Evaluation of the anti diabetic activity of column fractions obtained from the bark extract of *Soymida febrifuga* A. Juss

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ABSTRACT

Diabetes has become a worldwide problem afflicting humans irrespective of age. Even though a number of synthetic drugs are available for the treatment of diabetes, plant drugs are generally preferred due to the assumption that they have less side effects and low cost. This study reports the hypoglycaemic and antihyperglycaemic actions of the methanolic bark extract of *Soymida febrifuga* A. Juss. (fam: Meliaceae) in euglycaemic and alloxan-induced diabetic rats, respectively. The results revealed that three column fractions obtained from the methanol extract of *S. febrifuga* possess maximum hypoglycaemic and antihyperglycaemic activities 6 h after treatment. At a dose of 200 mg/kg, the relatively nonpolar column fraction obtained by using 20% chloroform in acetone as an eluent was by far the most potent fraction which showed comparable activity with that of the standard drug glibenclamide (10 mg/kg). At a dose of 200 mg/kg, the same column eluate showed maximum antihyperglycaemic effect reducing blood glucose level by 33.00%. At a dose of 10 mg/kg, the reference drug glibenclamide brought about 33.46% reduction of blood glucose level. In light of the results obtained from the current study, it could be concluded that the bark of *S. febrifuga* has genuine antidiabetic activity.

Keywords *Soymida febrifuga*, Meliaceae, methanol extract, euglycaemic, hypoglycaemic effect, alloxan-induced antihyperglycaemic activity.

INTRODUCTION

Interest in herbal medicines is growing day-by-day because nature can cure many diseases. Diabetes has become a very common ailment afflicting humans irrespective of age. It is a worldwide problem, and India is not exceptional. Even though a number of synthetic drugs are available for the treatment of diabetes, plant drugs are generally preferred due to the assumption that they have less side effects and low cost.

Soymida febrifuga A. Juss. (fam: Meliaceae) commonly called Indian Red Wood is an endemic plant that grows wild in all dry deciduous forests of Andhra Pradesh, India.[1] The plant is easily identified by its grayish green paripinnate alternate leaves, and the red petioles and veins that the young leaves have. Traditionally, it is used for the treatment of diseases like rheumatoid arthritis, asthma and vaginal infections.[2] It is often claimed that the extracts of the plant have good activity against ulcers, tridosha fevers, leprosy, dysentery and diarrhea.[3] It has also been reported that the extracts of the plant have antimalarial activity similar to that of the cinchona bark. Decoction of the crushed bark is used for tongue sores, fixing loose teeth, gum infection and cough.[4]

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DOI: 10.5530/pj.2012.31.7

Simonsen et al.[5] showed that the bark extract of *S. febrifuga* possess significant *in vitro* antiplasmodial activity against *Plasmodium falciparum*. The extract also showed a dose dependent anti-inflammatory activity in Carrageenan-induced rat paw oedema, which was comparable to NSAIDs like naproxen, ibuprofen, and piroxicam.[6] Methyl angolensate, a natural tetranortriterpenoid isolated from *S. febrifuga* root calluses has been reported to have anticancer activity against T-cell leukemia, and chronic myelogenous leukemia.[7]

Several chemical constituents including quercetin, myricetin, quercetin 3-*O*-L-rhamnoside, lupeol, luteolin and tetranortriterpenoids have been isolated from the different parts of *S. febrifuga*.[8,9] Some of these constituents have been shown to possess antioxidant activity.[10]

In view of the wide medicinal uses of the various parts of *S. febrifuga*, it was deemed prudent to assess the effect of the bark extract of the plant on blood glucose levels in euglycaemic as well as in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Materials

Plant materials

The bark of *S. febrifuga* was collected from the forest areas of Rajahmundry, East Godavari District, Andhra Pradesh, India. Immediately after collection the plant materials were authenticated by Dr. VS Raju, Department of Botany, Kakatiya University, and voucher specimens were deposited at KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, for future reference.

Chemicals and drugs

Glibenclamide was a generous gift from Dr. Reddy's Foundation, Hyderabad, India. Alloxan monohydrate was purchased from Sigma-Aldrich, Germany. Assay kits (GOD-POD) were purchased from Beacon Diagnostics Ltd., Navasari, India. All other chemicals and solvents used were of analytical grade procured from local suppliers.

Animals

Wistar albino rats weighing 180–200 g were purchased from Mahaveer agencies, Hyderabad, India, and used for the studies after obtaining permission from institutional animal ethical committee (CPCSEA Reg. No. 146/1999). Experimental animals were housed in standard polypropylene cages, maintained under standard laboratory conditions (12 hour light/dark cycle; at an ambient temperature of 25 ± 5ºC; 35%–60% of relative humidity) and fed with standard rat pellet diet and water *ad libitum*.

Methods

Extraction and phytochemical screening

Barks of *S. febrifuga* were dried in shade and powdered in a mechanical grinder. Chloroform, methanol and aqueous extracts were prepared by maceration with sufficient amounts of the respective solvents for 7 days with intermittent stirring. The contents of the flask were then filtered and the mark further macerated with fresh solvents for an additional 3 days. After filtration, the combined extracts were concentrated to dryness under reduced pressure, first kept in desiccators, weighed and then stored in a refrigerator for future use. Preliminary phytochemical screening was carried out on the methanol bark extract of *S. febrifuga* following standard procedures.[11,12,13] The extract was then subjected to column chromatography using hexane, benzene, chloroform, acetone and methanol. Among the collected fractions only three fractions, namely, the 20% chloroform in acetone (AFSF), the 60% acetone in methanol (MF_1SF) and the 20% acetone in methanol (MF_2SF) were used for bioactivity studies after carrying out preliminary activity tests.

Acute toxicity study

Acute toxicity study was carried out according to the method described by Glombitza et al.[14] and Ghosh et al.[15] Overnight fasted Wistar albino rats were divided into groups, each consisting of 6 animals, and orally fed separately with the column fractions AFSF, MF_1SF and MF_2SF at dose levels of 100, 500, 1000 and 2000 mg/kg body weight, respectively. The rats were observed continuously for 2 h for behavioural, neurological and autonomic profiles. After a period of 24 and 72 h, observations were made for any death that might have occurred.[16]

Assessment of hypoglycaemic activity in euglycaemic rats

The experiment was conducted according to the procedure described in the literature.[17,18] A total of 66 normoglycaemic rats fasted for 18 h were divided into 11 groups of 6 mice per group and treated as follows: Group I - 5% gum acacia (Control group); Group II - glibenclamide, 10 mg/kg (Standard group); Group III - AFSF 100 mg/kg; Group IV - AFSF 200 mg/kg; Group V - AFSF 400 mg/kg; Group VI - MF_1SF 100 mg/kg;

Group VII - MF_1SF 200 mg/kg; Group VIII - MF_1SF 400 mg/kg; Group IX - MF_2SF 100 mg/kg; Group X - MF_2SF 200 mg/kg; Group XI - MF_2SF 400 mg/kg. Immediately before the experiment begins, initial fasting blood samples were taken from the animals of all the groups. This was followed by oral administration of different doses of the column fractions of *S. febrifuga* or the reference drug glibenclamide (10 mg/kg) suspended in 5% gum acacia. The effects of the column fractions and reference drug on fasting blood glucose level were monitored for 24 h. Blood samples were drawn from the retro-orbital plexus of the treated rats at 0 h (initial fasting blood sample) and 2, 4, 6, 8, 12 and 24 h after the treatment. The samples were analyzed on autoanalyser (Selectra Junior, Merck) for blood glucose content using glucose oxidase-peroxidase method.[19]

Assessment of antihyperglyacemic activity in alloxan-induced diabetic rats

Diabetes was induced in normoglycaemic overnight fasted Wistar albino rats by a single intraperitoneal injection of alloxan monohydrate (125 mg/kg) dissolved in saline. Blood glucose level of the animals was checked after 72 h. Animals with blood glucose level >250 mg/dl were considered diabetic and were used for the study. Diabetic rats were divided into 11 groups of six animals per group as shown above, and treated orally as described in the literature.[20,21] Blood samples were collected from all the animals at the time intervals of 0 (fasting blood sample), 2, 4, 6, 8, 12 and 24 h after treatment to estimate blood glucose levels.

Estimation of serum biochemical parameters

Serum triglycerides and serum cholesterol were estimated as described by Trinder et al.[19] Serum total proteins were measured according to the method of Lowry et al.[22] and serum insulin levels were determined by chemiluminescence assay.[23,24]

Blood samples collected from the experimental animals were subjected to centrifugation at 3000 rpm for 10 min to separate the serum. For the estimation of the above mentioned biochemical parameters, 0.5 µl of serum sample was transferred to each of the pediatric sample cups. The cups and the working reagent bottles (25 ml) corresponding to the biochemical parameters were then placed at their respective positions in the rotor system of the autoanalyzer. Thirty min after programming, each of the test parameters, the corresponding values of the different serum samples displayed on the computer were recorded.

Statistical analysis

All values were expressed as mean ± SD. The data were statistically evaluated using one way analysis of variance (ANOVA) followed by post hoc Dunnet's t-multiple comparison test using GraphPad Prism 4 computer software.[25] Values corresponding to $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

Preliminary phytochemical screening revealed the presence of alkaloids, triterpenoids and saponins in AFSF, triterpenoids in MF_1SF, and alkaloids, triterpenoids and saponins in MF_2SF. In the present study, none of the fractions produced behavioural, neurological and autonomic changes in rats nor did they cause death during the first 72 h of the study period indicating that the extracts are safe at the tested doses.

Effect of fractions on blood glucose levels in euglycaemic rats

Effects of the column fractions of *S. febrifuga* bark extract on blood glucose levels in euglycaemic rats are shown in Table 1. At doses of 200 and 400 mg/kg, AFSF produced a significant ($P < 0.05$) hypoglycaemic effect 2 h after treatment, while the same doses of MF_1SF and MF_2SF showed similar effects 4 h after treatment. All fractions showed significant hypoglycaemic effect at all test doses with a P value < 0.01 at 4, 6 and 8 h of the study. AFSF (200 mg/kg) and the reference drug glibenclamide (10 mg/kg) exhibited significant effect with P value < 0.05, 2 h after treatment. Maximum percentage reduction in blood glucose levels ranging from 34.4 to 54.1 was obtained 6 h after administration of the fractions. However, maximum hypoglycaemia of 54.1, 43.0 and 37.8% was observed 6 h after administration of 200 mg/kg of AFSF, MF_2SF and MF_1SF, respectively. Percentage reduction of blood glucose level by the 200 mg/kg of AFSF (54.1) was comparable to that of the reference drug, glibenclamide (10 mg/kg; 54.8).

Effect of fractions on fasting blood glucose levels in alloxan-induced diabetic rats

Effect of the column fractions obtained from *S. febrifuga* bark extract on blood glucose levels of alloxan-induced diabetic rats is shown in Table 2. At doses of 100, 200 and 400 mg/kg, all the fractions significantly ($p < 0.01$) lowered blood glucose levels 2 h after treatment, and the effect continued up to 24 h of the study period. However, all the fractions showed maximum effect, 6 h after treatment with the highest effect observed for the

Table 1. Effect of column fractions obtained from the bark extract of *Soymida febrifuga* on blood glucose levels in euglycaemic rats.

			Blood glucose level (mg/dl)						
			0 h	2 h	4 h	6 h	8 h	12 h	24 h
I	Control	—	80.5 ± 9.51	80.6 ± 7.62 (0.12%)	78.4 ± 8.01 (2.60%)	78.3 ± 9.10 (2.73%)	79.3 ± 8.01 (1.49%)	77.5 ± 8.29 (3.70%)	80.8 ± 8.08 (0.37%)
II	Gliben-clamide	10	84.1 ± 8.06	60.7 ± 3.50 (27.82%)*	51.5 ± 6.20 (38.70%)**	38.0 ± 6.10 (54.80%)**	48.5 ± 8.40 (42.30%)**	61.3 ± 8.00 (27.10%)**	73.5 ± 7.20 (12.60%)
III	AFSF	100	90.2 ± 20.1	67.9 ± 10.10 (24.70%)	60.3 ± 11.50 (33.20%)**	48.8 ± 10.90 (45.90%)**	54.6 ± 10.30 (39.47%)**	63.6 ± 10.10 (29.50%)**	74.6 ± 13.52 (5.30%)
IV	AFSF	200	85.6 ± 7.90	61.7 ± 5.90 (27.90%)*	53.9 ± 5.60 (37.00%)**	39.3 ± 7.10 (54.10%)**	51.1 ± 7.30 (39.10%)**	62.8 ± 7.30 (26.60%)**	73.7 ± 6.90 (13.90%)
V	AFSF	400	85.0 ± 8.20	62.9 ± 5.80 (26.00%)*	53.6 ± 8.60 (36.90%)**	40.8 ± 7.10 (52.00%)**	50.8 ± 6.00 (40.20%)**	63.2 ± 7.30 (25.60%)**	79.6 ± 6.90 (6.35%)
VI	MF_1SF	100	85.5 ± 13.90	71.6 ± 12.30 (16.25%)*	66.6 ± 12.21 (22.10%)**	55.6 ± 12.20 (34.90%)**	63.5 ± 10.30 (25.70%)**	70.5 ± 9.10 (17.50%)**	76.5 ± 11.90 (10.50%)
VII	MF_1SF	200	85.0 ± 8.39	66.8 ± 11.30 (21.40%)*	61.5 ± 8.50 (27.60%)**	44.8 ± 7.40 (47.30%)**	56.8 ± 6.80 (33.10%)**	66.5 ± 7.50 (21.70%)**	74.1 ± 6.50 (12.90%)
VIII	MF_1SF	400	86.5 ± 10.53	67.9 ± 8.10 (21.50%)*	59.9 ± 10.70 (30.60%)**	46.3 ± 8.90 (45.90%)**	59.5 ± 8.60 (31.20%)**	68 ± 8.10 (21.30%)**	81.6 ± 8.50 (5.60%)
IX	MF_2SF	100	86.4 ± 13.40	70.6 ± 12.12 (18.20%)*	67.3 ± 10.10 (22.40%)**	56.6 ± 12 (34.40%)**	64.5 ± 11.30 (25.30%)**	70.3 ± 11.10 (18.90%)**	77.3 ± 12.10 (10.80%)
X	MF_2SF	200	85.3 ± 10.20	65.5 ± 11.20 (22.90%)*	59.3 ± 8.90 (30.50%)**	48.55 ± 7.80 (43.00%)**	59.3 ± 10.80 (30.50%)**	65 ± 6.50 (23.50%)**	79.8 ± 19.00 (6.40%)
XI	MF_2SF	400	85.0 ± 8.21	66.4 ± 10.80 (21.60%)*	60.4 ± 6.31 (28.90%)**	50.67 ± 10.10 (41.00%)**	60.9 ± 9.70 (28.30%)**	65.9 ± 7.30 (22.40%)**	80.2 ± 9.50 (5.60%)

AFSF = 20% chloroform in acetone fraction; MF1SF = 60% acetone in methanol fraction; MF2SF = 20% acetone in methanol fraction; All values are expressed as mean + SD, n = 6; Figures in parenthesis indicate the percentage reduction of blood glucose levels when compared to 0 h value(s), *p < 0.05; **p < 0.01 when compared with control at the respective time interval.

Table 2. Effect of column fractions obtained from the bark extract of *Soymida febrifuga* on blood glucose levels in alloxan-treated rats.

			Blood glucose levels (mg/dl) at different hours						
			0 h	2 h	4 h	6 h	8 h	12 h	24 h
I	Control	—	279.4 ± 2.5	280.4 ± 2.3 (0.35%)	281.3 ± 3.1 (0.68%)	281.4 ± 6.1 (0.72%)	281.8 ± 3.1 (0.85%)	280.4 ± 3.2 (0.35%)	280.6 ± 2.8 (0.43%)
II	Gliben-clamide	10	280.3 ± 1.6	235.0 ± 4.8 (16.60%)*	214.0 ± 5.0 (23.65%)*	186.5 ± 3.2 (33.46%)*	198.3 ± 4.2 (29.25%)*	216.0 ± 9.3 (22.90%)*	239.1 ± 14.2 (14.69%)*
III	AFSF	100	280.4 ± 2.1	240 ± 1.6 (14.40%)*	219.0 ± 2.1 (21.8%)*	190.8 ± 2.2 (31.90%)*	200.0 ± 2.3 (28.60%)*	221.5 ± 3.2 (21.00%)*	242.4 ± 2.3 (13.50%)*
IV	AFSF	200	280.5 ± 3.9	236.5 ± 3.2 (15.60%)*	215.3 ± 4.5 (23.30%)*	187.7 ± 3.3 (33.08%)*	199.4 ± 3.2 (28.90%)*	217.6 ± 4.3 (22.40%)*	239.9 ± 5.8 (14.40%)*
V	AFSF	400	284.3 ± 3.5	253.0 ± 3.5 (11.00%)*	229.8 ± 2.3 (19.16%)*	196.6 ± 4.8 (30.84%)*	214.5 ± 4.2 (24.55%)*	220.5 ± 2.5 (22.35%)*	253.5 ± 6.3 (10.80%)*
VI	MF_1SF	100	280.6 ± 2.4	250.6 ± 4.3 (10.70%)*	225.0 ± 7.8 (19.80%)*	199.6 ± 9.3 (28.80%)*	204.4 ± 8.5 (27.20%)*	229.6 ± 8.8 (18.10%)*	246.6 ± 8.8 (12.10%)*
VII	MF_1SF	200	280.5 ± 1.4	240.2 ± 4.01 (14.40%)*	220.4 ± 4.8 (21.40%)*	190.6 ± 5.1 (32.00%)*	202.4 ± 8.3 (27.80%)*	221.0 ± 10.3 (21.20%)*	243.3 ± 10.3 (13.30%)*
VIII	MF_1SF	400	282.3 ± 1.6	258.1 ± 3.6 (8.60%)*	214.9 ± 2.1 (23.80%)*	199.9 ± 5.6 (29.10%)*	215.3 ± 4.6 (23.75%)*	223.3 ± 4.6 (20.90%)*	245.0 ± 3.8 (13.50%)*
IX	MF_2SF	100	280.3 ± 1.5	255.4 ± 4.1 (8.70%)*	227.1 ± 6.8 (18.90%)*	198.3 ± 3.6 (29.20%)*	205.3 ± 5.9 (26.70%)*	227.5 ± 8.3 (18.70%)*	247.5 ± 5.8 (11.60%)*
X	MF_2SF	200	281.4 ± 1.6	242.1 ± 2.9 (13.80%)*	221.4 ± 4.3 (21.30%)*	189.6 ± 5.8 (32.50%)*	200.7 ± 8.5 (28.50%)*	223.5 ± 10.4 (20.40%)*	244.4 ± 10.2 (13.10%)*
XI	MF_2SF	400	282.5 ± 1.5	256.2 ± 3.5 (9.30%)*	216.40 ± 1.8 (23.39%)*	199.4 ± 5.9 (29.50%)*	215.5 ± 3.6 (23.70%)*	220.5 ± 4.6 (21.94%)*	249.5 ± 2.8 (11.60%)*

AFSF = 20% chloroform in acetone fraction; MF_1SF = 60% acetone in methanol fraction; MF_2SF = 20% acetone in methanol; All values are expressed as mean ± SD, n = 6; Figures in parenthesis indicate the percentage reduction of blood glucose levels when compared to 0 h value(s); *P < 0.01 when compared with control at the respective time interval.

200 mg/kg of AFSF, MF_2SF and MF_1SF, which showed 33.0, 32.5 and 32.0% reduction of blood glucose levels, respectively. Although significant blood glucose lowering effect was also achieved by the 400 mg/kg of each of the fractions, 6 h after treatment, the reduction was much less than that of the lower dose (200 mg/kg). The significant ($p < 0.01$) antihyperglycaemic effect achieved by the 200 mg/kg of AFSF was comparable to that of the

reference drug glibenclamide (10 mg/kg) at any time interval of the study period, with maximum percent reduction of 33.0 and 33.46, respectively.

Effect of fractions on body weight of alloxan-induced diabetic rats

The effect of the fractions on body weight of alloxan-induced diabetic rats study is summarized in Table 3. The observations made on the results of different parameters studied are as follows: There was a gradual decrease in body weight of animals in diabetic control group. Animals treated with fractions and reference drug showed a gradual and significant ($p < 0.01$) increase in body weight after 7 days of the treatment. The increase in body weight was observed until the end of the study period (21 days). The significant ($p < 0.01$) effect of all the fractions at a dose of 200 mg/kg on body weight was comparable to that of the reference drug, glibenclamide (10 mg/kg) at each time interval of the study.

A significant reduction in body weight observed in alloxan-induced diabetic rats was very likely due to increased excretion of glucose and reduced uptake of glucose by peripheral tissues and glycogen synthesis. The improvement in body weight of the animals when treated with the fractions is possibly due to potentiation of insulin secretion which reverses these effects. A significant reduction in body weight was observed in alloxan-induced diabetic rats. The decrease is likely due to increased excretion of glucose and reduced uptake of glucose by peripheral tissues and glycogen synthesis.[26] Improvement in body weight of the animals when treated with the fractions indicates a protective effect.

Effect of fractions on serum biochemical parameters in alloxan-induced diabetic rats

Results of the effects of column fractions on the various biochemical parameters are shown in Table 4.

Serum triglyceride level

In groups treated with fractions and reference drug, a significant ($p < 0.001$) decrease in serum triglyceride level was recorded at day 21 the study period. The percent reduction in serum triglyceride level in AFSF, MF_1SF and MF_2SF treated groups were 12.18, 12.55 and 12.26, respectively, whilst glibenclamide (10 mg/kg, b.w.)

Table 3. Effect of column fractions obtained from the bark extract of *Soymida febrifuga* on body weight in alloxan-induced diabetic rats.

		Body weight in grams			
		Day 1	Day 7	Day 14	Day 21
Diabetic control-I	–	220.6 ± 6.20	207.5 ± 9.40	190.4 ± 8.50	179.0 ± 5.90
Glibenclamide-II	10	219.3 ± 10.80	230.2 ± 9.03**	244.4 ± 8.90**	256.3 ± 10.80**
AFSF-III	200	219.9 ± 10.60	229.0 ± 8.90**	242.4 ± 5.30**	250.4 ± 8.30**
MF_1SF- IV	200	218.4 ± 11.50	225.8 ± 7.60*	238.4 ± 6.50**	246.5 ± 5.60**
MF_2SF -V	200	219.5 ± 10.60	227.6 ± 1.80**	239.0 ± 7.50**	249.5 ± 5.80**

AFSF = 20% chloroform in acetone fraction; MF_1SF = 60% acetone in methanol fraction; MF_2SF = 20% acetone in methanol fraction;
All values are expressed as mean ± SD, n = 6; Values given in the parenthesis are percent blood glucose reduction when compared to 1st day value(s); * P < 0.01; ** P < 0.001 when compared with control at the respective time interval.

Table 4. Effect of column fractions obtained from the bark extract of *Soymida febrifuga* on different serum biochemical parameters in alloxan-induced diabetic rats.

		Serum insulin levels in µIU/ml		Serum triglyceride levels (mg/dl)		Serum cholesterol (mg/dl)		Serum total protein (g/dl)	
		Day 1	Day 21	Day 1	Day 21	Day 1	Day 21	Day 1	Day 21
Diabetic control - I	–	10.7 ± 0.6	10.9 ± 0.60 (1.86%)	180.8 ± 1.50	181.2 ± 1.80 (0.22%)	122.4 ± 6.00	122.9 ± 3.50 (0.40%)	4.2 ± 0.5	4.3 ± 0.40 (0.70%)
Glibenclamide - II	10	11.2 ± 0.6	18.9 ± 1.90 (68.75%)	172.5 ± 2.90	152.3 ± 3.00** (11.70%)	121.2 ± 4.30	67.5 ± 10.30** (44.30%)	3.9 ± 0.2	5.8 ± 0.70** (48.70%)
AFSF- III	200	11.3 ± 0.3	19.0 ± 2.20** (68.10%)	171.5 ± 2.60	150.6 ± 2.60** (12.18%)	121.3 ± 3.20	71.4 ± 8.30** (41.13%)	4.1 ± 0.2	6.0 ± 0.70** (65.34%)
MF_1SF - IV	200	11.4 ± 0.4	19.1 ± 2.80** (64.60%)	170.5 ± 4.80	149.1 ± 3.90** (12.55%)	120.5 ± 4.04	72.1 ± 8.40** (40.16%)	4.4 ± 0.3	5.7 ± 0.50** (43.18%)
MF_2SF - V	200	11.3 ± 0.6	19.4 ± 4.0** (71.68%)	174.6 ± 4.90	153.2 ± 4.20** (12.26%)	120.1 ± 3.61	72.1 ± 7.40** (39.96%)	4.4 ± 0.3	5.3 ± 0.32* (41.14%)

AFSF = 20% chloroform in acetone fraction; MF_1SF = 60% acetone in methanol fraction; MF_2SF = 20% acetone in methanol fraction; All values are expressed as mean ± SD, n = 6; Values given in the parenthesis are the percent increase or decrease in respective parameter level; * P < 0.05; ** P < 0.001 when compared with control at the respective time interval.

reduced serum triglyceride level by 11.7% in the positive control group.

Serum cholesterol level

At a dose of 200 mg/kg, all the fractions significantly ($p < 0.001$) lowered serum cholesterol level at day 21 of the study period. The percent reduction in serum cholesterol level in AFSF, MF_1SF and MF_2SF groups were 41.13, 40.16 and 39.96, respectively. The percent reduction of glibenclamide was 44.3. The statistically significant ($p < 0.01$) antihypercholesterolemic effect of the fractions was comparable to that of the reference drug.

Serum total protein level

At a dose of 200 mg/kg, all the fractions showed a significant increase in serum total protein level by 65.0, 43.18 and 41.14%, respectively. In glibenclamide treated group, the increase was 48.7%. Though the statistically significant effect of the fractions and reference drug were comparable to each other, the effect of AFSF was greater than that of the other two fractions and glibenclamide.

Serum insulin

In AFSF, MF_1SF and MF_2SF groups, a significant ($p < 0.001$) increase in serum insulin level was observed after 21 days. The percent increase in serum insulin level was 68.1 and 64.6 and 71.8 for AFSF, MF_1SF and MF_2SF, respectively, which was very close to the effect shown by the reference drug, glibenclamide.

Several plants with proven hypoglycaemic effects have been reported to contain compounds like terpenoids[27], glycosides[28,29], alkaloids[30] and saponins.[31] Preliminary phytochemical screening indicated the presence of one or more of these classes of compounds in the fractions studied. The observed hypoglycaemic effect may be a result of individual compound or a combination of these constituents. The hypoglycaemic activity exhibited by these fractions could be due to one or more of the following. The fractions may: (1) potentiate pancreatic secretion of insulin from β-cell of islets of Langerhan's (2) simulate uptake of glucose by peripheral tissues (3) inhibit endogenous glucose production (4) stimulate gluconeogenesis in liver and muscles.

There is an association between diabetes and hyperlipidemia. Insulin is responsible for activation of the lipolytic enzyme lipoprotein lipase which hydrolyses triglyceride under normal conditions. Therefore, destruction of β-cells results in decreased plasma insulin and ultimately hyperlipidemia. Significant control of plasma lipid levels by the tested fractions suggests that they produce the action by possibly improving secretion of insulin.[32] The effect of the fractions on cholesterol and triglyceride levels may be due to decrease in activity of enzymes involved in cholesterol biosynthesis or low levels of lipolysis which are in turn controlled by insulin.[33] The above findings confirm that the tested fractions have antidiabetic activity and are capable of correcting the altered biological parameters.

CONCLUSION

As the percentage of diabetic toll increases day by day, there is an urgent need for natural antidiabetics and hypoglycaemic agents to be explored. In the present study, the various column fractions obtained from the bark extract of *S. febrifuga* showed significant hypoglycaemic and antihyperglycaemic activites in normal healthy and alloxan-induced diabetic rats, respectively. At a dose of 200 mg/kg, the 20% chloroform in acetone eluate showed the maximum activity that was comparable to that of glibenclamide. It can therefore be concluded that there is a possibility of getting effective compounds from the bark extract of *S. febrifuga*, which can be of value in the fight against diabetes. Needless to say that further *in vivo* activity and chronic toxicity studies are required before determining the possible therapeutic value of the plant.

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