease in blood sugar levels may be attributed to stimulation of the residual pancreatic mechanism or to a probable increase in the peripheral utilization of glucose (33). Normoglycemic studies, however, revealed MELS to have no effect on euglycemia. This implies that the extract is probably acting through any of the extrapancreatic mechanisms rather than stimulating insulin secretion from β cells and results in antihyperglycemic action rather than hypoglycemic effect, i.e. does not affect normal blood sugar level, which may be beneficial in case of misdosing.

Induction of diabetes with STZ is associated with the characteristic loss of body weight, which is due to the increased muscle wasting and loss of tissue proteins (34). MELS administration to STZ diabetic rats reversed the weight loss.

Serum enzymes including SGPT, SGOT and ALP are used in the evaluation of hepatic disorders. An increase in these enzyme activities reflects active liver damage or inflammatory hepatocellular disorders (35). In accordance with these findings, STZ induction has a significant role in the alteration of liver functions since the activities of SGPT, SGOT and ALP were significantly higher than normal values. On the other hand, treatment with MELS, like that with glibenclamide, caused significant reduction in the activities of these enzymes, showing the protective effect of the extract.

Diabetes is associated with profound alteration in the plasma lipid and lipoprotein profile and therefore is associated with an increased risk of coronary heart disease. Under normal circumstances, insulin activates enzyme lipoprotein lipase and hydrolyses triglycerides. Insulin deficiency results in failure to activate the enzymes, thereby causing hyper-triglyceridemia (36). The significant control of the serum lipid levels in the MELS treated diabetic rats may be directly attributed to the improvement in insulin levels upon MELS treatment.

Hyperglycemia results in the generation of free radicals, which can exhaust antioxidant defenses thus leading to disruption of cellular functions, oxidative

damage to membranes and enhanced susceptibility to lipid peroxidation, as reflected by the increased level of lipid peroxide in the liver, pancreas and kidney tissues of STZ control rats. A significant reduction of this lipid peroxide level in MELS treated animals is indicative of its ability to reduce body glucose concentration and its subsequent oxidative damage.

GSH is normally present at high concentrations in the cells and is a direct scavenger of free radicals, thereby protecting the cells against the toxic effects of oxidative stress. Antioxidant enzyme catalase (CAT) is involved in detoxification of hydrogen peroxides and thus protects the tissue from highly reactive hydroxyl radicals. Diabetic animals in the present study showed lowered levels of GSH and CAT in the liver, pancreas and kidney tissues, reflecting the exhaustion of the endogenous antioxidant defenses. Treatment with MELS, however, increased both the reduced glutathione content and CAT activity, and thus may help avoid the free radical induced complications in diabetes mellitus (35,37,38).

The above antihyperglycemic and antioxidant properties of MELS were supported by the comparative histopathologic studies of the pancreas, liver and kidney tissues of diabetic control animals as well as the extract and standard drug treated animals (Figs. 2, 3 and 4).

In order to establish the relationship between the chemical content and the antidiabetic activity, total phenol and flavonoid contents of the extract were determined and the results obtained from the experiment revealed the high concentration of both the phenolic and flavonoid contents. The beneficial effects of several flavonol glycosides, rutin, quercetin, anthocyanins, and various flavonoid rich extracts of various plants are already known to have antidiabetic activity, especially against type 2 diabetes mellitus (39,40). These suggest that in the present study, there can also be a correlation between the rich phenolic and flavonoid contents of the extract and its potent antidiabetic activity.

From the present investigation, therefore, it can be concluded that MELS supplementation is quite beneficial in controlling the blood glucose level, without producing hypoglycemia; additionally, it improves lipid metabolism and represents a protective mechanism against the development of atherosclerosis, and prevents diabetic complications from lipid peroxidation by improving the antioxidant status in experimental diabetic rats. Hence, the aerial parts of *L. siceraria* methanol extract can be considered as a potent source of antidiabetic agents, which may be attributed to the flavonoid and polyphenolic content of the extract. However, further studies are ongoing to isolate the bioactive principle(s) from it.

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