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Evaluation of the Anti-Diabetic Activity of Sophora Interrupta: Pharmacological Screening Against Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Introduction: Diabetes Mellitus (DM) is a highly prevalent metabolic disorder characterized by chronic hyperglycaemia. Though multiple conventional therapies are available these therapies are reported to have side effects.

Aim: To evaluation of the anti-diabetic activity of *Sophora interrupta* by using the streptozotocin-induced diabetic rat model. Plants are potential sources of phytoconstituents with varied pharmacological activities.

Methodology: The leaves and the stem bark of *Sophora interrupta*(SI) were collected and initial results of the phytochemical screening revealed that all the bark and leaf extract of the plant showed the presence of flavonoids, Saponins, steroids, alkaloids, tannins, phenolic compounds, triterpenoids and carbohydrates.

Results: The results of acute toxicity studies demonstrated that animals did not display any drug-related behavioural, physiological and psychological changes. The streptozotocin model was used for the induction of diabetes. In diabetic rats, decreased bodyweight, High-density lipoprotein (HDL), reduced glutathione (GSH), Superoxide dismutase (SOD), Catalase (CAT) and increased level of Blood glucose, total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL) and TBARs was observed which was normalized by 200 mg/kg and 500 mg/kg extract of the plant.

Conclusion: It shows that the ethanolic extract of SI showed significant antioxidant and antidiabetic activity directing it as a better therapeutic regimen in the treatment of diabetes and associated complications.

Key Words: Plant extract, High-density lipoprotein, Reduced glutathione, Total cholesterol, Triglycerides, Low-density lipoprotein, Very low-density lipoprotein

INTRODUCTION

DM is a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both.[1]Diabetes is the sixth leading cause of deaths worldwide.[2]

Several pathophysiological processes are involved in the development of DM. These range from autoimmune destruction of the pancreatic β-cells with consequent insulin deficiency to abnormalities that result in resistance to insulin action. Deficiency and insufficient action of insulin on target tissues lead to carbohydrates, fats and proteins metabolism abnormalities.[3]

Oxidative stress has been suggested as a contributory factor in the pathogenesis of DM(4). Diabetes increases the production of tissue-damaging reactive oxygen species (ROS) by glucose autoxidation and/or non-enzymatic protein glycosylation (5). Hyperglycemia has been found to increase the production of ROS such as superoxide anion ($O2^{-}$), and hydrogen peroxide (H_2O_2) which reduce nitrogen oxide (NO) bioavailability in cultured endothelial cells, and in vascular tissue (6). Endothelial dysfunction is a well-documented characteristic phenomenon in DM (5–7), and is attributed to decreased vasorelaxant, and increased contractile responses to physiological, and pharmacological stimuli.[4]

In conventional medical practice, the present therapies of DM are reported to have side effects. For instance; sulfonylurea causes weight gain due to hyperinsulinemia, biguanide cause body weakness, fatigue, lactic acidosis and alpha-glucosidase

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inhibitor may cause diarrhoea while thiazolidinediones may increase LDL-cholesterol level.[8]

This raises the need for other sources of these inhibitors that have fewer side effects.[9] Therefore, because of the side effects associated with the present antidiabetic drugs, there are a need to develop effective, safe and cheap drugs for diabetes management. Such effective, safe and cheap drugs could be obtained by using medicinal plants which have been used by humans to prevent or cure diseases including diabetes since the dawn of civilization.[10]

Medicinal plants are used by almost 80% of the world's population for their basic health care because of their low cost and ease in availability.[11]The use of herbal medicinal plants has always played a positive role in the control or prevention of diseases such as diabetes, heart disorders and various cancers.[12] Some medicinal plants have been used in the production of various drugs singly or in combination and even as principal raw material for the production of other conventional medicines.[13] Extracts from the other common species have also been used as medicine in treating various illnesses.[14] Therefore, traditional medicine offers promising solutions to face the globally increasing demands for new therapeutic agents. Insufficient data exist for most plants to guarantee their quality, efficacy and safety.[15] However, the adverse effects of phytotherapeutic agents are less frequent compared with synthetic drugs, but well-controlled clinical trials have now confirmed that such effects exist.[16] In the present study, the pharmacognostic and pharmacological profile *Sophora interrupta*(SI) plant is explored.SI plant shows a plethora of pharmacological effects including its role in cancer treatment.[17,19] as anti-ulcer. [20] anthelmintic.[17] hepatoprotective [21] and antioxidant activity. This cumulative data shows that SI plants have a wide spectrum of therapeutic potential. Thus, the beneficial effects of individual plant extracts in STZ-induced diabetes were assessed in the present study.

MATERIALS AND METHODS

Collection and Authentication of *Sophora interrupta* Plant

Fresh leaves and bark of **SI** were collected from Tirumala hills, Chittoor district from the state of Andhra Pradesh. The plant materials were taxonomically identified and authenticated by Dr. MadhavaChetty, Asst. Professor, Dept. of Botany, S.V. University, Tirupathi Andhra Pradesh, India and the sample voucher specimen and herbarium have been preserved in the Dept. of Pharmacognosy, Luqman College of Pharmacy Gulbarga, Karnataka.

Preliminary phytochemical screening of SI plant extracts[22,23]

The phytochemical screening of leaf and bark of FD, SI and CM were carried out for detection of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, protein and free amino acids, saponins, sterols, acidic compound, steroids, fixed oil and fats and terpenoids.[23-27]

Experimental animals:

Age-matched young Wistar rats weighing about 200-250 g were employed in the study. The animals have housed at approximately 24±1°C temperature and humidity of 55±5% with a 12-hour light/dark cycle. The animals were fed with a standard diet (standard chow from Ashirwad Industries, Ropar, India) and water ad libitum. The animals were acclimatized for at least 3-4 days before the initiation of the experiment and were observed for any sign of disease. The animals were maintained under proper conditions throughout the study. The experimental protocol was approved by the Institutional Animal's Ethics Committee. The animals were sacrificed after a predetermined period of the treatment as per the study design to evaluate various parameters.

Acute toxicity study

Acute toxicity includes the effects of a single dose of a chemical/substance (or several doses within 24 hours) on the whole body, usually manifested over 14 days. In the current study, acute toxicity of plant extracts were as per Organisation for Economic Co-operation and Development (OECD) guidelines. Acute toxicity study was performed following OECD guidelines 425.[28] No adverse effect or mortality was detected in albino rats up to 3 gm/kg, per oral of extracts during the 24 to 72 hr observation periods. For this period the rats were continuously observed for 5 hr for any gross behavioural, neurological or autonomic toxic effect and lethal Fly after 24 to 72 hrs.

Induction of diabetes

Animals were injected with a single dose of streptozotocin (STZ; 65 mg/kg, *i.p.*) prepared in fresh citrate buffer (pH 4.5). The development of diabetes was confirmed after 72 h of the STZ injection. The animals having fasting blood glucose levels of more than 250 mg/dL were selected for the study.[29]

Experimental Protocol

Experimental animals were divided into five different groups (eight animals each). The plant extracts were evaluated for their antidiabetic effect at a dose of 500 mg/kg per oral (p.o.) and glipizide (4 mg/kg, p.o.).

Group I (Untreated normal control rats): Normal control rats received only a normal diet and water during the experimental period but without any therapy.

Group ll (Plant extract-treated normal rats): Normal rats treated with a single dose of aqueous extract of SI orally at a dose of 500 mg/ kg daily one time, for 14 consecutive days.

Group III (Diabetic control rats): Rats of this group were STZ-induced diabetic models and were served as diabetic controls throughout the experimental period but without any therapy.

Group IV, V, VI (Plant extract treated diabetic rats): Diabetic models of rats treated with a single dose of aqueous extract of SI orally at a dose of 100, 200, 500 mg/kg daily one time, for 14 consecutive days.[30]

Group VII (Glipizide Treated Diabetic Group): The diabetic rats after 1 week of STZ administration were treated with glipizide (4 mg/kg, p.o.) for 2 weeks.

Estimation of body weight

The body weight of each animal was measured before induction of STZ and periodically till the end of the study.

Biochemical estimation:

Blood samples were collected (under light anaesthesia) by retro-orbital puncture method after overnight fasting and analyzed for Blood glucose level, lipid profile [Serum total cholesterol (TC), triglycerides (TG), low-density lipoproteins (LDL), very-low-density lipoproteins (VLDL) and high-density lipoproteins (HDL)]. Biochemical estimation was carried out using available laboratory kits of Erba Diagnostics Pvt. Ltd.

Estimation of serum glucose

Blood glucose level was estimated after 72 hours of STZ administration to confirm diabetes. Fasting blood glucose level was estimated on the 0^{th} day, 30^{th} day and 75^{th} day.

Assessment of Blood lipid profile

The total cholesterol was estimated by cholesterol oxidase peroxidase CHOD-POD (cholesterol oxidase-peroxidase) method (30) and serum triglyceride was estimated by glycerophosphate oxidase peroxidase GOD-POD method.[30,31] The HDL was assayed by cholesterol oxidase peroxidase CHOD-POD method using manufacturer kit. Serum VLDL and LDL concentrations were calculated according to the Friedewald equation.

LDL cholesterol = Total cholesterol (TC) – High-density lipoprotein (HDL) -Triglycerides (TG)/5.

Assessment of oxidative stress in serum samples

The oxidative and antioxidant parameters in serum samples were assessed by estimating TBARS (thiobarbituric acid reactive substance), GSH (glutathione), CAT (catalase) and SOD (superoxide dismutase) levels. Lipid peroxidation was determined by TBARS concentrations, which was spectrophotometrically measured at 532 nm. SOD and GSH-Px levels were determined using kits. SOD activity was measured by the method of Misra and Fridovich. The GSH level was estimated using the methods described by Ellman. A standard curve was plotted using the reduced form of glutathione (0.1–1 M), and the results were expressed as mM/g protein. Serum CAT activity was assayed using the Spectrophotometric method (36) at 620 nm and expressed as micromoles of hydrogen peroxide decomposed/min/milligram protein.

Statistical Analysis

Data were presented as mean± S.EM. For continuous variables, a student t-test was used to differentiate the mean difference. For comparison between more than 2 groups, the data were processed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. *$p < 0.05$ was considered significant. Statistical analysis was performed using SPSS version 21.

RESULTS

Preliminary Phytochemical screening in the leaves and barks extracts of SI Plant

Preliminary phytochemical screening of the crude, 1:10 and 1:100 extracts of all the four solvents (n-hexane, chloroform, ethanol and water) was performed to characterize the classes of compounds that are present in the leaf and bark of SI plant (Table 1). This qualitative screening included the tests were performed using reference methods (37,38). The results of the phytochemical screening revealed that all the bark and leaf extracts of the plant showed the presence of flavonoids, Saponins, steroids, alkaloids, tannins, phenolic compounds, triterpenoids and carbohydrates. All four extracts were found positive for alkaloids, flavonoids, phenols tannins, carbohydrates, saponins, cardiogylcoside and proteins. On the other hand, steroids and Anthraquinones are not present in either of the extracts. Moreover, ethanolic extract of leaf of SI (ELESI), aqueous extract of leaf of SI (WELSI), ethanolic extract of bark of SI (EBESI) and aqueous extract of bark of SI (WEBSI) found richer in these phytoconstituents as compare to hexane and chloroform extracts. Glycosides, anthraquinones, and reducing sugars are not present in either of the extract.

Table 1: Preliminary phytochemicals constituents in the SI plant

Phytochemicals	Plants extracts							
	N-hexane		Chloroform		Ethanol		Water	
	Stem bark	Leaf	Stem bark	Leaf	Stem bark	Leaf	Stem bark	Leaf
Alkaloids	+	+	+	+	++	+++	++	+++
Anthroquinones	-	-	-	-	-	-	-	-
Flavonoids	+	+	+	+	++	+++	++	++
Phenolics	+	+	+	+	++	+++	++	++
Tannins	+	+	+	+	+++	+++	++	++
Carbohydrates	+	+	+	+	+	+	+	+
Steroids	-	-	-	-	-	-	-	-
Saponins	+	++	+	+	+	+	++	++
Cardioglycosides	+	++	+	+	++	+++	++	++
Proteins	+	+	+	+	++	++	++	++

Note: "+++" indicates positive (Most Abundance), "++" indicates positive (Moderately present), "+" slightly positive, "–" completely absent.

Quantitative estimation of total phenolic, flavonoids and tannin content in the leaf and bark extracts of FD, SI and CM plants

The results for the total phenol, tannin and flavonoid estimation of all four extracts of SI are tabulated in **table 2.** The total phenolic, tannin and flavonoids content of n-hexane, Chloroform, ethanol and aqueous extracts in SI leaf part was ranged from 1.5 -27.5, 1.8-2.7 and 2.3-29.2 g GAE/100 g extract and in bark part was ranged from 3.2 -32.1, 2.6-3.4 and 3.1-35.3 g GAE/100 g extract respectively.

Table 2: Quantitative estimation of Phytoconstituents in leaf and bark extract of FD plant

Sample	Total Phenolic mgGAE/g of extract	Total Tannins mgGAE/g of extract	Total Flavonoids mgRE/g of extract
Leaf			
n-hexane	1.5 ± 0.4	1.8 ± 1.3	2.3 ± 1.1
Chloroform	3.3 ± 1.5	1.9 ± 2.1	3.2 ± 0.6
Ethanol	32.1 ± 4.9	3.4 ± 1.0	35.3 ± 3.3
Water	22.12 ±2.7	2.5 ± 0.7	29.4 ± 2.8
Bark			
n-hexane	3.2 ± 1.3	3.3 ± 1.4	3.1 ± 0.9
Chloroform	7.2 ± 2.4	2.6 ± 0.9	7.4 ± 2.6
Ethanol	27.5 ± 2.4	2.7 ± 1.4	29.2 ± 1.9
Water	19.1 ± 0.4	2.0 ± 0.7	22.1 ± 4.2

Values are the mean of 3 replicate determinations + SD. GAE- Gallic acid equivalent, RE- rutin equivalent.

Acute Toxicity Study

The acute toxicity studies of *SI* leaves extract was carried out as per OECD guideline no. 423. The limit test dose used for the study was 2000 mg/kg. There was no gross evidence of any abnormality observed up to a period of 4-6 hrs or mortality up to a period of 24hrs at the maximum tolerated dose level of 2000 mg/kg body weight p.o. Results demonstrated that animals did not display any drug-related changes in behaviour, breathing, skin effects, water consumption, and impairment in food intake, temperature, autonomic and neurological. Therefore, the extract seems to be safe at a dose level of 2000 mg/kg, and the LD50 was considered to be >2000 mg/kg.

Effect of ethanolic leaf extract of SI (ELESI) on body weight

No difference in the initial body weight was observed in any experimental group. Two-way ANOVA revealed that STZ

subjected rats gained less body weight than normal rats. After 28 days of STZ, a prominent decrease in body weight was found as compared to normal rats (table 3). The higher dose of ELESI and standard drugs significantly prevent the decrease in body weight at 14 and 28 days. Lower dose treatment did not show the pronounced difference in body weight as compared to diabetic control rats.

Table 3: Effect of oral administration of ELESI on body weight (g) in normal and STZ-induced diabetic rats

Groups	Treatment	Mean body weight in gram			
		0th day	7th day	14th day	28th day
Group I (normal control Rats)	Vehicle	227.37 ± 5.32	242.48±5.58	256.73±4.35	270.55±4.44
Group ll (Plant extract per se)	500 mg/kg	230.55 ± 6.15	239.67±4.74	260.750±4.71	274.23±5.21
Group III (Diabetic control)	STZ only	230.14 ± 5.94	214.71±5.17	180.46±5.55**	154.83±4.68**
Group lV (Plant extract)	STZ plant extract (100 mg/kg)	229.70 ± 4.07	218.65±3.61	189.72±6.29	1156.88±9.25a
Group V (Plant extract)	STZ plant extract (200 mg/kg)	230.63 ± 3.77	227.49±5.45	232.35±4.68a	230.45±5.16*
Group V (Plant extract)	STZ plant extract (500 mg/kg)	228.96 ± 0.64	228.90±0.57	230.30±0.67a	232.34±0.14a

Effect of extract on body weight. Data are mean ± SEM; Data were analyzed using one-way ANOVA followed by Tukey's multiple tests; *P<0.01 as compared to Vehicle control Group; [a]P<0.05 as compared to Diabetic control group.

Effect of extract on Blood Glucose level

The administration of extracts or standard treatment such as glipizide (4 mg/kg, p.o 2 weeks) to normal rats did not produce any significant *per se* effects on various parameters assessed at the end of 4 weeks of treatment in the present study. Fasting blood glucose level was significantly elevated ($p < 0.05$) after 3 days of STZ treatment concerning control level. The results showed that rats in the control group showed no significant change in blood glucose levels is observed at 7, 14, 21 and 28 days of the experiment (Table 4). No significant changes in blood glucose levels were observed after oral administration of 500 mg/kgbwt of SI in normal animals at 28 days of the experiment when compared to control. However, treatment with a single dose of STZ at a dose of 180 mg/kgbwt after 3 days caused a significant increase ($p < 0.05$) in the blood glucose levels of rats. Whereas, oral administration of 100 mg/kgbwt, 200mg/kgbwt and 500mg/kgbwt of ethanolic leaf extract of SI for 28 days showed significant reduction ($p<0.05$) in blood glucose levels when compared to STZ treated group (Table 6). Treatment with Glipizide (4 mg/kg body weight, 4 weeks) significant decreased the glucose level at 7, 14, 21 and 28 days when compared with diabetic control rats.

Table 4: Effect of oral administration of ELESI on blood glucose in normal and STZ-induced diabetic rats

Groups	Treatment	Blood glucose level (mg/dl)				
		Day 0	Day 7	Day 14	Day 21	Day 28
(Group-I) Normal	Vehicle	92.30 ± 5.21	94.80±1.55	91.39±2.1	90.41±6.21	93.12±8.15
(Group-II) Plant per se	500 mg/kg	94.55 ± 5.34	89.82±7.77	94.78±4.22	84.35±3.68	85.13±2.78
(Group-III) Diabetic control	STZ only	94.58 ± 7.70	349.80±7.81***	330.45±8.16***	336.12±14.3***	312.21± 6.32***
(Group-IV) Plant extract 100mg/kg	STZ+ extract (100 mg/kg)	92.32 ± 5.55	355.23 ± 6.62	339.45±10.18	324.72±6.90	298.98±10.76
(Group-V) Plant extract 200mg/kg	STZ+ extract (200 mg/kg)	90.67 ± 6.62	302.43±6.85	290.43±8.72	241.37±18.66aa	204.48± 11.54aaa
(Group-VI) Plant extract 500 mg/kg	STZ+ extract (500 mg/kg)	90.95 ± 8.75	294.56±11.23	268.86±13.56a	181.78±12.54aaa	149.42± 15.24aa
Standard Treatment (GROUP-VII)	Glipizide (4 mg/kg)	88.72±6.14[a]	210.18±6.97[a]	177.43±4.56[a]	113.35±4.35[a]	99.63±7.43[a]

Effect of extract on serum glucose. Data are mean ± SEM; Data were analyzed using one-way ANOVA followed by Tukey's multiple tests; *p<0.01 as compared to Vehicle control Group; [a]p<0.05 as compared to Diabetic control group.

3.6 Effect of ELEFD on serum lipid profile

The results showed that the serum TC, TG, LDL and VLDL levels increased to 167.39 mg/dl, 178.13 mg/dl, 103.54 mg/dl, 35.62 mg/dl and decreased levels of HDL (28.22 mg/dl) in STZ-induced diabetic rats. When treated with standard drug glipizide lipid levels were reduced significantly (Table 5). Among the plant extract, high doses of SI (200 and 500mg/kg) worked effectively and significantly altered the lipid parameters in diabetic rats in a dose-dependent manner. However, a lower dose did not produce a significant effect on the TC, TG, HDL, LDL and VLDL levels in diabetic rats.

Table 5: Effect of ELSESI *on* blood lipid profile in normal and STZ-induced diabetic rats.

Groups	Treatment	Total Cholesterol (mg/ dl)	Triglycerides (mg/dl)	HDL Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)	VLDL (mg/dl)
(Group-I) Normal	Vehicle	95.19±4.20	110.25±4.72	46.72±4.32	26.42±2.46	22.05±1.36
(Group-II) Plant per se	500 mg/kg	90.77±5.48	111.35±5.56	42.54±5.54	25.96±6.44	22.27±4.67
(Group-III) Diabetic control	STZ only	167.39±6.55***	178.13±3.35***	28.22±5.55***	103.54±5.44***	35.62±1.55***
(Group-IV) Plant extract 100mg/kg	STZ+ extract (100 mg/kg)	159.32±11.32	167.44±6.98	30.23±5.67	95.60±7.25	33.48±3.31
(Group-V) Plant extract 200mg/kg	STZ+ extract (200 mg/kg)	137.15±7.22a	156.68±5.92a	40.19±3.57a	65.62±5.45aa	31.33±2.45a
(Group-VI) Plant extract 500 mg/kg	STZ+ extract (500 mg/kg)	108.54±6.87aaa	144.88±6.76aaa	42.76±4.98aaa	36.80±5.32aaa	28.97±5.55aaa
(GROUP-VII) Standard treatment	Glipizide (4 mg/kg)	113.68±4.22aa	123.18±5.97aaa	44.24±5.56aaa	44.80±4.35aaa	24.63±2.43aaa

Effect of extract on serum lipid. Data are mean ± SEM; Data were analyzed using one-way ANOVA followed by Tukey's multiple tests; *P<0.01 as compared to Vehicle control Group; aP<0.05 as compared to Diabetic control group.

Effect of plant extracts on serum oxidative/antioxidant parameters

The levels of oxidative markers detected in the serum of normal and diabetic control rats are summarized in table 6. STZ administration resulted in a profound increase in TBARS levels, as compared with normal controls. Chronic administration of 200mg/kg and 500mg/kg of plant extracts of SI significantly reduced the elevated levels of TBARS in serum of diabetic rats in comparison to the levels observed in vehicle-treated diabetic control rats. However, plant extract at a lower dose (100mg/kg) did not notably influence the TBARS level in STZ rats.

The antioxidant enzyme status in serum samples was assessed by measurements of Glutathione (GSH), Superoxide dismutase (SOD), catalase (CAT) levels. Serum GSH, SOD and CAT levels were decreased in diabetic rats when compared with the normal control group, indicative of impairment in anti-oxidant status. Treatment with plant extract of SI (200 & 500 mg/kg,) and glipizide restored serum GSH, SOD and CAT levels as compared to the control group (table 6). Further, a lower dose (100 mg/kg, p.o.) did not produce any effect on the antioxidant enzyme levels in diabetic rats.

Table 6: Effect of ELESI *on oxidant*/anti-oxidant profile in normal and STZ-induced diabetic Rats

Groups	Treatment	MDA (nM/mg)	Glutathione (µg/mg)	Catalase (µM /min /mg)	SOD (Unit/min//mg)
(Group-I) Normal	Vehicle	10.50±0.80	25.77±3.10	19.13±1.80	14. 47±1.87
(Group-II) Plant per se	500 mg/kg	10.65±1.04	26.16±3.54	19.24±4.45	14. 03±3.17
(Group-III) Diabetic control	STZ only	21.30±1.86***	11.67±2.26***	11.70±0.75***	5.70±1.50***
(Group-IV) Plant extract 100mg/kg	STZ+ extract (100 mg/kg)	19.98±0.35	12.99±2.51	12.11±0.35	7.86±2.11
(Group-V) Plant extract 200mg/kg	STZ+ extract (200 mg/kg)	15.56±2.15aa	17.20±1.19aa	14.27±1.21aa	8.66±3.09a

Table 6: *(Continued)*

Groups	Treatment	MDA (nM/mg)	Glutathione (µg/mg)	Catalase (µM /min /mg)	SOD (Unit/min//mg)
(Group-VI) Plant extract 500 mg/kg	STZ+ extract (500 mg/kg)	14.18±1.20aaa	21.62±2.54aaa	15.06±2.22aaa	11.82±0.06aaa
(GROUP-VII) Standard treatment	Glipizide (4 mg/kg)	11.11±1.67aaa	23.12±2.37^{aaa}	18.99±2.55aaa	13.92±2.09aaa

Effect of extract on *oxidant*/anti-oxidant profile. Data are mean ± SEM; Data were analyzed using one-way ANOVA followed by Tukey's multiple test; *P<0.01 as compared to Vehicle control Group; ^{a}P<0.05 as compared to Diabetic control group.

DISCUSSION

Medicinal plants have a long-standing history in many indigenous communities and continue to provide useful tools for treating various diseases. The practices of traditional medicine are based on hundreds of years of beliefs and observations, which predate the development and spread of modern medicine.[39] It is reported that the compounding of highly standardized herbal products concerning chemical composition and pharmacological activity is considered an important approach in this field. Considering the above-mentioned aspects, this comprehensive study examines the prominent features such as pharmacognostic, Phytochemical and pharmacological activity of leaves and bark of SI plant.

In the present study, the results of the phytochemical screening revealed that all the bark and leaf extract of the SI plant showed the presence of flavonoids, Saponins, steroids, alkaloids, tannins, phenolic compounds, triterpenoids and carbohydrates whereas, glycosides, anthraquinones, and reducing sugars were absent. Moreover, quantitative estimation of total tannins, total phenolic and total flavonoids compounds were also performed in the leaf and bark of all three plants. The ELESI show the presence of the higher amount of total phenol, total flavonoid and tannins components as compared to other extracts. This report eventually justifies the preliminary phytochemical studies and also helps in finding suitable extracts for the pharmacological study.

Extracts of the SI plant was further investigated for their beneficial effects in the management of STZ-induced diabetes. In the present study, diabetes was induced by the administration of STZ. The results, based on biochemical parameters, were compared with normal control, diabetic control and positive control rats treated with glibenclamide. The result of the present study showed significant changes in biochemical parameters of the experimentally induced diabetes. In support of earlier reports, increased fasting blood glucose (FBG) level was seen in STZ induced diabetic rats compared to the control group.[40,41] FBG was significantly attenuated by treatment with all three plant extracts ELESI, which is consistent with our previous report. Plant extracts normalize the glucose level similar to the standard drug (glimepiride) and exhibited a potent anti-diabetic effect. The decrease in the level of FBG might be attributed to the insulin secretion from residual pancreatic cells or regeneration β-cell. Moreover, an increase in insulin secretion from remnant β-cells and an increase in the peripheral utilization of glucose may also contribute to the anti-hyperglycemic action of plants. So, this study divulged the association between glucose level and insulin levels.

Along with hyperglycemia, induction of diabetes with STZ is associated with characteristic weight loss. Oral administration of ELESI improved body weight in diabetic rats. The decreased body weight was due to protein metabolism and muscle wasting. After treatment with plant extracts, diabetic animals showed improvement in body weight. This increased body weight might be linked to insulin secretion which improves glucose levels in diabetic animals.

The correlation between hyperglycemia and dyslipidemia is well known.[42,43] Increase level of lipids leads to atherosclerosis which may cause diabetes and complications of diabetes.[43] In our study, a significant increase in TC, TG, VLDL and LDL levels was observed along with a significant reduction in HDL levels in diabetic rats. Administration of plant extracts effectively increased serum HDL and reduced the level of TC, TG, LDL and VLDL cholesterol. Interestingly, plant extracts at higher doses shown quite a similar result as to the glimepiride treated group in the reduction of TC, TG, LDL and VLDL indicating equivalent hypolipidemic activity similar to available standard drugs. Moreover, in the case of HDL, the highest dose of both the extracts showed more pronounced effects as compared to standard drug glimepiride.[44,45,46]

Moreover, the aetiology of diabetes involves various factors like increased oxygen free radical, alteration in antioxidant enzymes, nonenzymatic protein glycosylation, impaired glutathione metabolism and lipid peroxidation.[47] STZ-induced hyperglycemia elevates ROS generation and depresses antioxidant defence resulting in cellular disruption and increased lipid peroxidation.[48] Thus, oxidative stress can be diminished *via the* diminution of free radical generation.

Also, the potential in-vitro antioxidant activity of plant extracts was estimated by DPPH radical scavenging and

reducing power activity method. The WBESI showed dose-dependent increased in scavenging activity on free radicals in our present study.

Polyphenolic compounds such as flavonoids, phenolic acids and tannins are considered to be the major contributors to the antioxidant activity of fruits and vegetables of medicinal plants. Phenol and phenolic compounds such as flavonoids have been shown to possess significant antioxidant activities and their effects on human nutrition and health are considerable.[49] Hence, the leaf and bark extracts of FD, SI and CM could be a good source of antioxidants.

In our study, various antioxidant enzyme levels like SOD, CAT, and GSH were significantly decreased in diabetic rats, whereas the level of TBARS increased significantly It is previously reported that the activity of antioxidant enzymes is reduced in serum and tissue homogenate of diabetic rats.[50] In this study, treatment with ethanolic leaf extract of all three plants reduced TBARS level and increased SOD, CAT as well as GSH levels, due to its potential antioxidant activity. Reduction in the level of TBARS after treatment shows the effective antioxidant activity of ELESI. Thus, cumulative results show the antidiabetic potential of plans.

CONCLUSION

The study evaluated the anti-diabetic activity of Sophora interrupta by using the streptozotocin-induced diabetic rat model and reveals that the ethanolic extract of SI showed significant antioxidant, antihyperlipidemic and antidiabetic activity suggesting an alternate and promising therapeutic regimen in the treatment of diabetes and secondary complications.

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