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RESEARCH ARTICLE

In vivo assessment of antidiabetic and antioxidant activities of methanol extract of *Smilax zeylanica* leaves in wistar rats

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Abstract The aim of the present study was to evaluate the antidiabetic and antioxidant effect of methanol extract of *Smilax zeylanica* leaves on streptozotocin (STZ) induced diabetic rats. Experimental diabetes was induced by a single intraperitoneal (i.p.) injection of STZ (60 mg/kg body weight) dissolved in 0.1 M cold citrate buffer (pH 4.5). Diabetic rats exhibited increased plasma glucose levels with significant decrease in serum insulin levels. There was an apparent reduction in body weight and significant increase in aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total bilirubin levels with concomitant decrease in serum protein levels. In addition, there was significant elevation in serum urea and creatinine levels in diabetic control animals compared to the control animals. Moreover, with reference to lipid peroxidation and antioxidant systems, there was significant increase in levels of lipid peroxidation in liver and kidney tissues of diabetic animals with concomitant decrease in activities of antioxidant enzymes viz., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR), glutathione-S-transferase (GST) and non-enzymatic antioxidants glutathione (GSH), vitamin- C and vitamin-E in liver and kidney tissues. Treatment with *Smilax zeylanica* leaf extract at dose levels of 200 mg/kg and 400 mg/kg for a period of 28 days showed a significant ameliorative effect on all biochemical parameters studied. The extract treatment also increased the body weight of diabetic rats significantly compared to diabetic control rats. On other hand, the extract treatment decreased the extent of lipid peroxidation and was able to normalize the activities of enzymatic and non-enzymatic antioxidants in liver and kidney tissues. Histological examination of pancreas, liver and kidney too correlated with biochemical observations. Furthermore, the extract did not produce any deleterious effects in extract alone treated groups. The phytochemical and quantitative analysis revealed that the extract was rich in total phenolics and flavonoids. These results suggest that, the antidiabetic and antioxidant potential of *Smilax zeylanica* leaf extract might be due to stabilization and increase in all the components of antioxidant system attributed to antioxidant and free radical scavenging activity.

Keywords *Smilax zeylanica* leaves · Streptozotocin · Antidiabetic · Lipid peroxidation · Antioxidant activity

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Introduction

Over the past decade, there has been substantial interest in oxidative stress and their potential role in development of diseases. Oxidative stress in the body is produced during normal metabolic process as well as induced by a variety of environmental and chemical factors. To protect the cells from oxidative damage, mammalian cells are equipped with both enzymatic and non-enzymatic antioxidants (Freeman and Crapo 1982). However, the balance between oxygen free radical production and antioxidant defense are disturbed by several conditions. This imbalance causes cell dysfunction and destruction resulting in tissue damage. It is evident from the experimental and clinical studies, that oxidative stress plays a major role in the pathogenesis of diabetes mellitus. In diabetes mellitus, free radicals are formed disproportionately by glucose oxidation, non enzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. (Kawamura et al. 1992; Morgan et al. 2002). Unusual elevated levels of free radicals and simultaneous decline in antioxidant defense mechanisms leads to damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance.

In recent years, antioxidant supplements have attracted the focus of attention as potentials in prevention of diseases caused

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by oxidative damage and natural products with antioxidant property has been of considerable interest. The plant kingdom has become a target for the search of new drugs and many phytochemicals isolated from plant sources are used for prevention and treatment of diseases (Mary et al. 2002). It has been reported that compounds with antioxidant effect can regenerate β cells and protect pancreatic islets from oxidative damage (Alvarez et al. 2004). Traditionally many herbal medicines and plant products are used for the treatment of complicated diseases because of their therapeutic potential. Among the herbal resources belonging to Smilacaceae, the genus *Smilax* Linn. consist of about 350 species in the world (Shao et al. 2007), out of which 24 species are found in India (Saldhana and Wicolson 1976; Ramaswamy et al. 2001; Santapau and Henry 1976). In south India, the genus is represented by 4 species viz., *Smilax zeylanica* Linn., *Smilax aspera* Linn., *Smilax perfoliata* Roxb. and *Smilax wightii* A. DC (Gamble 2004). Many of them have been long used as medicinal herbs, especially as traditional Chinese medicines in China (Abdala et al. 2008). The extracts of *Smilax china* L. tubers are known to show antitumor and antioxidant activities on mice and rats (Wang et al. 1996). *Smilax zeylanica* Linn. is distributed in tropical and subtropical hills of Asia between an altitude of 500–1,800 m. This perennial climbing shrub is extensively used in traditional system of medicine against veneral diseases (Oommachan and Masih 1991), skin disorders, sores, swellings, abscess (Ambasta 2006; Nadkarni 1976) and also applied for rheumatism and pain in lower extremities (Kirtikar and Basu 1991). Species of *Smilax* Linn. contain dioscin (spirostanol triglycoside), smilagenin and sarasapogenin (1–3 %) (Evans 2002). The root of *Smilax zeylanica* Linn. contain diosgenin, a steroidal saponin glycoside (Sen 1984). Based on the widespread use of *Smilax* species in traditional medicine and the traditional importance of *Smilax zeylanica* Linn it was decided to select the plant for investigation.

Materials and methods

Chemicals

Streptozotocin (STZ) was purchased from Sigma Chemical Company St. Louis, MO, USA. All other chemicals and reagents used were of analytical grade.

Plant material

The fresh leaves of *Smilax zeylanica* Linn were collected in the month of September from yercaud hills, Tamilnadu, India. The plant material was Taxonomically indentified, confirmed and authenticated by Botaniocal Survery of India, Coimbatore, Tamilnadu (BSI/SRC/5/23/2011-12/Tech-1256) and the voucher specimen was retained in our laboratory for further reference. The collected leaves were shade dried and the dried materials were crushed to coarse powder with mechanical grinder. The powder was stored in an airtight container for extraction.

Extraction

The powdered leaves of *Smilax zeylanica* were extracted with methanol using soxhlet apparatus for 72 h. After completion of extraction, methanol was removed by distillation. The residue obtained was air dried. The percentage yield of methanol extract was 22.03 % w/w. The dried methanol extract was stored in air tight glass container for further investigation.

Phytochemical screening

The extract obtained was subjected to preliminary phytochemical screening (Khandelwal and Kokate 1995).

Quantitative estimation of bioactive compounds

Estimation of total phenolic content

The total phenolic content was determined spectrophotometrically using the Folin–Ciocalteu method. This test is based on the oxidation of phenolic groups by phosphomolybdic and phosphotungstic acids (FC reagent). This reagent, based on the Slinkard and Singleton (1977), and the early work of Singleton and Rossi (1965) is a colorimetric oxidation/reduction method for phenolic compounds. The products of the metal oxide reduction have a blue color that exhibits a broad light absorption with a maximum at 764 nm. The intensity of light absorption at that wavelength is proportional to the concentration of phenols. Briefly, a 20 μL of the diluted sample was added to 100 μL of Folin–Ciocalteu reagent. After 8 min, 300 μL of saturated sodium carbonate solution (25 %) was added. The absorbance was measured at 764 nm. The calibration curve was prepared with gallic acid solutions ranging from 10 to 1,000 μg/ml, and the results are given as gallic acid equivalents (GAE).

Determination of total tannin content

The total tannin content was determined by modified method of Polshettiwar et al. (2007). The sample (0.1 ml) was mixed with 0.5 ml of Folin-Denis reagent followed by 1 ml of sodium carbonate (Na_2Co_3) (0.5 % w/v) solution and distilled water (up to 5 ml). The absorbance was measured at 755 nm within 30 min of the reaction against the blank. The total tannin in the extract was expressed as the equivalent to tannic acid (g TAE/g extract).

Determination of total flavonoids

Flavonoid content was estimated using aluminium chloride colorimetric method. Various concentrations of extract in methanol

were mixed with 0.1 ml of 10 % aluminium chloride (w/v), 0.1 ml of 1 M potassium acetate and 2.8 ml distilled water. The mixture was allowed to stand at room temperature for 30 min. The absorbance of reaction mixture was read at 415 nm. Results are expressed as mg/g quercetin equivalent (Chang et al. 2002).

Acute oral toxicity study of *Smilax zeylanica* leaf extract

Healthy, young adult albino wistar rats (150–200 g) were used for the study. The animals were obtained from Agricultural University, Mannuthy, Thrissur, kerala (328/99/CPCSEA) and were housed in polypropylene cages. The animals were maintained under standard laboratory conditions (25°±2 °C; 12 h light and dark cycle). The animals were fed with standard diet and water ad libitum. Ethical clearance (for handling of animals and the procedures used in study) was obtained from the Institutional Animal Ethical Committee (887/ac/05/CPCSEA) before performing the study on animals.

Experimental design

Acute oral toxicity study of *Smilax zeylanica* leaf extract was carried out as per Organization for Economic Cooperation and Development (OECD) guideline 425 (Up and Down procedure). Limit test was performed to determine the safety of extract. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention given during the first 4 h), and daily thereafter for a total of 14 days. After the experimental period, the animals were weighed and humanely killed and their vital organs including heart, lungs, liver, kidneys, spleen, adrenals, sex organs and brain were grossly examined (OECD 2000).

Evaluation of extract on fasting blood glucose level in normoglycemic rats

Overnight fasted healthy male rats were divided into three groups of six animals each. Pre-treatment fasting blood glucose levels of each group were evaluated. Group I served as control received vehicle (0.5 % w/v carboxy methyl cellulose (CMC), 2 ml/kg b.w.) Group II and group III animals received methanol extract of *Smilax zeylanica* leaves orally in doses of 200 mg/kg and 400 mg/kg b.w. respectively. Blood samples were collected from the tail vein at 30, 60, 120 and 240 min after oral administration of test samples and the blood glucose levels were determined by glucose oxidase–peroxidase method.

Evaluation of extract on streptozotocin-induced diabetic rats

Induction of diabetes

Diabetes was induced by single intraperitoneal (i.p.) injection of streptozotocin at a dose of 60 mg/kg b.w. dissolved in a freshly prepared 0.1 M cold citrate buffer (pH 4.5) (Erejuwa et al. 2010; Pandit et al. 2010) to overnight fasted rats (deprived of food for 16 h allowed free access to water). In order to stave off the hypoglycaemia during the first day, the animals were given 5 % glucose solution for 24 h following streptozotocin injection. Three days after streptozotocin injection, the blood glucose levels were measured and the animals with blood glucose above 250 mg/dl were considered to be diabetic. To avoid statistical difference in blood glucose levels among the diabetic animals in a group, randomization in selection of diabetic animals were carried out. The selected animals were grouped for the study.

Treatment protocol

The animals were divided into six groups ($n=6$). Group I served as vehicle control received 0.5 % w/v CMC, 2 ml/kg (non-diabetic normal animals). Group II and group III non-diabetic animals received methanol extract of *Smilax zeylanica* leaves at doses 200 mg/kg and 400 mg/kg respectively for 28 days. Group IV served as diabetic control received vehicle 0.5 % w/v CMC, 2 ml/kg orally for 28 days. Group V and group VI diabetic animals received methanol extract of *Smilax zeylanica* leaves at dose levels 200 mg/kg and 400 mg/kg once daily orally for 28 days. Fasting blood glucose levels were measured before treatment and on day 7, 14, 21 and 28 days of treatment. Body weight of all experimental animals was recorded daily for 28 days.

Sample collection

At the end of the experimental period (28 days), blood samples were collected from retro-orbital plexus under anaesthesia. Serum was separated by centrifugation and subjected to biochemical analysis.

Biochemical analysis

Fasting glucose level was estimated by glucose oxidase–peroxidase method (Triender 1969) and the results were expressed as mg glucose/dl. Serum transaminases (AST and ALT) were determined by the method of Reitman and Frankel (1957). The activity of serum alkaline phosphatase (ALP) was estimated by the method of Kind and King (1954). Serum bilirubin (SB) and total protein (TB) were estimated by the methods of Malloy and Evelyn (1937) and Wooten (1964) respectively. Serum insulin levels were determined using RIA kit (Linco, St, Charles Mo). Serum urea was determined according to urease-colorimetric method described by Patton and Crouch (1977) and serum creatinine was measured according to the method described by Siest et al. (1985).

After blood collection the animals were sacrificed by cervical decapitation and the organs, liver, kidney and pancreas were excised. The isolated liver and kidney were washed in

ice cold saline to remove blood stains, blotted to dryness and examined for any deductable changes. A portion of liver and kidney were then homogenized for biochemical assays.

Preparation of tissue homogenate

The tissues were weighed and 10 % tissue homogenate was prepared with 0.025 M Tris–HCl buffer, pH 7.5. After centrifugation at 10,000×*g* for 10 min, the clear supernatant was used to measure thiobarbituric acid reactive substances (TBARS). For the determinations of vitamin E level, the tissues were weighed and lipids were extracted from tissues by the method of Folch et al. (1957) using chloroform–methanol mixture ($CHCl_3$: CH_3OH) (2:1; v/v). The extract used for the estimation of vitamin E. For the estimation of non-enzymatic and enzymatic antioxidants, tissues were minced and homogenized (10 % w/v) in 0.1 M phosphate buffer (pH 7.0) and centrifuged for 10 min and the resulting supernatant was used for enzyme assays.

Estimation of lipid peroxidation (LPO)

The levels of thiobarbituric acid reactive substances (TBARS) in the liver and kidney homogenate were measured by the method of Ohkawa et al. (1979) as a marker for lipid peroxidation. An aliquot of liver homogenates was mixed with 0.2 ml of 8.1 % Sodium dodecyl sulphate (SDS), 1.5 ml of 20 % acetic acid and 1.5 ml of 0.8 % thiobarbituric acid (TBA), then the volume was adjusted to 4.0 ml with distilled water. After boiled at 95° for 60 min, the reaction solution was extracted with 1.0 ml of distilled water and 5.0 ml of n-butanol and pyridine (15:1 v/v). The absorbance at 532 nm of the organic layer was determined after centrifugation.

Estimation of superoxide dismutase (SOD) activity

0.5 ml of tissue homogenate was diluted with 1 ml of water. In this mixture, 2.5 ml of ethanol and 1.5 ml of chloroform (all reagents chilled) were added and shaken for 1 min at 4 °C then centrifuged. The enzyme activity in the supernatant was determined. The assay mixture contained 1.2 ml of sodium pyrophosphate buffer (0.025 M, pH 8.3), 0.1 ml of 186 μM phenyl methane sulphate (PMS), 0.3 ml of 30 μM nitro blue tetrazolium (NBT), 0.2 ml of 780 μM nicotinamide adenine dinucleotide reduced (NADH), appropriately diluted enzyme preparation and water in a total volume of 3 ml. Reaction was started by the addition of nicotinamide adenine dinucleotide reduced (NADH). After incubation at 30 °C for 90 s the reaction was stopped by the addition of 1 ml glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4 ml of n-butanol. The intensity of the chromogen in the butanol layer was measured at 560 nm against butanol blank. A system devoid of enzyme served as control. One unit of the enzyme activity is defined as the enzyme reaction, which gave 50 % inhibition of nitro blue tetrazolium (NBT) reduction in one minute under the assay conditions (Kakkar et al. 1984).

Estimation of catalase activity

The reaction mixture (1.5 ml) contained 1.0 ml of 0.01 M phosphate buffer (pH 7.0), 0.1 ml of tissue homogenate and 0.4 ml of 2 M H_2O_2. The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5 % potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). Then the absorbance was read at 620 nm; CAT activity was expressed as μM of H_2O_2 consumed/min/mg protein (Sinha 1972).

Glutathione peroxidase (GSH-Px)

GSH-Px activity was measured by the method of Rotruck et al. (1973). To 0.2 ml of buffer, 0.2 ml of ethylenediaminetetraacetic acid (EDTA), 0.1 ml of sodium azide and 0.5 ml of tissue homogenate were added. To that mixture, 0.2 ml of glutathione solution and 0.1 ml of hydrogen peroxide were added. The contents were mixed well and incubated at 37 °C for 10 min along with the control tubes containing all the reagents but no enzyme. After 10 min, the reaction was arrested by the addition of 0.4 ml of 10 % trichloroacetic acid (TCA). 0.2 ml of tissue homogenate was added to the control tubes. The tubes were centrifuged and supernatant was assayed for glutathione content by adding Ellman's reagent.

Glutathione-S-transferase (GST)

GST activity was measured by the method of Habig et al. (1974). The reaction mixture containing 1 ml of buffer, 0.1 ml of 1-chloro-2, 4-dinitrobenzene (CDNB), 0.1 ml of homogenate and 1.7 ml of distilled water was incubated at 37 °C for 5 min. The reaction was then started by the addition of 1 ml of glutathione. The increase in absorbance was followed for 3 min at 340 nm. The reaction mixture without the enzyme was used as blank.

Glutathione reductase (GR) activity

Glutathione reductase that utilizes nicotinamide adenine dinucleotide phosphate (NADPH) to convert metabolized glutathione (GSSG) to the reduced form was assayed by the method of Horn and Burns (1978).

Reduced glutathione (GSH)

GSH was estimated by the method of Ellman (1959). 0.5 ml of tissue homogenate was precipitated with 2 ml of 5 % trichloroacetic acid (TCA). After centrifugation, 1 ml of supernatant was taken and added 0.5 ml of Ellman's reagent (19.8 mg of 5,5′ dithio (bis) nitrobenzoic acid in 100 ml of 1 % sodium

Table 1 Total Phenolic content (TPC), Total Tannin content (TTC) and Total Flavonoid content (TFC) in methanol extract of *Smilax zeylanica* leaves

Extract	TPC (mg of GAE/g of extract)	TTC (mg of TAE/g of extract)	TFC (mg of quercetin/g of extract)
Methanol extract of *Smilax zeylanica* leaves	620±2.36	750±2.85	1,137.5±12.45

Values are means ± SD from three determinations. Total Phenolic content (*TPC*) is expressed as milligram of gallic acid equivalent per gram of extract. Total tannin content (*TTC*) is expressed as milligrams of tannic acid equivalents per gram of dry extract. Total Flavonoid content (*TFC*) is expressed as milligrams of quercetin equivalents per gram of dry extract

citrate) and 3 ml of phosphate buffer. Standards were treated in a similar way and the colour developed was read at 412 nm.

Estimation of ascorbic acid (vitamin C)

0.5 ml of tissue homogenate was mixed thoroughly with 1.5 ml of 6 % TCA and centrifuged for 10 min at 3500*g*. After centrifugation, 0.5 ml of the supernatant was mixed with 0.5 ml of dinitrophenylhydrazine (DNPH) reagent and allowed to stand at room temperature for an additional 3 h then added 2.5 ml of 85 % sulphuric acid and allowed to stand for 30 min. Then the absorbance was read at 530 nm. A set of standards containing 10–50 μg of ascorbic acid were taken and processed similarly along with a blank. Ascorbic acid values were expressed as μg/mg tissue (Omaye et al. 1979).

Estimation of vitamin E

0.1 ml of lipid extract, 1.5 ml of ethanol and 2 ml of petroleum ether were added, mixed and centrifuged for 3,000 rpm for 10 min. The supernatant was evaporated to dryness at 80 °C then 0.2 ml of 2, 2-1-dipyridyl solution and 0.2 ml of ferric chloride solution was added and mixed well. This was kept in dark for 5 min and added 2 ml of butanol. Then the absorbance was read at 520 nm. Standards of α-tocopherol in the range of 10–100 μg were taken and treated similarly along with blank containing only the reagent. The values were expressed as μg/mgtissue (Barker et al. 1951).

Histopathological examination

A portion of liver, kidney and the isolated pancreas were fixed in 10 % formalin and embedded in molten paraffin wax and were ultra sectioned (5–6 μm thickness), stained with hematoxylin and eosin and were examined under light microscope for histopathological changes.

Statistical analysis

Results were expressed as mean ± standard error of mean (SEM). The results were analysed for statistical significance by one way ANOVA followed by dunnett's test (Graphpad Software Inc,La Jolla, CA. Trial version). The criterion for statistical significance was set at $P<0.05$.

Results

Percentage yield of extract

The percentage yield of extract obtained from extraction of *Smilax zeylanica* leaves using methanol as solvent was found to be 22.03 % w/w.

The phytochemical examination of methanol extract of *Smilax zeylanica* leaves revealed the presence of carbohydrates, alkaloids, phenolics, tannins, flavonoids, glycosides and alkaloids.

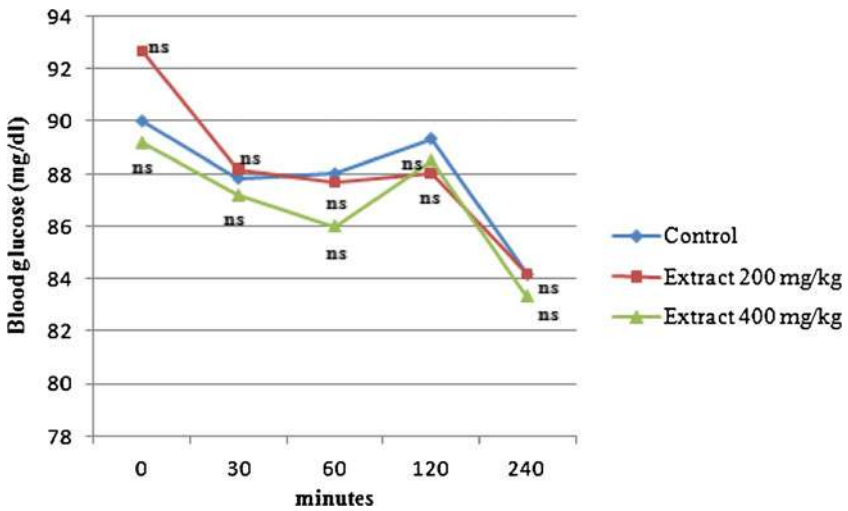


Fig. 1 Effect of *Smilax zeylanica* leaf extract on blood glucose levels in normoglycaemic rats. All values are expressed as mean ± S.E.M for six animals. *ns* denotes non-significance at various time intervals compared with values of normal control group

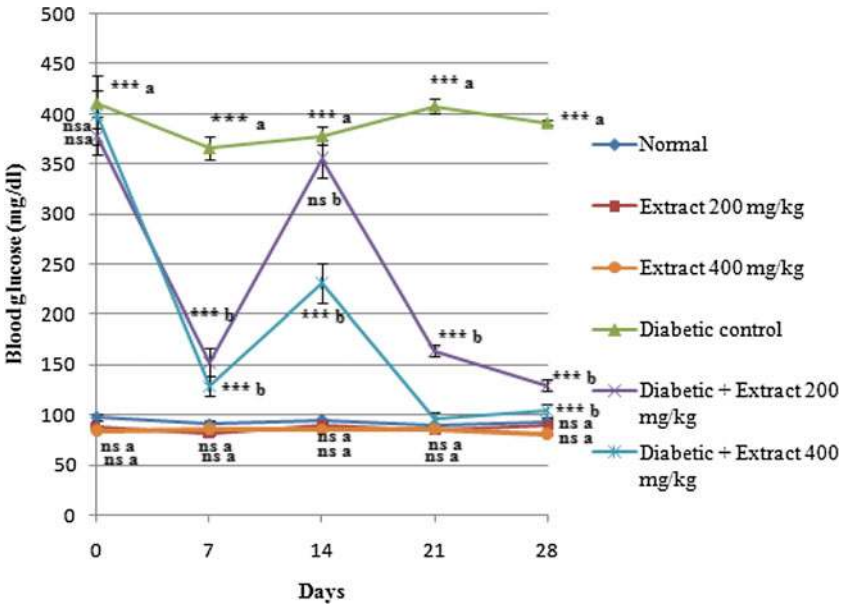


Fig. 2 Effect of *Smilax zeylanica* leaf extract on fasting blood glucose levels in control and STZ induced diabetic rats. All values are expressed as mean ± S.E.M, $n=6$ in each group. One -way ANOVA followed by Dunnett's test was used to compare experimental groups. [a] Values are significantly different from control group; *ns* non significant; $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$. [b]Values are significantly different from diabetic control group; *ns* non significant; $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$

Quantitative estimation of bioactive compounds

The total phenolic content in methanol extract was found to be 620±2.36 mg GAE/gm of dry extract. The total tannin content was found to be 750±2.85 mg tannic acid equivalents per g of dry extract and the flavonoid content was 1,137±12.45 mg quercetin equivalent/gm of extract (Table 1).

Acute oral toxicity study

Acute oral toxicity study was carried out as per OECD guideline 425. From the limit test results it was observed that, the *Smilax zeylanica* leaf extract is safe upto a dose level of 2,000 mg/kg. There was no mortality and the experimental animals did not show any toxic effect throughout the observation period of 14 days.

Effect of *Smilax zeylanica* leaf extract on normoglycaemic rats

As a preliminary activity assessment, the methanol extract was administered to normal animals at two dose levels to determine the acute effects on blood glucose concentrations. The fasting blood glucose levels of each group of animals were recorded at

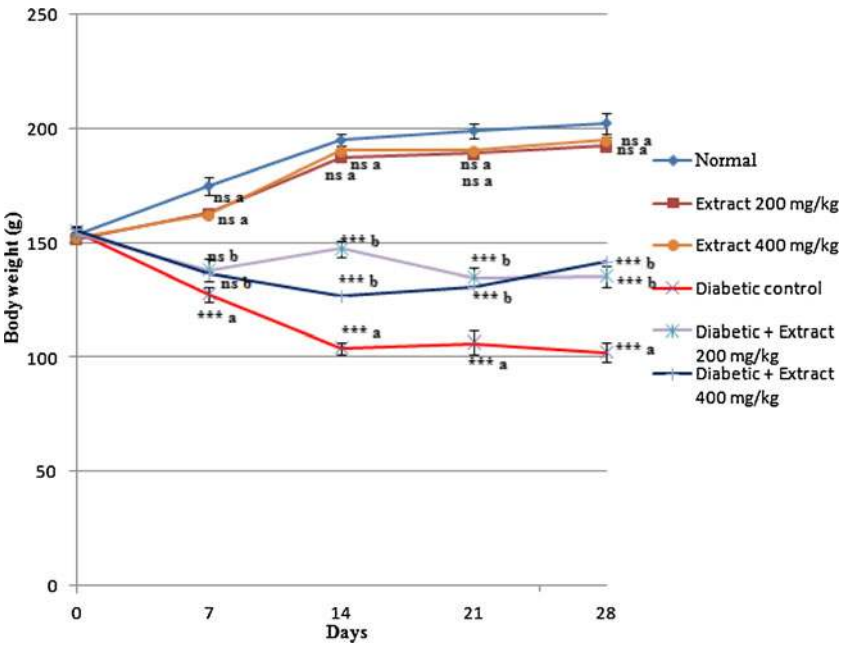


Fig. 3 Effect of *Smilax zeylanica* leaf extract on body weight of control and experimental rats. Average body weight of different animal groups at various intervals. Results are given as mean ± S.E.M for six animals. There was no significant difference in final body weight between the control (normal) group and extract alone treated groups (ns-a). STZ induced diabetic control animals showed a significant decrease in body weight ($^{***}p<0.001$) compared to control animals (**a**). The group V and group VI diabetic animals treated with extract 200 mg/kg and 400 mg/kg showed a significant increase ($^{***}p<0.001$) in final body weight compared to untreated animals (**b**)

Table 2 Effect of *Smilax zeylanica* leaf extract on biochemical parametes in control and experimental groups of rats

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TB (mg/dl)	TP (mg/dl)
Group I					
(Control) (0.5 %CMC) 2 ml/kg	35.50±2.18	28.67±2.30	67.00±3.67	0.38±0.04	7.8±0.11
Group II					
(Extract 200 mg/kg)	25.00±2.51$^{ns, a}$	19.33±1.28$^{ns, a}$	59.17±2.68$^{ns, a}$	0.41±0.04$^{ns, a}$	7.55±0.18$^{ns, a}$
Group III					
(Extract 400 mg/kg)	29.50±1.31$^{ns, a}$	24.33±1.08$^{ns, a}$	64.33±1.87$^{ns, a}$	0.43±0.04$^{ns, a}$	7.35±0.14$^{ns, a}$
Group IV					
Diabetic control	154.2±3.51$^{***, a}$	147.5±3.59$^{***, a}$	1,044.00±36.95$^{***, a}$	5.7±0.57$^{***, a}$	6.06±0.08$^{***, a}$
Group V					
(Diabetic + Extract 200 mg/kg)	130.0±12.58$^{*, b}$	75.83±9.60$^{***, b}$	852.5±64.03$^{***, b}$	2.63±0.05$^{***, b}$	7.30±0.12$^{***, b}$
Group VI					
(Diabetic + Extract 400 mg/kg)	99.67±2.59$^{***, b}$	45.17±2.07$^{***, b}$	150.00±10.25$^{***, b}$	1.63±0.05$^{***, b}$	7.51±0.06$^{***, b}$

All values are expressed as mean ± S.E.M, $n=6$ in each group. One -way ANOVA followed by Dunnett's test was used to compare experimental groups

[a] Values are significantly different from control group; *ns* non significant; $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$

[b] Values are significantly different from diabetic control group; *ns* non significant; $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$

various time intervals. It was observed that, there was no remarkable change in blood glucose level on normoglycaemic rats (Fig. 1).

Effect of *Smilax zeylanica* leaf extract on fasting blood glucose levels in normal and STZ induced diabetic rats

The effect of repeated daily dose of *Smilax zeylanica* leaf extract in normal and STZ induced diabetic rats is presented in Fig. 2. The fasting blood glucose levels of each group of animals were recorded before treatment and on day 7, 14, 21 and day 28 of experimental study. The fasting blood glucose levels were significantly high ($p<0.001$) throughout the experimental period compared to group I control normal rats. There was no significant difference (ns) in fasting blood glucose between group V and group VI compared with group IV diabetic control animals before administration of extract (day 0). A significant decrease ($p<0.001$) in fasting blood glucose levels were observed on day 7 in group V diabetic animals treated with *Smilax zeylanica* leaf extract 200 mg/kg and group VI animals treated with *Smilax zeylanica* leaf extract 400 mg/kg. On day 14, the fasting blood glucose levels in group V animals treated with extract 200 mg/kg were increased and it was observed that there was no significant difference (ns) in fasting blood glucose levels compared to group IV diabetic control animals. Though group VI animals treated with extract 400 mg/kg showed an increase in fasting blood glucose levels on day 14, the results were significant compared to diabetic control animals ($p<0.001$). Significant decrease ($p<0.001$) in blood glucose levels were observed on day 21 and day 28 in treatment groups compared to diabetic control animals.

The percentage decrease in blood glucose levels in group V animals treated with *Smilax zeylanica* leaf extract 200 mg/kg on day 7, 14, 21 and day 28 was found to be 58.56 %, 10.23 %, 55.55 % and 64.99 % respectively and with 400 mg/kg the percentage decrease in blood glucose on day 7, 14, 21 and day 28 was found to be 67.12 %, 42.36 %, 75.48 % and 73.55 % respectively.

Non-diabetic animals in group II and group III treated with *Smilax zeylanica* leaf extract at dose levels 200 mg/kg and

Table 3 Effect of *Smilax zeylanica* leaf extract on serum insulin levels in control and experimental groups of rats

Groups	Serum insulin μ IU/ml
Group I (Control)	
(0.5 %CMC) 2 ml/kg	29.98±0.85
Group II	
(Extract 200 mg/kg)	29.50±0.87$^{ns, a}$
Group III	
(Extract 400 mg/kg)	30.67±0.47$^{ns, a}$
Group IV	
Diabetic control	11.20±0.56$^{***, a}$
Group V	
(Diabetic + Extract 200 mg/kg)	15.33±0.89$^{**, b}$
Group VI	
(Diabetic + Extract 400 mg/kg)	21.57±0.92$^{***, b}$

All values are expressed as mean ± S.E.M, $n=6$ in each group. One -way ANOVA followed by Dunnett's test was used to compare experimental groups

[a] Values are significantly different from control group; *ns* non significant; $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$

[b] Values are significantly different from diabetic control group; *ns* non significant; $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$

Table 4 Effect of *Smilax zeylanica* leaf extract on blood urea and creatinine levels in control and experimental groups of rats

Groups	Blood urea (mg/dl)	Creatinine (mg/dl)
Group I (Control)		
(0.5 %CMC) 2 ml/kg	51.17±4.75	1.63±0.04
Group II		
(Extract 200 mg/kg)	57.00±3.09$^{ns, a}$	1.60±0.05$^{ns, a}$
Group III		
(Extract 400 mg/kg)	57.83±1.90$^{ns, a}$	1.48±0.07$^{ns, a}$
Group IV		
Diabetic control	135.7±5.85$^{***, a}$	2.68±0.08$^{***, a}$
Group V		
(Diabetic + Extract 200 mg/kg)	120.00±3.71$^{*, b}$	2.23±0.07$^{***, b}$
Group VI		
(Diabetic + Extract 400 mg/kg)	64.00±1.41$^{***, b}$	1.88±0.07$^{***, b}$

All values are expressed as mean ± S.E.M, $n=6$ in each group. One -way ANOVA followed by Dunnett's test was used to compare experimental groups

[a] Values are significantly different from control group; *ns* non significant; $*P<0.05$; $**P<0.01$; $***P<0.001$

[b] Values are significantly different from diabetic control group; *ns* non significant; $*P<0.05$; $**P<0.01$; $***P<0.001$

400 mg/kg did not exhibit any change in fasting blood glucose levels.

Effect of *Smilax zeylanica* leaf extract on body weight

Average body weights of different animal groups at various intervals are shown in Fig. 3. There was no significant difference in final body weight between the control group (202.5±4.42 g) and extract alone treated groups (Extract 200 mg/kg- 190.8±0.83 g, Extract 400 mg/kg- 195.0±1.29 g). The final body weight of diabetic control animals (102.5±4.23 g) were significantly less ($p<0.001$) compared to control animals (202.5±4.42 g). The group V and group VI diabetic animals treated with extract 200 mg/kg and 400 mg/kg showed a significant increase ($p<0.001$) in final body weight (Extract 200 mg/kg- 132.5±1.70 g and Extract 400 mg/kg- 141.7±4.59 g) compared to untreated animals. It shows the *Smilax zeylanica* leaf extract prevented the body weight loss in diabetic animals and maintained the growth rate near normal.

Effect of *Smilax zeylanica* leaf extract on biochemical parameters

The effect of *Smilax zeylanica* leaf extract on the activities of marker enzymes and serum protein levels in serum of control and experimental groups are shown in Table 2. A significant increase ($P<0.001$) in AST (154.2±3.51), ALT (147.5±3.59), ALP

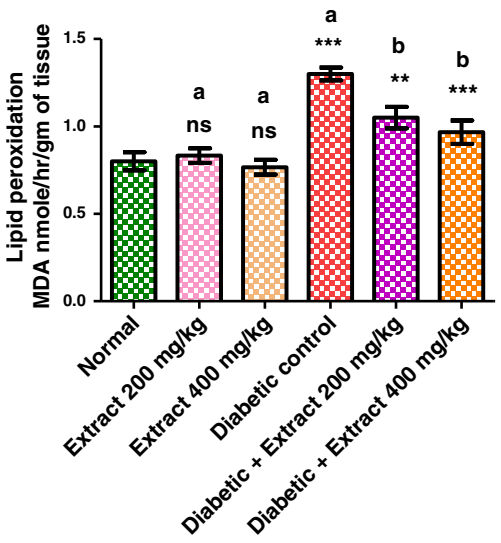


Fig. 4 Effect of *Smilax zeylanica* leaf extract on lipid peroxidation in liver homogenate of experimental animals. Results are expressed as mean ± S.E.M. [a]values are significantly different from control group; *ns* non significant; $*P<0.05$; $**P<0.01$; $***P<0.001$. [b]values are significantly different from diabetic control animals; *ns* non significant; $*P<0.05$;$**P<0.01$;$***P<0.001$. (ANOVA, followed by Dunnett's test)

(1,044.00±36.95 IU/L) and total bilirubin (TB) levels (5.7±0.57 mg/dl) were observed in group IV diabetic control animals compared to group I control animals with AST (35.50±2.18), ALT (28.67±2.30), ALP (67.00±3.67 IU/L) and TB levels (0.38±0.04 mg/dl). The animals treated with *Smilax zeylanica* leaf extract 200 mg/kg and 400 mg/kg showed a significant decrease in AST, ALT, ALP and TB levels from (130±12.58 to 99.67±

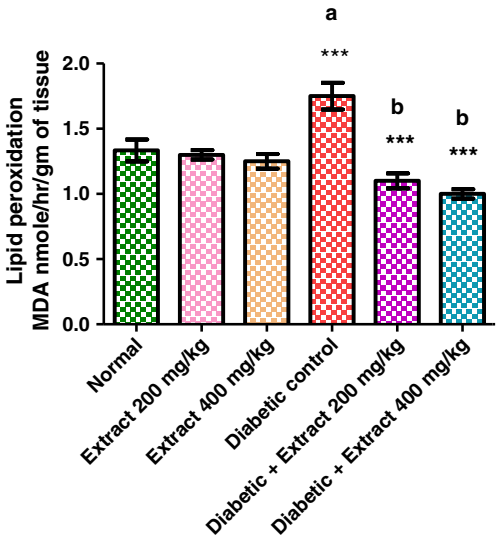


Fig. 5 Effect of *Smilax zeylanica* leaf extract on lipid peroxidation in kidney homogenate of experimental animals. Results are expressed as mean ± S.E.M. [a]values are significantly different from control group; *ns* non significant; $*P<0.05$; $**P<0.01$; $***P<0.001$. [b]values are significantly different from diabetic control animals; *ns* non significant; $*P<0.05$; $**P<0.01$;$***P<0.001$. (ANOVA, followed by Dunnett's test)

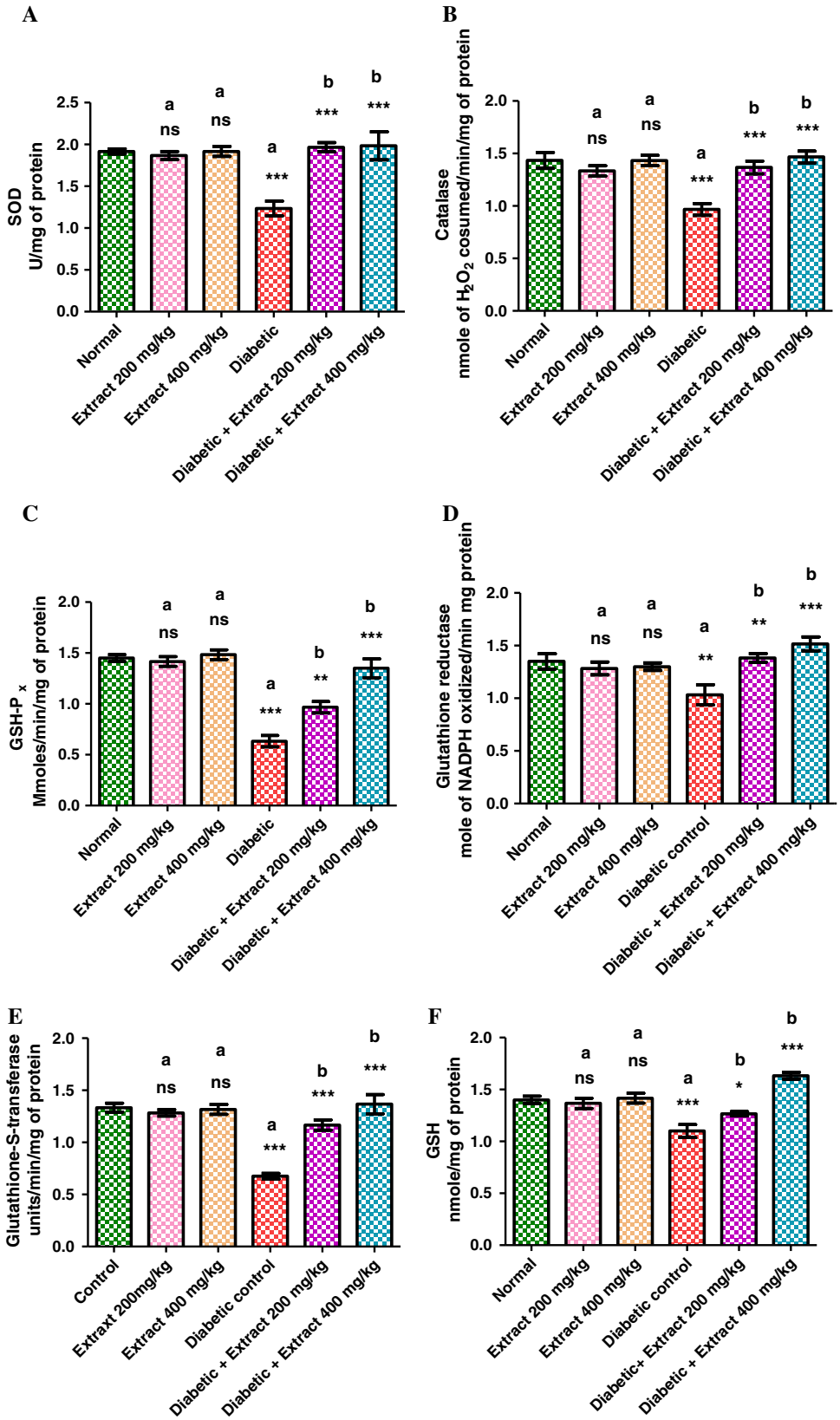


Fig. 6 Effect of *Smilax zeylanica* leaf extract on the status of enzymatic and non-enzymatic antioxidants in liver homogenate of control and experimental group of animals. Results are expressed as mean ± S.E.M. [a]values are significantly different from control group; *ns* non significant; $*P<0.05$; $**P<0.01$; $***P<0.001$. [b]values are significantly different from NDEA induced untreated group; *ns* non significant; $*P<0.05$;$**P<0.01$;$***P<0.001$. (ANOVA, followed by Dunnett's test)

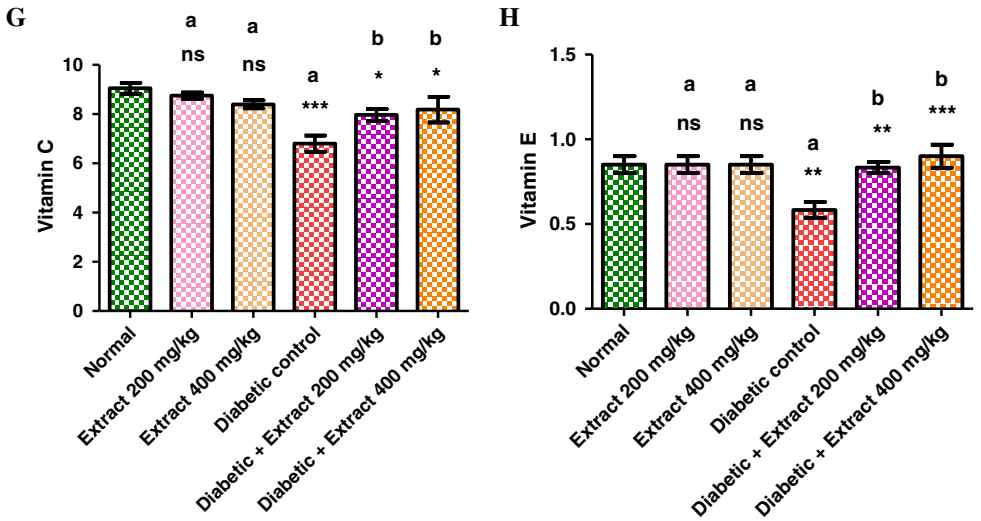


Fig. 6 (continued)

2.59) ($p<0.05$ to $p<0.001$), (75.83±9.60 to 45.17±2.07) ($p<0.001$), (852.00±64.03 to 150.00±10.25 IU/L) ($p<0.001$) and (2.63±0.05 to 1.63±0.05 mg/dl) ($p<0.001$) respectively compared to diabetic control animals. The total protein levels were decreased ($P<0.001$) in diabetic control animals (6.06±0.08 mg/dl) compared to group I control animals (7.8±0.11 mg/dl). However, upon treatment with *Smilax zeylanica* leaf extract 200 mg/kg and 400 mg/kg the protein levels were significantly increased from (7.30±0.12 to 7.51±0.06 mg/dl) ($p<0.01$ to $p<0.001$). Non-significant alteration in serum marker enzymes in extract alone treated group II and group III animals when compared with control group I animals indicates the non-toxic nature of *Smilax zeylanica* leaf extract.

Effect of *Smilax zeylanica* leaf extract on serum insulin levels

Table 3 presents the effect of *Smilax zeylanica* leaf extract on serum insulin levels in control and experimental animals after 28 days of treatment. It was observed that, in group IV diabetic control animals the serum insulin levels were significantly reduced (11.20±0.56) ($p<0.001$) when compared to group I normal control animals (29.98±0.85 μ IU/ml). However, administration of *Smilax zeylanica* leaf extract at dose levels 200 mg/kg and 400 mg/kg significantly increased ($p<0.001$) the serum insulin levels to 15.33±0.89 and 21.5±0.92 μ IU/ml respectively compared to diabetic control animals.

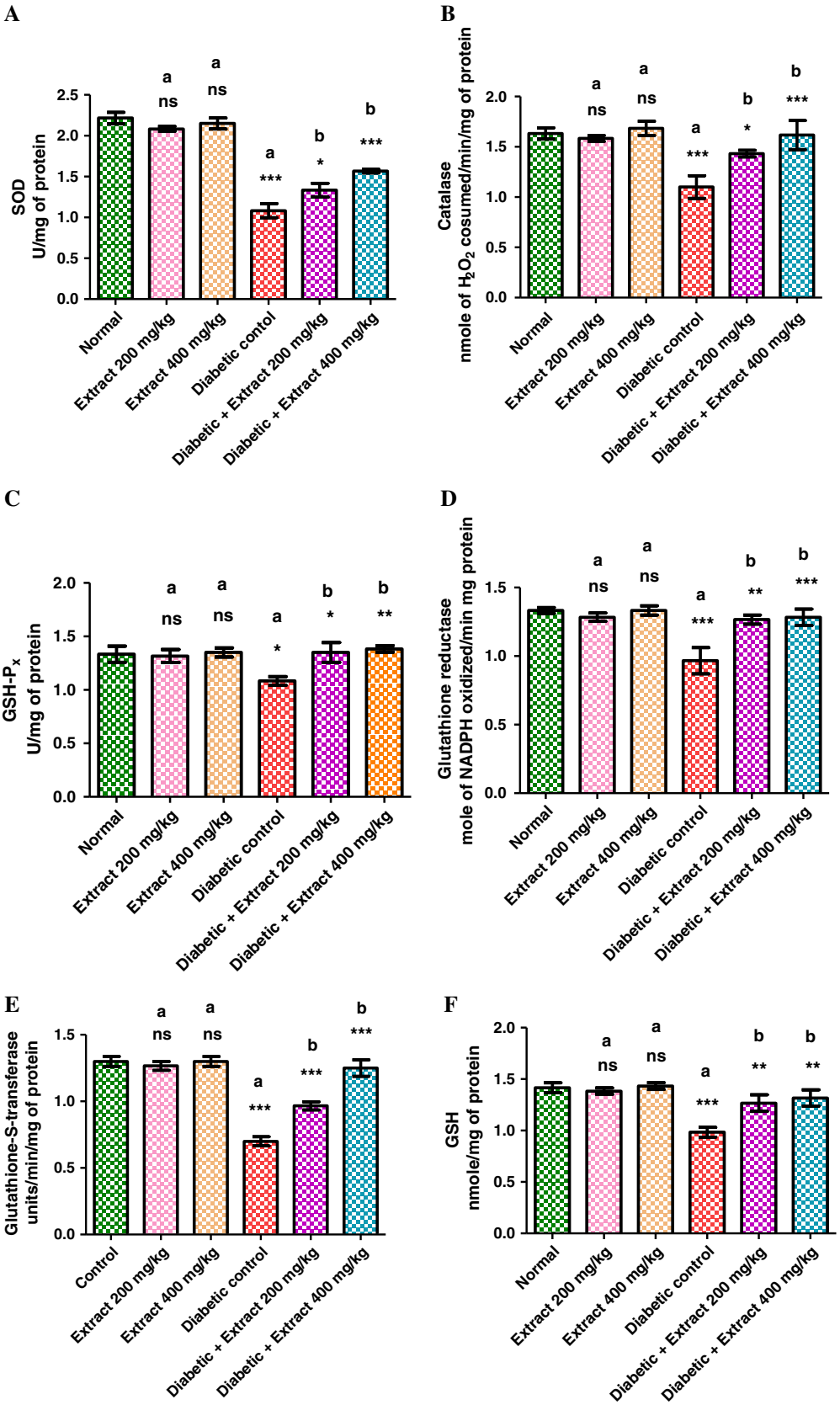
Effect of *Smilax zeylanica* leaf extract on serum urea and creatinine levels

The effect of *Smilax zeylanica* leaf extract on serum urea and creatinine levels of control and experimental groups are shown in table 4. Serum urea and creatinine levels were significantly increased ($p<0.001$) in group IV diabetic control animals with urea level 135.7±5.85 mg/dl and creatinine level 2.68±0.08 mg/dl compared to group I normal control animals with urea level 51.17±4.75 and creatinine level 1.63±0.04. In contrast, treatment with *Smilax zeylanica* leaf extract 200 mg/kg and 400 mg/kg significantly reduced the levels of serum urea and creatinine. The serum urea levels were reduced to 120.00±3.71 mg/dl and 2.23±0.07 mg/dl on treatment with *Smilax zeylanica* leaf extract 200 mg/kg and on treatment with *Smilax zeylanica* leaf extract 400 mg/kg the urea and creatinine levels were reduced to 64.00±1.41 and 1.88±0.07 mg/dl respectively.

Effect of *Smilax zeylanica* leaf extract on LPO and levels of antioxidant enzymes

The lipid peroxidation levels in liver and kidney homogenate of control and experimental animals are illustrated in Figs. 4 and 5. A significant increase ($p<0.001$) in the production of MDA was observed in liver (1.30±0.03) and kidney homogenate (1.75±0.10) in group IV diabetic control animals compared to group I normal control animals with MDA levels in liver homogenate (0.80±0.05) and kidney homogenate (1.33±0.20). Administration of *Smilax zeylanica* leaf extract at dose levels doses 200 mg/kg and 400 mg/kg showed a significant reduction in LPO as evidenced by a significant fall in MDA levels to 1.05±0.06 ($p<0.001$) and 0.96±0.06 ($p<0.001$) in liver homogenate and 1.10±0.05 ($p<0.01$) and 1.00±0.03 ($p<0.001$) in kidney homogenate respectively.

Fig. 7 Effect of *Smilax zeylanica* leaf extract on the status of enzymatic and non-enzymatic antioxidants in kidney homogenate of control and experimental group of animals. Results are expressed as mean ± S.E.M. [a]values are significantly different from control group; *ns* non significant; $*P<0.05$; $**P<0.01$; $***P<0.001$. [b]values are significantly different from diabetic control group; *ns* non significant; $*P<0.05$;$**P<0.01$;$***P<0.001$. (ANOVA, followed by Dunnett's test)


A
SOD
U/mg of protein
B
Catalase
nmole of H_2O_2 cosumed/min/mg of protein
C
GSH-P_x
U/mg of protein
D
Glutathione reductase
mole of NADPH oxidized/min mg protein
E
Glutathione-S-transferase
units/min/mg of protein
F
GSH
nmole/mg of protein
Normal
Control
Extract 200 mg/kg
Extract 400 mg/kg
Diabetic contol
Diabetic control
Diabetic + Extract 200 mg/kg
Diabetic + Extract 400 mg/kg
ns
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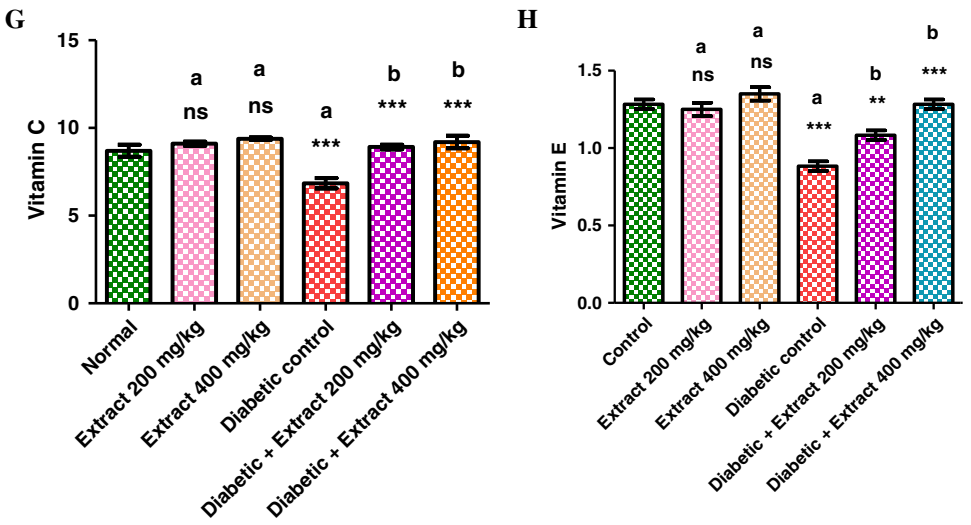

Fig. 7 (continued)

Figure 6a–h portrays the activities and the levels of antioxidants in liver of control and experimental animals and the results were expressed as U/mg of protein. A significant decrease ($p<0.001$) in activities of SOD (1.23±0.08), catalase (0.96±0.05), GSH-Px (0.63±0.05), GST (0.67±0.02) and GR (1.03±0.09) ($p<0.01$) were noted in liver of group IV diabetic control animals compared to group I normal control animals with SOD (1.91±0.03), catalase (1.43±0.07), GSH-Px (1.45±0.03), GST (1.33±0.04) and GR (1.35±0.07). The levels of non-enzymatic antioxidants GSH (1.10±0.06), vitamin-C (6.80±0.33) and vitamin-E (0.58±0.04) were also significantly decreased ($p<0.001$) in liver of group IV diabetic control animals compared to group I normal animals with GSH (1.40±0.03), vitamin-C (9.05±0.21) and vitamin-E (0.85±0.05). However, administration of *Smilax zeylanica* leaf extract at dose levels 200 mg/kg and 400 mg/kg significantly increased the levels of SOD, catalase, GSH-Px, GR and GST to near normal levels. The SOD levels in liver of animals treated with extract 200 mg/kg and 400 mg/kg were significantly increased ($p<0.001$) to 1.96±0.05 and 1.98±0.16 respectively compared to group IV diabetic animals. On treatment with extract 200 mg/kg and 400 mg/kg the catalase, GSH-Px, GR and GST levels were significantly increased from (1.36±0.06) to (1.46±0.05) ($p<0.001$), (0.96±0.05) to (1.35±0.09) ($p<0.01$ to $p<0.01$), (1.38±0.04) to (1.51±0.06) ($p<0.01$ to $p<0.001$) and (1.16±0.04) to (1.36±0.09) U/mg protein ($p<0.001$), respectively compared to group IV diabetic control animals. Similarly, administration of extract 200 mg/kg and 400 mg/kg significantly increased the non-enzymatic antioxidants, GSH, vitamin C and vitamin E levels from (1.26±0.02) to (1.63±0.03) (<0.05 to $p<0.001$), (7.96±0.24) to (8.18±0.51) ($p<0.05$) and (0.83±0.03) to (0.90±0.06) ($p<0.01$ to $p<0.001$), respectively compared to group IV diabetic control animals.

Figure 7a–h portrays the activities and the levels of antioxidants in kidney of control and experimental animals. A significant decrease ($p<0.001$) in activities of SOD (1.08±0.08), catalase (1.10±0.11), GR (0.96±0.09), GST (0.70±0.03) and GSH-Px (1.08±0.04) ($p<0.05$) were noted in kidney of group IV diabetic control animals compared to group I normal control animals with SOD (2.21±0.07), catalase (1.63±0.05), GR (1.33±0.02), GST (1.30±0.03) and GSH-Px (1.33±0.07). The levels of non-enzymatic antioxidants GSH (0.98±0.04), vitamin-C (6.80±0.33) and vitamin-E (0.58±0.04) were also significantly decreased ($p<0.001$) in liver of group IV diabetic control animals compared to group I normal animals with GSH (1.40±0.03), vitamin-C (6.85±0.29) and vitamin-E (0.88±0.03). However, administration of *Smilax zeylanica* leaf extract at dose levels 200 mg/kg and 400 mg/kg significantly increased the levels of SOD, catalase, GSH-Px, GR and GST to near normal levels. The SOD levels in liver of animals treated with extract 200 mg/kg and 400 mg/kg were significantly increased to 1.33±0.08 ($p<0.05$) and 1.56±0.02 ($p<0.001$) respectively compared to group IV diabetic animals. On treatment with extract 200 mg/kg and 400 mg/kg the catalase, GSH-Px, GR and GST levels were significantly increased from (1.43±0.03) to (1.67±0.14) ($p<0.05$ to $p<0.001$), (1.35±0.09) to (1.38±0.03) ($p<0.05$ to $p<0.01$), (1.26±0.03) to (1.28±0.06) ($p<0.01$ to $p<0.001$) and (0.96±0.03) to (1.25±0.06) ($p<0.001$) U/mg protein, respectively compared to group IV diabetic control animals. Similarly, administration of extract 200 mg/kg and 400 mg/kg significantly increased the non-enzymatic antioxidants, GSH, vitamin C and vitamin E levels from (1.26±0.08) to (1.31±0.07) ($p<0.01$), (8.91±0.13) to (9.20±0.35) ($p<0.001$) and (1.08±0.03) to (1.28±0.03) ($p<0.01$ to $p<0.001$), respectively compared to group IV diabetic control animals.

The extract did not produce any deleterious effect on the antioxidant defense system in normal animals which is evidenced from the non-significant alteration of the enzymatic and non-enzymatic antioxidants along with the maintained rate of lipid peroxidation in group II and group III animals

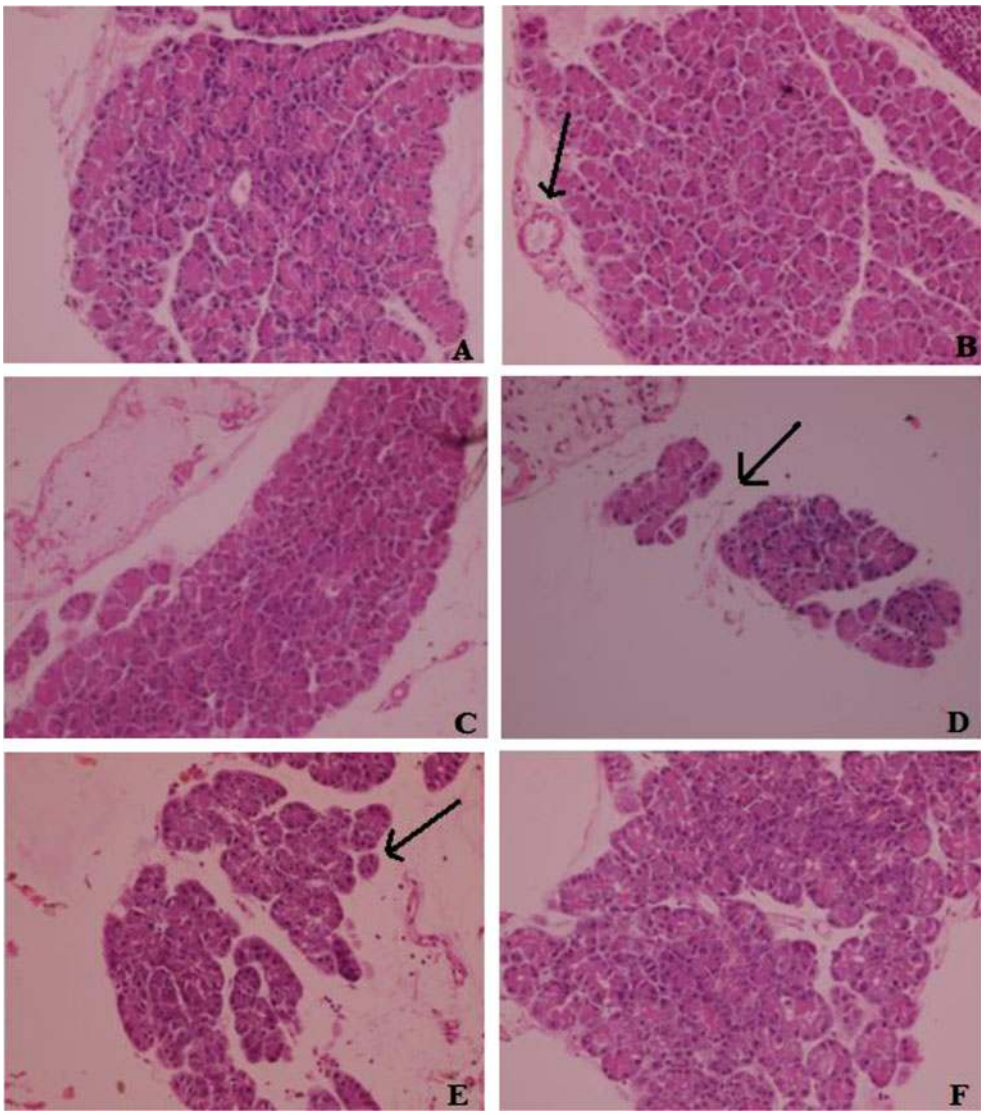

Fig. 8 Histopatholgical image of pancreas. **a** (40×) H and E stained section of pancreas from group I animals showing pancreatic acini with normal morphology. **b** and **c** (40×) H and E stained section of pancreas from group II and group III animals given extract alone in dose level 200 mg/kg and 400 mg/kg respectively for 28 days showing pancreatic parenchyma with normal pancreatic acini and lymph node (indicated by *arrows*) with normal histology indicating the non-toxic nature of extract. **d** (40×) H and E stained section of pancreas from group IV streptozotocin induced diabetic control animals showing atropy and degenerative changes in pancreatic acini (indicated by *arrows*) with infilteration of inflammatory cells (not shown in figure). **e** (40×) H and E stained section of pancreas from group V diabetic animals treated with *Smilax zeylanica* leaf extract 200 mg/kg for 28 days showing regeneration of islet cells (indicated by *arrows*). **f** (40×) H and E stained section of pancreas from group VI diabetic animals treated with *Smilax zeylanica* leaf extract 400 mg/kg for 28 days showing normal pancreatic parenchyma with pancreatic acini

when compared with the normal control group I animals. The results indicate that, the level of lipid peroxidation which increased in liver of STZ induced diabetic animals were lowered in extract treated animals and in contrast the antioxidant status which was found to be decreased in STZ induced diabetic animals were improved to near normal upon *Smilax zeylanica* leaf extract administration. This indicates that *Smilax zeylanica* leaf extract contributes to exert antioxidant defense mechanism.

Histopathology

Section of pancreas from group I control normal animals showed pancreatic acini with normal morphology (Fig. 8a). The section of pancreas from group II and group III animals showed normal pancreatic parenchyma with pancreatic acini. Lymph node with normal histology was noted depicting the non-toxic nature of *Smilax zeylanica* leaf extract (Fig. 8b and c). Section of pancreas from group IV diabetic control animals showed atrophy and degenerative changes in pancreatic acini with infiltration of inflammatory cells (Fig. 8d). Section of pancreas from group V diabetic animals treated with *Smilax zeylanica* leaf extract showed mild intracellular oedema in pancreatic acini with a mild degeneration (Fig. 8e). Section of pancreas from group VI animals treated with *Smilax zeylanica* leaf extract showed normal pancreatic parenchyma with pancreatic acini. Pancreatic tissue appeared unremarkable (Fig. 8f).

Histopathological examination of liver sections from control animals revealed normal architecture (Fig. 9a). Hepatocytes and portal traids appeared normal. The liver sections of group II and group III animals given extract alone for 28 days showed normal architecture. The hepatic parenchyma showed normal hepatocytes and the portal triads appeared normal with well preserved architecture depicting the non-toxic nature of *Smilax zeylanica* leaf extract (Fig. 9b and c). Section of liver from

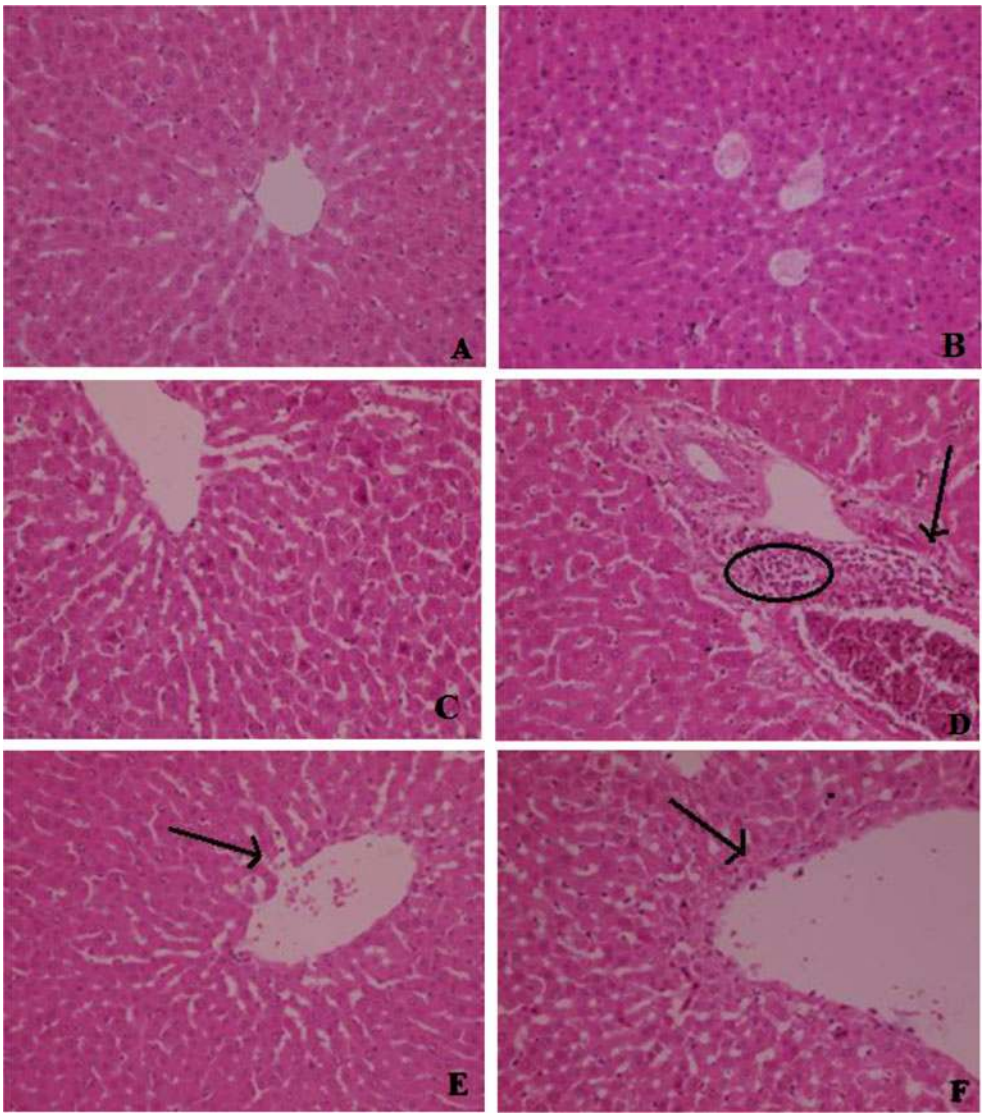

Fig. 9 Histopatholgical image of liver tissues. **a** (40×) H and E stained section of liver tissue from group I animals showing normal architecture. **b** and **c** (40×) H and E stained section of liver from group II and group III animals given extract alone in dose level 200 mg/kg and 400 mg/kg respectively for 28 days showing normal hepatocytes and portal traids indicating the non-toxic nature of extract. **d** (40×) H and E stained section of liver from group IV streptozotocin induced diabetic control rats showing dialation and congestion in central vein and sinusoids (indicated by *arrows*) with infilteration of lymphomononuclear cells in portal region (indicated by *oval shaped*). **e** (40×) H and E stained section of liver from group V diabetic animals treated with *Smilax zeylanica* leaf extract 200 mg/kg showing mild sinusoidal dialation (indicated by *arrows*). **f** (40×) H and E stained section of liver from group VI diabetic animals treated with *Smilax zeylanica* leaf extract 400 mg/kg showing normal architecture with hepatocytes around central vein (indicated by *arrows*)

group IV diabetic control animals showed dialation and congestion of central vein and sinusoids. The portal region showed focal infilteration of lymphomononuclear cells (Fig. 9d). Section of liver from group V diabetic animals treated with *Smilax zeylanica* leaf extract 200 mg/kg showed mild sinusoidal dialation. The hepatocytes appeared normal (Fig. 9e). Section of group VI animals treated with *Smilax zeylanica* leaf extract 400 mg/kg showed normal liver architecture with mild dialation in central vein and sinusoids (Fig. 9f).

Histopathology of kidney from group I control normal animals showed renal parenchyma with normal glomeruli and tubules (Fig. 10a). Section of kidney from group II and group III animals were normal depicting the non-toxic nature of *Smilax zeylanica* leaf extract (Fig. 10b and c). Section of kidney from group IV diabetic control animals showed glomeruli with capillary wall thickening and increased eosinophilic cells in cytoplasm of proximal tubular cells (Fig. 10d). Section of kidney from group V animals treated with *Smilax zeylanica* leaf extract 200 mg/kg showed glomeruli with mild capillary wall thickening (Fig. 10e). In animals treated with *Smilax zeylanica* leaf extract 400 mg/kg, kidney section showed normal renal parenchyma with normal glomeruli and tubules (Fig. 10f).

Discussion

The present study was undertaken to evaluate the antidiabetic and antioxidant status of methanol extract of *Smilax zeylanica* leaves on streptozotocin induced diabetic rats. Streptozotocin, a 2-deoxy-D-glucose with an N-nitrosomethylurea moiety at second carbon atom experimentally produces diabetes in laboratory animals. The presence of 2-deoxy-D-glucose facilitates uptake of streptozotocin into the pancreatic β cells through GLUT 2 (Thulesen et al. 1997) and the N-nitrosomethylurea moiety triggers DNA fragmentation in pancreatic β cells through

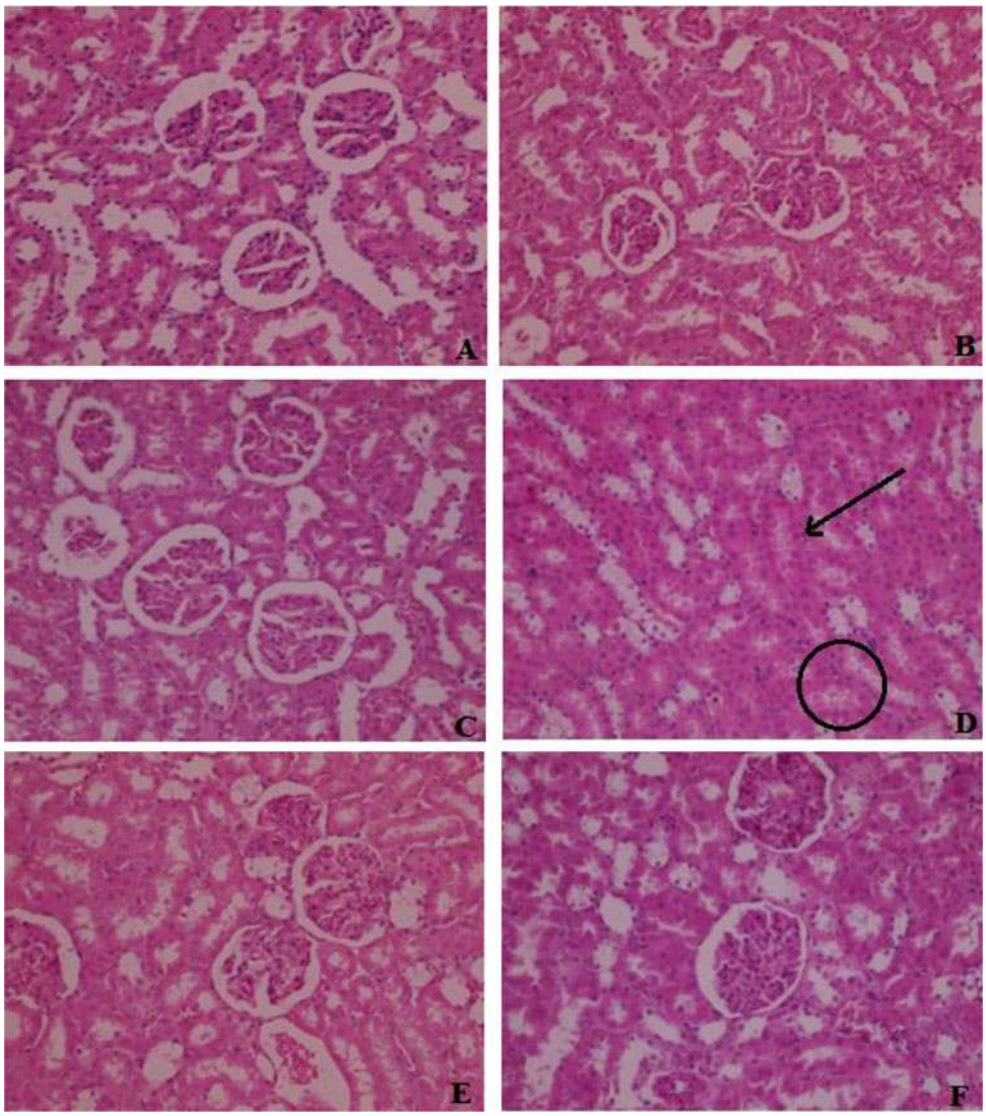

Fig. 10 Histopatholgical image of kidney. **a** (40×) H and E stained section of kidney from group I animals showing renal parenchyma with normal glomeruli. **b** and **c** (40×) H and E stained section of kidney from group II and group III animals given extract alone in dose level 200 mg/kg and 400 mg/kg respectively for 28 days showing normal kidney architecture denotes the non-toxic nature of extract. **d** (40×) H and E stained section of kidney from group IV streptozotocin induced diabetic control animals showing glomeruli with capillary wall thickening (indicated by *arrows*) and increased infiltration of eosinophilic cells (indicated in *circle*). **e** (40×) H and E stained section of kidney from group V diabetic animals treated with *Smilax zeylanica* leaf extract 200 mg/kg showing glomeruli with mild capillary wall thickening. **f** (40×) H and E stained section of kidney from group VI diabetic animals treated with *Smilax zeylanica* leaf extract 400 mg/kg showing renal parenchyma with normal glomeruli and tubules

formation of alkylating free radicals leading to hasty necrosis of the β cells (Coskun et al. 2008). In our study, the difference observed between initial and final fasting blood glucose levels of different groups under investigation revealed a significant increase in blood glucose in diabetic control group as compared to normal animals at the end of the 28 day experimental period. Administration of methanol extract of *Smilax zeylanica* leaves to diabetic rats showed a significant decrease in the levels of blood glucose. The possible mechanism by which *Smilax zeylanica* leaf extract brings about its hypoglycaemic action in diabetic rats might be due to stimulation of surviving β cells leading to increase in insulin secretion. This was confirmed by increased levels of serum insulin in diabetic rats treated with *Smilax zeylanica* leaf extract. STZ-induced diabetes is characterized by a severe loss in body weight due to increased muscle wasting (Swanston-Flatt et al. 1990). Diabetic rats treated with methanol extract showed increased in body weight as compared to the diabetic control rats which may be due to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis. Administration of methanol extract to normal rats did not significantly alter body weight, blood glucose and serum insulin during the 28 day experimental period. Marked elevation in AST and ALT in diabetic control animals indicated the hepatocellular damage. Because AST and ALT are cytoplasmic in location, they are released into circulation after cellular damage and rupture of plasma membrane (Wroblewski 1959; Sallie et al. 1991). Hence AST and ALT are considered as sensitive markers employed in diagnosis of hepatic damage. Treatment with *Smilax zeylanica* leaf extract significantly reduced the levels of AST and ALT in diabetic animals. This indicates that *Smilax zeylanica* leaf extract tends to prevent liver damage in diabetes by maintaining integrity of plasma membrane, thereby suppressing the leakage of enzymes through membrane. Elevated levels of serum urea and creatinine levels observed in diabetic control animals might be due to increased protein breakdown and renal dysfunction associated

with diabetes (Almadal and Vilstrup 1988). The decrease in serum urea and creatinine levels on treatment with *Smilax zeylanica* leaf extract suggest the gluconeogenesis in control with renal protection.

As is widely known, oxidative stress plays an important role in the causation of diabetes and lipid peroxidation is one of the major mechanisms of cell injury caused by free radicals. Enormous amount of free radicals generated in diabetes mellitus reacts with lipids causing lipid peroxidation (Praveen et al. 2010). The products of lipid peroxidation include malondialdehyde, that interacts with various molecules leading to oxidative stress. In the present study, significant increase in the levels of lipid peroxidation observed in liver and kidney of diabetic control animals might be due to the excessive production of free radicals and due to reduction in antioxidant defense. Significantly reduced levels of lipid peroxidation in liver and kidney of extract treated groups clearly shows the effectiveness of *Smilax zeylanica* leaf extract in controlling lipid peroxidation. The presence of flavonoids may contribute this effect because they are proved to be a potential inhibitor of lipid peroxidation (Siegers and Younes 1981). The enhanced formation of lipid peroxides is further evidenced by decrease in activities of antioxidant enzymes in liver and kidney of diabetic control animals compared with normal control animals. Antioxidants constitute the foremost defense that limit the toxicity associated with free radicals. Oxidative stress coupled with decreased antioxidant status in diabetes mellitus increase the deleterious effects of free radicals (Atli et al. 2004).

SOD is said to act as the first line of defense against superoxide radical generated as a by-product of oxidative phosphorylation. SOD mediated dismutation of superoxide radical ($O_2^{\cdot-}$) generates hydrogen peroxide (H_2O_2). Accumulation of excess of H_2O_2 causes toxic effects on cellular system. In this regard GSH-Px and catalase converts H_2O_2 into water (Li Shijun et al. 2000). Glutathione peroxidase (GSH-Px) catalyses the reduction of H_2O_2 at the expense of reduced GSH, thereby protecting cells against oxidative damage. Catalase detoxifies H_2O_2 into H_2O and O_2 (Murray et al. 2003). Thus SOD, catalase and glutathione peroxidase (GSH-Px) act mutually and constitute the enzymatic defense mechanism against ROS (Bhattacharjee and Sil 2006). In the present study decrease in the activities of SOD, catalase and GSH-Px in liver and kidney of diabetic control animals could be attributed to excessive utilization of enzymes in detoxification of peroxides and hydroperoxides generated oxidative stress in diabetes mellitus. Restoration in the levels of lipid peroxidation upon treatment with *Smilax zeylanica* leaf extract might have resulted in the recoupment in the activities of the above antioxidant enzymes to normalcy. SOD, catalase and GSH-Px require several secondary enzymes like glutathione reductase and cofactors like GSH, NADPH, to function at high efficacy. Glutathione reductase catalyzes the NADPH dependent reduction of glutathione disulphide to glutathione thus maintaining glutathione levels (Katiyar et al. 1993). GST catalyzed GSH conjugation is an important mechanism for the detoxification process. In the present study the activities of glutathione reductase and Glutathione-S-transferase were significantly reduced in liver and kidney of diabetic control animals. Upon treatment with *Smilax zeylanica* leaf extract, the glutathione reductase and GST levels were significantly increased. Increase in glutathione reductase can protect the liver and kidney by maintaining the basal level of GSH, which is important for many other GSH dependent detoxification reactions.

GSH, vitamin C and vitamin E are well known non-enzymatic antioxidant defense system of cells, act synergistically to scavenge free radicals in biological system. GSH is found to be present in high concentration in cells, protects cells against free radical attack (Farombi et al. 2000). GSH acts directly as free radical scavenger by donating a hydrogen atom and thereby neutralizing hydroxyl radical. It reduces peroxides and maintains protein thiols in the reduced state (Nwanjo and Oze 2007). Glutathione peroxidase uses GSH as a substrate to catalyze the reduction of hydroperoxide and H_2O_2 (Bebe and Panemangalore 2003). Reduced glutathione (GSH) in tissues maintains the cellular levels of vitamin C and Vitamin E in active form. Vitamin C and vitamin E act synergistically in scavenging wide variety of ROS. Vitamin C protects the cell membrane from oxidative damage induced by aqueous radicals (Allen 1991). It removes free radicals from cytosol and plays a vital role in protecting lipoprotein molecules from oxidative damage by regenerating the reduced form of vitamin E (Das 1994). Vitamin E is a well recognized, important free radical scavenger in the cell membrane limits LPO by terminating chain reaction initiated in the membrane lipids (Wiseman 1996). GSH acts synergistically with vitamin E against oxidative stress (Chaudiere 1994). Vitamin C also scavenges and detoxifies free radicals in combination with Vitamin E and GSH (George 2003). The decreased level of these non-enzymatic antioxidants observed in liver and kidney tissues of diabetic control animals might be due to excessive utilization of these antioxidants for quenching enormous free radicals produced. Treatment with *Smilax zeylanica* leaf extract effectively restored the depleted levels of non-enzymatic antioxidants GSH, vitamin C and vitamin E. Increase in GSH levels in turn also contributes to the recycling of other antioxidants such as vitamin C and vitamin E. Moreover, the pathological changes observed in histology of liver, kidney and pancreas in diabetic rats were reduced in diabetic rats treated with *Smilax zeylanica* leaf extract.

Phytochemical exploration and quantitative analysis led to the conclusion that the methanol extract of *Smilax zeylanica* was rich in total phenolics and total flavonoids, which are known hypoglycaemic agents, powerful antioxiadnts and their ability to regenerate β cells of pancreas. Thus, the significant antidiabetic effect of *Smilax zeylanica* leaf extract could be due to the presence of more than one active principles and their synergistic properties.

Conclusion

From the above findings, we conclude that *Smilax zeylanica* leaf extract has the ability to ameliorate oxidative stress in streptozotocin induced diabetic rats as evidenced by improved glycemic and antioxidant status along with decreased lipid peroxidation. In addition, it protects histological changes from oxidative injury through its antioxidant properties.

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