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Evaluation of antihyperglycemic and antioxidant activities of *Saraca asoca* (Roxb.) De Wild leaves in streptozotocin induced diabetic mice

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ABSTRACT

Objective: To investigate the antihyperglycemic and antioxidant properties of the petroleum Ether, chloroform and methanol extract of *Saraca asoca* (Roxb.) De Wild (*S. asoca*) leaves in mice. **Methods:** Swiss albino mice were made diabetic by a single dose of streptozotocin (150 mg/kg i.p.). Blood glucose levels and body weights of mice were measured using on weekly intervals i.e day 0, 7, 14 and 21 after daily administration of all extracts at doses of 250 and 500 mg/kg. Other biochemical parameters such as serum cholesterol, triglycerides, HDL-cholesterol, VLDL-cholesterol, LDL-cholesterol, urea, creatinin and proteins levels were also measured at the end of study. The effects of all extracts were also noticed on vital organs e.g. liver, kidney and pancreas. The antioxidant potential of all extracts was also determined by DPPH and H₂O₂ radical scavenging *in vitro* methods. **Results:** Oral administration of the extracts for 21 days caused a significant ($P < 0.01$) reduction in blood glucose levels in diabetic mice. Among all the extracts, methanol extract showed better results. The body weight of diabetic animals was also improved after daily administration of extracts. All the extracts also improved other altered biochemical parameters associated with diabetes. Furthermore, the extracts have favorable effects on the histopathological changes of the pancreas, liver and kidney in STZ induced diabetic mice. All the extracts showed significant ($P < 0.05$) antioxidant activity at the dose of 500 µg/ml. **Conclusions:** *S. asoca* possesses antihyperglycemic and antioxidant properties as well improves body weight, liver profile, renal profile and total lipid levels. It can justify folklore uses of the plant in diabetes.

1. Introduction

Herbal medicines are the oldest remedies known to mankind. In the present scenario, the demand for herbal products is growing exponentially throughout the world and major pharmaceutical companies are currently conducting extensive research on plant materials for their potential medicinal value. In many journals, national and international, we find an increasing number of research publications based on herbal drugs. Many analysis-based studies regarding pharmacological research in India have been conducted in the past^[1].

Diabetes is a metabolic disorder characterized by hyperglycemia resulting deficiency of insulin secretion by pancreas, ineffectiveness of produced insulin, or both^[2]. It is the most important non-infective epidemic to hit the

globe in the present millennium. The number of people suffering from diabetes worldwide is increasing at an alarming rate. It is predicated that about 366 million people are likely to be diabetic by the year 2030^[3]. Hyperglycemia can be handled initially with oral synthetic agents and insulin therapy. However, these synthetic agents produce some serious side effects and are relatively expensive for developing countries^[4]. The toxicity of oral antidiabetic agents differs widely in clinical manifestations, severity, and treatment^[5]. In the natural system of medicine many plants have been claimed to be useful for the treatment of diabetes mellitus. The dependence of large rural population on medicinal plants for treatment of diabetes is because of its availability and affordability^[6]. In recent years, several authors evaluated and identified the antidiabetic potential of traditionally used Indian medicinal plants using experimental animals. Although a large number of medicinal plants have been tested for their antidiabetic effects, but it remains to be investigated in several other Indian medicinal plants. The excessive oxidative stress is observed in the diabetes^[7]. So, the present study was

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conducted to evaluate antihyperglycemic and antioxidant activities of *Saraca asoca* (*S. asoca*) leaves in streptozotocin induced diabetic mice.

Ashoka is the most ancient tree of India, generally known as a “ashok briksh”, botanist known as *Saraca asoca* (Roxb.), De.wild or *Saraca indica* belonging to family Caesalpinaceae[8]. The leaves of *S. asoca* are used in the treatment of diabetes but there was no significant proof for its antidiabetic efficacy. Also the plant contains many flavonoids and sterols/Triterpenoids as its main constituents[9–10], which are known bioactive principles for antidiabetic potential[11–12]. Flavonoids are also known to regenerate the damaged β –cells in diabetic mice[13–14]. From here it was thought worthwhile to find out the efficacy of leaves of *S. asoca* for treating diabetes.

2. Materials and methods

2.1. Collection of Plant material

The leaves of *S. asoca* were collected from the campus of Kurukshetra University, Kurukshetra in the month of October and November and were taxonomically identified and authenticated by Dr. BD Vashishta, Department of Botany, Kurukshetra University, Kurukshetra, India. The leaves were then separated, washed and shed-dried under room temperature.

2.2. Preparation of plant extracts

The plant material was powdered to coarse powder and extracted with petroleum ether (60–80°C) in Soxhlet apparatus at a temperature not exceeding 60°C. The defatted plant material was then extracted with chloroform and methanol at the same temperature. The extracts were concentrated under reduced pressure in rotary evaporator to yield a crude semi-solid mass. It was then dried and used.

2.3. Preliminary phytochemical screening

A portion of residue from each extract was subjected to phytochemical analysis to test the presence of carbohydrates, glycosides, alkaloids, flavonoids, tannins, sterols and triterpenoids in the leaves extracts[15].

2.4. Animals

Experiments were performed using Swiss albino male mice (25–35 g). Animals were maintained under standard environmental conditions i.e ambient temperature of (22±3)°C, relative humidity 30–70%, an artificial dark and light cycle of 12 h each, fed with a standard pellet mice diet from Ashirwad Industries, Chandigarh, India and water was supplied *ad libitum*. The Animal Ethical Committee, Kurukshetra University reviewed the entire animal protocols prior to conducting the experiments.

2.5. Acute Toxicity Studies

Animals were fasted for 3–4 h prior to dosing. Following

the period of fasting, all extracts at doses of 200, 300, 600, 1000, 1500 and 2000 mg/kg b.w. were administered to six groups with 6 rats each. Animals were observed individually after dosing at least once during the first 30 min. Periodically during the first 24 h, with special attention given during the first 4 h. Time of onset and length of recovery period were observed[16]. Additional observations include change in skin and fur, eyes and mucous membranes, and also somatomotor activity and behaviour pattern. Attention was given to observations of tremors, convulsions, salivation, diarrhoea, sleep and coma[17–18].

2.5. Induction of diabetes

Individual dose of the streptozotocin (STZ) to be injected in overnight fasted animals was extemporaneously prepared in ice cold citrate buffer (pH 4.5). STZ (150 mg/kg) was injected intraperitoneally to the animals. Control mice received an equivalent amount of citrate buffer. Twelve day after the STZ injection, mice with fasting blood glucose levels greater than 200 mg/kg were considered diabetic and included in study[19–20].

2.6. Treatment protocol

Diabetic animals were randomly assigned into following groups of six animals each.

Group I: Normal control received distilled water.

Group II: Diabetic control received vehicle (Tween 80, 5% v/v and distilled water)

Group III: Diabetic mice received glibenclamide (10 mg/kg)

Group IV: Diabetic mice received petroleum ether (250 mg/kg)

Group V: Diabetic mice received petroleum ether (500 mg/kg)

Group VI: Diabetic mice received chloroform (250 mg/kg)

Group VII: Diabetic mice received chloroform (500 mg/kg)

Group VIII: Diabetic mice received methanol (250 mg/kg)

Group IX: Diabetic mice received methanol (500 mg/kg)

The drug solutions or vehicle were administered orally by gastric intubation once daily at 11 o'clock for 21 days. The effect of vehicle, extract and standard drug on blood glucose and body weight was determined in animals at 0, 7, 14, 21 day after oral administration.

2.7. Biochemical estimation

Blood samples were collected by cardiac puncture and retro-orbital plexus method from all animals into the EDTA sprinkled tubes and were centrifuged at 3000 rpm for 20 min. Serum was separated and stored at –20°C until analysis was performed. Serum samples were analyzed for cholesterol, HDL, total proteins, urea, creatinine and triglycerides using the diagnostic kit (ERBA Diagnostics Mannheim, Germany) in Autoanalyzer. LDL and VLDL were calculated using following formulae:

$$\text{LDL} = \text{TC} - [\text{TG}/5 + \text{HDL}]^{[21]}$$

$$\text{VLDL} = \text{TC} - [\text{LDL} + \text{HDL}]^{[22]}$$

2.8. Antioxidant assay

2.8.1. Evaluation method of scavenging of hydrogen peroxide (H_2O_2)

A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations (100, 300 and 500 μ g/mL) of extracts were added to a H_2O_2 solution (0.6 mL, 40 mM). Absorbance of H_2O_2 at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without H_2O_2 [23–24]. The percentage scavenging of H_2O_2 of extract and standard compounds was calculated using the following formula

$$\% \text{ Scavenged}[H_2O_2] = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the sample and standards.

2.8.2. Evaluation method by 1,1-diphenyl-2-picrylhydrazyl (DPPH) antioxidant assay

A stock solution of DPPH (33 mg in 1 L) was prepared in methanol and 5 mL of this stock solution was added to 1 mL of test solution at different concentrations (100, 300, 500 μ g/mL). After 30 minutes, absorbance was measured at 517 nm and compared with standards (100, 300, 500 μ g/mL). BHA and ascorbic acid were used as reference[25]. Scavenging activity was expressed as the percentage inhibition calculated using the following formula:

$$\text{Percentage anti-radical activity} = [\text{Abs. (control)} - \text{Abs. (sample)}] / \text{Abs. (control)} \times 100$$

2.9. Histopathology

For histopathological study, animals from all groups were anaesthetized with mild ether anaesthesia and dissected. Liver, kidney and pancreas are excised out of the animal's body and put immediately into 10% formalin solution in a stoppered container. These samples were then sent to diagnostic lab (Hindustan lab., Kurukshetra) fixation (using Bouin's solution), dehydration, embedding (in paraffin), sectioning (with standard microtome) and staining (Haematoxylin or eosin). The slides so prepared were then examined by pathologist and the pictures were clicked with the help of a binocular microscope fixed with a camera.

2.10. Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests to determine level of significance. A value of $P < 0.01$ was considered significant results are expressed as mean \pm SEM.

3. Results

Preliminary phytochemical analysis of *S. asoca* leaves showed the presence of flavonoids, tannins, saponins, sterols and triterpenoids which are known bioactive principles[11, 26]. Flavonoids are also known to regenerate the damaged β -cells in diabetic mice[14, 27]. The overall study showed the LD_{50} of oral toxicity of all extracts to be above 2 000 mg/kg b.w. in mice. So, the extracts are safe for long term administration. The effects of vehichloroform, Glibenclamide and all methanol, petroleum ether and chloroform extracts on blood glucose levels in normal and diabetic mice after treatment of 21 days are shown in Table 1, in which all extracts showed significant reduction ($P < 0.01$). It was observed that standard drug glibenclamide lowered the blood glucose levels significantly bringing it back to normal which is an indication of the presence of some β cells, as glibenclamide is known to stimulate insulin secretion from β -cells[12]. A significant reduction in average weight was observed in STZ induced diabetic mice (Table 1). The decrease in weight in diabetes was due to continuous excretion of glucose and decrease in peripheral uptake of glucose and glycogen synthesis[28]. Increase in body weight and decrease in blood glucose might be due to improving the glycemic control mechanisms and insulin secretions from remnant pancreatic-cells in diabetic animals. Standard drug and different extracts showed dose-related reductions in the serum concentrations of TC, TG, LDL, VLDL, TG, SU and SC but caused the reverse effect on the serum concentration of HDL and TP (Table 2). Percentage anti radical activity of all extracts is summarized in Table 3. All the extracts exhibited good but varying levels of antioxidant activity in both DPPH and H_2O_2 radical scavenging assay.

Table 1

Effect of *S. asoca* extracts on blood glucose and body weight. (mean \pm SEM)

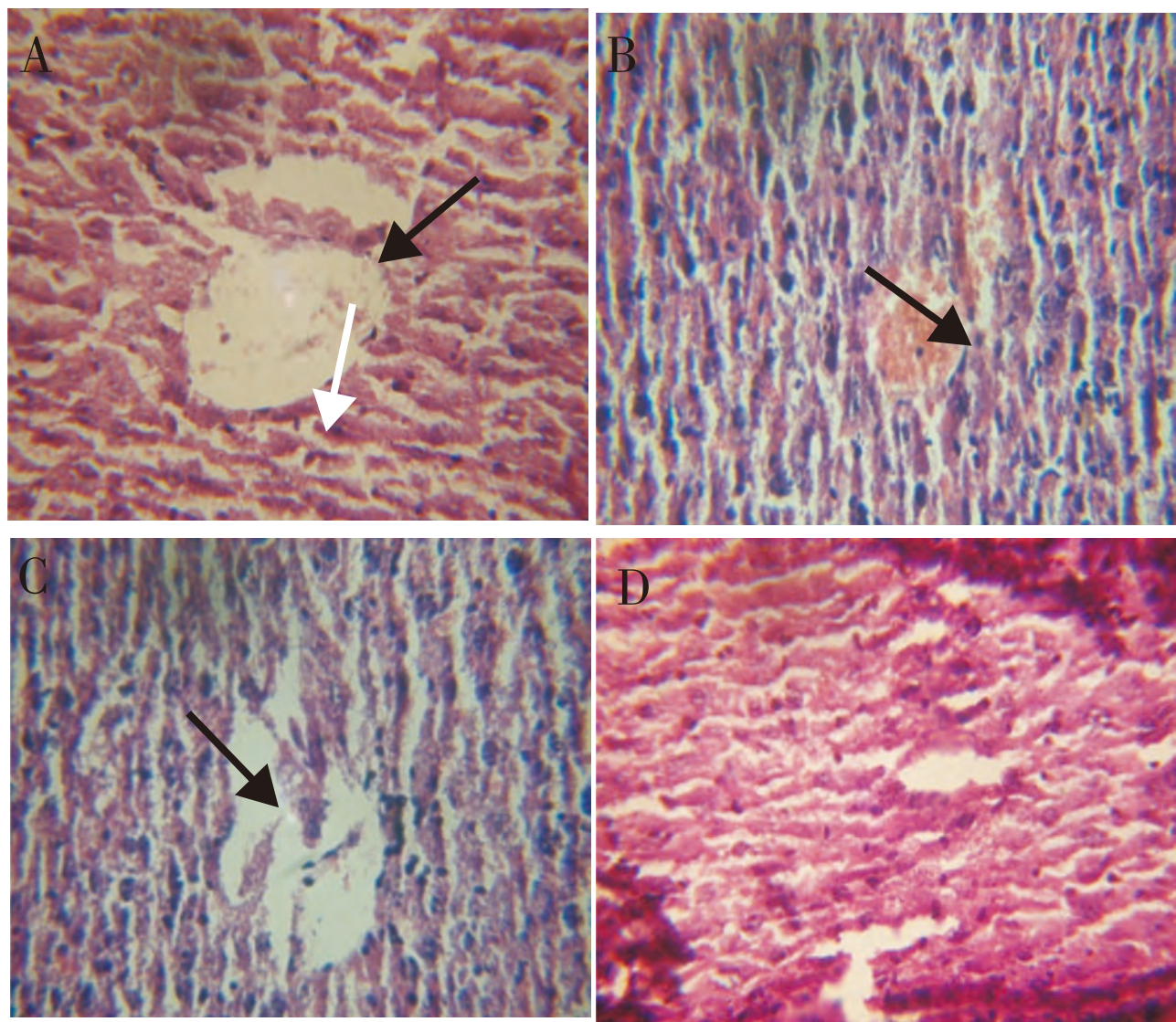
Groups	Blood glucose (mg/dL)				Body weight (g)			
	Day 0	Day 7	Day 14	Day 21	Day 0	Day 7	Day 14	Day 21
Group I	85.20 \pm 3.200	83.0 \pm 2.387	84.2 \pm 2.478	85.20 \pm 2.437	30.2 \pm 0.860	30.0 \pm 1.095	29.6 \pm 0.812	30.4 \pm 0.748
Group II	223.60 \pm 12.343	258.60 \pm 17.201	274.80 \pm 17.862	302.40 \pm 21.018	29.4 \pm 0.748	28.6 \pm 0.970	27.2 \pm 0.734	26.4 \pm 0.509
Group III	218.8 \pm 11.968	155.0 \pm 14.557**	113.20 \pm 8.737**	96.00 \pm 4.712**	28.4 \pm 0.812	28.6 \pm 0.509*	29.2 \pm 0.663**	29.6 \pm 0.509**
Group IV	212.60 \pm 13.212	241.40 \pm 13.801	221.40 \pm 10.605	208.60 \pm 20.585*	30.2 \pm 1.356	28.2 \pm 1.158	28.8 \pm 1.158	28.4 \pm 0.927
Group V	222.20 \pm 24.461	159.6 \pm 13.604**	169.20 \pm 18.502**	155.40 \pm 8.617**	29.0 \pm 0.836	28.2 \pm 0.583	27.8 \pm 0.663	29.4 \pm 0.678**
Group VI	241.40 \pm 22.713	275.60 \pm 35.765	289.80 \pm 41.278	233.60 \pm 21.949*	30.0 \pm 0.836	28.6 \pm 0.979	27.8 \pm 0.860	28.4 \pm 0.871
Group VII	204.4 \pm 8.658	220.40 \pm 11.021	214.40 \pm 10.294	195.80 \pm 9.173*	28.0 \pm 0.707	26.6 \pm 0.812	26.4 \pm 0.509	27.2 \pm 0.583*
Group VIII	267.20 \pm 21.542	383.20 \pm 65.136	314.80 \pm 65.925	226.2 \pm 42.254**	31.4 \pm 1.166	29.8 \pm 1.241	30.6 \pm 1.327	31.4 \pm 0.927**
Group IX	334.60 \pm 45.793	336.60 \pm 51.048	250.60 \pm 39.937	179.4 \pm 11.426**	29.0 \pm 0.707	28.4 \pm 0.927	28.8 \pm 0.583	29.6 \pm 0.748**

Group I: Normal control; Group II: Diabetic control; Group III: Standard with glibenclamide 10 mg/kg; Group IV: Petroleum ether 250 mg/kg; Group V: Petroleum ether 500 mg/kg; Group VI: Chloroform 250 mg/kg; Group VII: Chloroform 500 mg/kg; Group VIII: Methanol 250 mg/kg; Group IX: Methanol 500 mg/kg.

* $P < 0.05$, ** $P < 0.01$

Table 2Effect of *S. asoca* extracts on cholesterol levels. (mean \pm SEM)

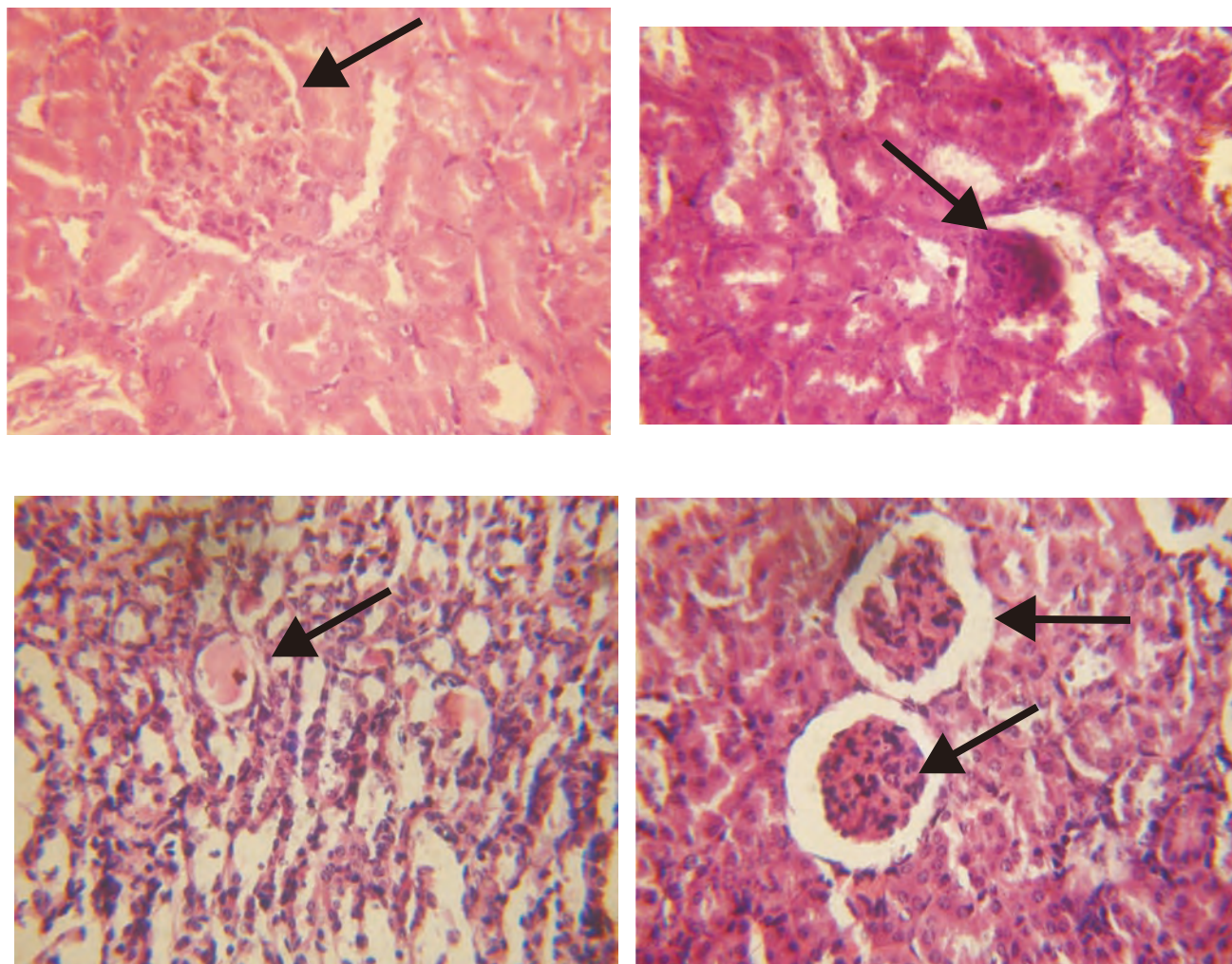
Groups	Cholesterol levels					Other biochemical parameters		
	Cholesterol	TG	HDL	VLDL	LDL	Creatinine (mg/dl)	Urea (mg/dl)	Total protein (g/dl)
Group I	87.33 \pm 1.35	96.50 \pm 1.64	33.01 \pm 0.63	19.30 \pm 1.32	35.10 \pm 2.85	0.73 \pm 0.22	32.03 \pm 1.36	7.00 \pm 1.63
Group II	198.67 \pm 2.55	172.33 \pm 1.81	22.00 \pm 1.24	34.40 \pm 4.35	129.20 \pm 12.3	1.70 \pm 1.55	73.88 \pm 2.27	4.00 \pm 0.11
Group III	90.67 \pm 1.42 ^{**}	91.33 \pm 0.68 ^{**}	32.43 \pm 3.24 ^{**}	18.24 \pm 0.32 ^{**}	37.00 \pm 2.43 ^{**}	0.61 \pm 1.42 ^{**}	28.68 \pm 1.61 ^{**}	8.33 \pm 3.21 ^{**}
Group IV	153.00 \pm 2.13	151.90 \pm 1.43	18.90 \pm 4.98	30.10 \pm 3.46	102.00 \pm 1.43	1.41 \pm 0.34	56.00 \pm 2.63	5.90 \pm 2.98
Group V	144.00 \pm 0.23	137.50 \pm 0.34	27.10 \pm 2.32	27.40 \pm 1.47 [*]	89.60 \pm 0.91	1.10 \pm 0.23	43.54 \pm 0.74	5.07 \pm 1.62
Group VI	191.32 \pm 3.45	168.80 \pm 2.32	29.00 \pm 2.76	33.70 \pm 0.61	108.50 \pm 1.39	1.52 \pm 2.75	53.80 \pm 1.02	6.03 \pm 4.54
Group VII	176.00 \pm 3.42	164.40 \pm 1.91	21.54 \pm 1.98	32.80 \pm 0.54 [*]	102.32 \pm 2.51	1.46 \pm 2.02	51.40 \pm 0.98	5.54 \pm 3.53
Group VIII	129.00 \pm 3.71	126.60 \pm 0.132	26.30 \pm 0.43 [*]	25.29 \pm 2.91	77.41 \pm 0.31	0.89 \pm 1.61 [*]	36.60 \pm 1.23	6.93 \pm 0.69 [*]
Group IX	121.20 \pm 0.41 [*]	106.20 \pm 3.59	34.30 \pm 2.72 ^{**}	21.31 \pm 2.30 ^{**}	54.37 \pm 0.31 [*]	0.63 \pm 0.41 ^{**}	31.20 \pm 3.67 [*]	7.06 \pm 3.92 ^{**}

^{*} $P < 0.05$, ^{**} $P < 0.01$ **Figure 1.** Histopathological changes in Liver

A: Normal control; showing the central vein (black arrow), with radiating cords of hepatocytes (white arrow); B: Diabetic control; Diabetic liver showing the periportal fatty infiltration with focal fat necrosis; C. Petroleum ether 500 mg/kg (leaves extract), showing regeneration in portal track; D: Methanol 500 mg/kg (leaves extract), portal track showing normal features

Table 3Antioxidant activity of *S. asoca* extracts

Extract	Rate of anti-radical activity by DPPH			Rate of scavenging of H ₂ O ₂		
	100 µg	300 µg	500 µg	100 µg	300 µg	500 µg
BHA	78.3	83.3	86.3	64.60	66.40	67.20
Ascorbic acid	78.2	85.4	88.7	49.30	53.00	56.00
Petroleum ether (250 mg/kg)	29.69	38.15	49.61	21.79	23.07	29.48
Petroleum ether (500 mg/kg)	32.25	39.37	46.71	38.46	43.58	47.43
Chloroform (250 mg/kg)	23.13	27.58	30.70	21.20	29.74	41.28
Chloroform (500 mg/kg)	24.24	42.82	44.04	37.17	44.87	47.43
Methanol (250 mg/kg)	59.17	62.5	73.19	38.46	47.43	58.97
Methanol (500 mg/kg)	38.48	51.72	61.84	55.12	58.97	60.94

**Figure 2.** Histopathological changes in kidney

In the normal liver, tissue section showed sinusoidal cords of hepatocytes with central vein and portal tracts.

A: Normal control, Showing glomeruli (black arrow), and proximal convoluted tubules B: Diabetic control: Shows tubular damage proteinuria and haemorrhage; C: Pet ether 500 mg/kg (leaves extract), Tubules shows proteinuria and glomerular damage. D. Methanol 500 mg/kg (leaves extract) Shows glomeruli and tubules without proteinuria and haemorrhage

The major alterations in liver were distortion in the arrangement of cells around the central vein, periportal fatty infiltration with focal necrosis of hepatocytes. Petroleum ether and methanolic extracts (250 and 500 mg/kg b. w.) treated brought back the cellular arrangement around the central vein and reduced necrosis. Also it helped to bring the blood vessels to normal condition (Figure 1). The excellent recovery of renal functions expected with

treatment of petroleum ether and methanolic flowers extract of *S. asoca* can be explained by regenerative capability of the renal tubules (Figure 2). The regenerative effect of the pancreatic cells by this plant via exocrine cells of pancreas may enlighten the positive effects of these agents on the production of insulin (Figure 3).

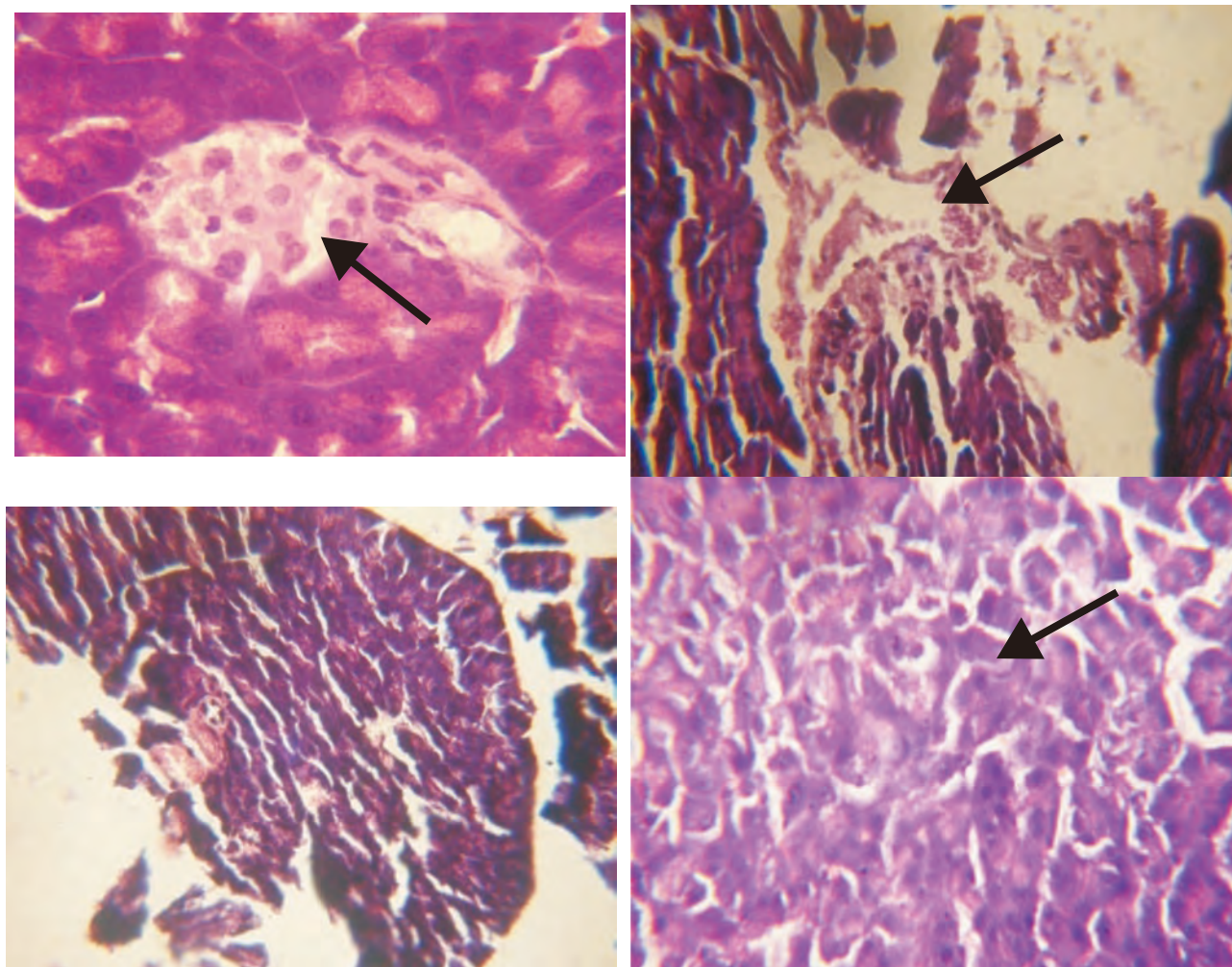


Figure 3. Histopathological changes in Pancreas

A: Normal control showing exocrine acini and endocrine islets; B: Diabetic control shows depleted islets shrunken in size; C: Petroleum ether 500 mg/kg (leaves extract) showing exocrine acini and small preserved islets; D: Methanol 500 mg/kg (leaves extract) recovering from infiltration of lymphocyte.

4. Discussion

STZ produces oxygen radicals in the body, which cause pancreatic injury and could be responsible for increased blood glucose in animals[29]. The present study indicates that petroleum ether, chloroform and methanol extract of *S. asoca* leaves showed antidiabetic properties against STZ induced diabetic model and also it proved to have antioxidant activity. A significant reduction ($P < 0.01$) was observed in petroleum ether (500 mg/kg) and methanol (250 and 500 mg/kg) as 28.452%, 17.845% and 42.047% respectively. Percentage scavenging of H_2O_2 by petroleum ether (500 mg/kg) and methanol (500 mg/kg) extract at 500 μ g concentration was found to be 47.43 and 60.94 and percentage anti radical activity of these extract by DPPH antioxidant assay at the same concentration was found to be 46.71 and 61.84. The presence of flavonoids contents might be the possible mechanism for antidiabetic activity of this plant.

Normal healthy animals were found to be stable in their body weight whereas diabetic animals showed reduction in body weight. In the study, the reduction of body weight was diminished by extracts treatment after 14 days of treatment in a dose dependent manner.

The reduction in the level of serum cholesterol, triglycerides, LDL and VLDL cholesterol confirms the

possibility that major functions of the extract are on the protection of vital tissues (kidney and liver) including the pancreas, thereby reducing the causation of diabetes in experimental animals. The portal tracts showed portal triad with portal vein, hepatic artery and bile duct, whereas the diabetic mice liver tissue section showed marked structural alterations in the liver as a result of absence of insulin. The results indicated a primary and secondary effect of diabetic state on the kidney of mice.

In petroleum ether and methanolic extracts (250 and 500 mg/kg b.w.) treated diabetic kidney, the damaged capillary loops with increase in the thickness of the wall, glomeruli and tubules without proteinuria and haemorrhage. The primary effect, the diabetes factor was associated with hyperglycemia and was responsible for dilation of proximal and distal tubules in the cortex. The secondary effect, named the individual response factor, was associated with inflammatory processes[30,31]. Diuresis is a common feature associated with diabetes which may be the reason for structural changes observed with glomerulus[32]. The ultra structure of diabetic pancreas showed considerable reduction in the islet langerhans and depleted islets. In petroleum ether and methanolic extracts (250 and 500 mg b.w.) treated pancreas the cells seem to have gathered together and small preserved islets similar to the normal.

The liver damage is partially reversed by both extracts.

The present study also indicates that *S. asoca* can partially inhibit renal toxicity as observed from serum creatinine. All the above observations suggest that *S. asoca* can be a promising antidiabetic and antioxidant drug.

Petroleum ether, chloroform and methanol extract of *S. asoca* leaves showed antidiabetic properties 250 and 500 mg/kg, b.w. *p.o.* exhibited significant antidiabetic and antioxidant properties evidenced by physical, biochemical and Histopathological parameter. Further study need to be done to elucidate the mechanism of action involved in antidiabetic and antioxidant activities.

As a conclusion, it could be speculated that the observed antihyperglycemic activity of *S. asoca* leaves might be related to the presence of flavonoids, sterols and triterpenoids and saponins as active constituents. The present investigation has also opened an avenue for further research especially with reference to the development of potent formulation for diabetes mellitus from *S. asoca* leaves.

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