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Original article

Anti-diabetic activities of (part) and fractions of *Stereospermum tetragonum* DC

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ABSTRACT

Objectives: To identify the active principles, determine the anti-diabetes activity of fraction of *Stereospermum tetragonum* root.
Materials and Methods: The efficacy was evaluated in streptozotocin induced type 2 diabetic rats and the anti-hyperglycemic activity was studied by glucose tolerance test. The major active compounds were isolated by solvent fractionation and chromatographic techniques and characterized with spectral data.
Results: The active fraction of *S. tetragonum* showed presence of anti-diabetes mellitus activity in type-2 diabetic rats. It did not significantly influence insulin release from cultured islets. Two active principles (active at 2 mg/kg dose) were isolated and characterized with spectral data. One of them was identified as an iridoid type glycoside and the other one was a lapachol like compound (derivative of naphthoquinone).
Conclusions: Two active principles from the anti-diabetes fraction of *S. tetragonum* root were isolated and identified as an iridoid glycoside and a naphthoquinone derivative.
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1. Introduction

Diabetes mellitus (DM) is a major disease affecting nearly 10% of the adult population of the world. In the recent past, many hypoglycemic agents are introduced; still DM and its complications continue to be a major medical problem.[1] There are many plants used in traditional medicine to treat DM, but almost all of these plants are not widely acceptable and satisfactory for treating DM. Many more plants are used in local health traditions, especially in rural areas, to treat DM. These plants are not known to the mainstream population.[2,3] So there is an urgent need to search and find out such plants and to determine their utility to develop valuable therapeutic agents with modern standards of safety and efficacy. In this connection, previous studies carried out in this laboratory using experimental animal models have shown anti-DM activity of *Artemisia pallen*,[4] *Cassia kleinii*,[5] and *Hemionitis arifolia*.[6] Recent preliminary studies have showed promising anti-hyperglycemic activity of the roots of *Stereospermum tetragonum* DC. (family: Bignoniaceae) in fed, not fasted, rats.[7] This plant root is used in folk medicine to treat DM in certain remote villages of Tamil Nadu, India. Hence the present study was carried out to determine the efficacy of the active fraction of *S. tetragonum* against streptozotocin-induced type-2 DM and to identify its active principles.

2. Materials and methods

2.1. Collection of plant materials

S. tetragonum root (family: Bignoniaceae) was collected from Tirunelveli and Chengalpatu districts of Tamil Nadu, India and identified by the taxonomist of TBGRI and a voucher specimen (TBGRI 8282) has been deposited in the institute herbarium.

2.2. Animals

Inbred Wister rats (150–200 g weight), reared in TBGRI animal house were used. Animals were caged in uniform hygienic conditions and fed with standard pellet diet (Lipton Indian Ltd, Bangalore) and water *ad libitum* as per the guide lines of Institute Animal Ethics Committee (IAEC). IAEC is controlled by Committee for the Purpose of Control and Supervision of Experiments on Animals [Approval No: B-31/03/2010/PPD-7 dated 31-03-2010].

2.3. Isolation of an active fraction (AF)

The water extract of *S. tetragonum* root powder was precipitated with absolute ethanol (1:1 v/v) and separated in to precipitate and soluble fractions. The activity was found in the soluble fraction.[7]

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This active fraction (AF) was used for efficacy evaluation against streptozotocin-induced type-2 diabetic rats and mechanism of action studies.

2.4. Determination of the efficacy of AF

Streptozotocin-induced type-2 diabetic rats were produced by injecting 80 mg/kg streptozotocin (i.p.) in citrate buffer on the 5th day of their birth and this model is called neonatal-5 STZ induced rat model of type-2 DM.[8] Eight weeks later, blood samples were drawn and glucose levels were determined to confirm induction of diabetes. The diabetic rats which showed blood glucose levels in the range of 11.5–12.0 mmol/L were selected for efficacy evaluation of the herbal drug.[9]

To evaluate the efficacy, the streptozotocin diabetic rats were divided into three groups of six each. The diabetic control group was given 1.5 mL of water, p.o., daily. The test group was given daily dose of AF (25 mg/kg). This dose was found to be the optimum dose in our glucose tolerance test (GTT). The third group received glibenclamide (0.5 mg/kg). Weight and sex matched six normal rats were kept as control group (4th group). Treatment was continued for 21 days. Blood samples were collected on days 1, 7, 14 and 21. On day 21, animals were killed after blood collection and liver samples were removed for glycogen estimation.[6]

2.5. Estimation of serum biochemical parameters

Serum glucose was estimated spectrophotometerically using a commercial assay kit (Monozyme, India ltd). Liver glycogen was estimated as described.[6] Lipid peroxides in blood serum of streptozotocin diabetic rats was estimated as per Okhawa et al.[10] Reduced glutathione in the serum was estimated as per standard method.[11] Vitamin C in the serum was estimated as described.[12]

2.6. Glucose tolerance test (GTT)

Normal fed rats were divided into indicated number of groups. Control group received the vehicle. The experimental groups received indicated doses of AF, sub-fractions or isolated compounds in an identical manner. Thirty minutes after herbal drug or vehicle (control) administration, the rats of all the groups were loaded with 60% glucose (3 g/kg, p.o.). Blood samples were collected by retro-orbital puncture under anesthesia,[13] just 1 min prior to herbal drug administration, and at 30 and 90 min after glucose loading. Serum glucose levels were measured immediately. Six fed animals were used in each group.[7]

2.7. Determination of the effect of AF on the release of insulin from rat pancreatic β-cells

Isolation of rat pancreatic β-cell islets and insulin release assay with or without the AF were carried out as per the method of Bhonde et al.[14]

The islets cultured for 48 h in RPMI-1640 medium supplemented with 10% fetal calf serum were checked for viability by Trypan blue exclusion method.

2.7.1. Insulin release assay

Ten cultured viable β-cell islets were placed in each of the well in a 24 well plate in such a manner to get triplicate of six groups. All these wells contained 1 mL of KRBH (pH-7.4), supplemented with 2.75 mmol/L of glucose. The islets were pre-incubated for 1 h at 37 °C in a CO_2 incubator, gassed with 5% CO_2 in air and supernatants were separated. After pre-incubation, the six groups were incubated again for 1 h with 1 mL of KRBH with or without glucose and *S. tetragonum* (AF). The first group (control) of β-cell islets was incubated in 1 mL of KRBH. The second group (glucose control group) was incubated in 1 mL of KRBH with 16.5 mmol/L glucose. Third and fourth groups of islets were incubated in 1 mL of KRBH and 10 and 40 μg/mL AF respectively. Fifth and sixth groups were incubated as third and fourth groups but with 16.5 mmol/L glucose. After incubation, the islets were spun down and supernatants were collected. The insulin released in the supernatant was measured by radio-immuno-assay method.[15]

2.8. Isolation of active principles

2.8.1. Separation of AF into 3 sub-fractions

The AF was subjected to silica gel-G (E. Merck Ltd. Mumbai) preparative thin layer chromatography (TLC) using butanol:acetic acid:water (8:2:2 v/v) as solvent system. [When the chromatograms were sprayed with ansaldehyde sulfuric acid reagent and heated at 110 °C for 5 min, the AF was resolved into 10 components]. The components separated on large scale preparative TLC plates were grouped into 3 sub-fractions namely sub-fraction-1 (bottom 3 bands/components), sub-fraction-2 (middle 4 bands), and sub-fraction-3 (top 3 bands). These three groups of bands were eluted separately with methanol and filtered free of silica gel and dried.[16] The three sub-fractions were tested for their glucose lowering activity by GTT in rats. [The first and third fractions showed significant glucose lowering effect].

2.8.2. Separation of active compounds from sub-fractions 1 and 3 by column chromatography

The sub-fraction-1 (3.2 g) was subjected to column chromatography [50 cm × 25 mm] using 70 g silica gel (60–120 mesh size) and eluting with increasing polarity of solvents. The sub-fraction was eluted with 100% chloroform [100 mL × 5], chloroform:methanol (95:5 v/v) [100 mL × 5], chloroform:methanol (90:10 v/v) [100 mL × 5], chloroform:methanol (85:15 v/v) [100 mL × 5], chloroform:methanol (80:20 v/v) [100 mL × 5], chloroform:methanol (75:25) [100 mL × 5], chloroform:methanol (60:40) [100 mL × 5], chloroform:methanol (40:60) [100 mL × 5], chloroform:methanol (25:75) [100 mL × 5] and methanol (100%). About 100 mL fractions were collected. The fractions collected were monitored by silica gel 60 F_{254} TLC. Similar fractions as judged from TLC were combined together. A pure compound was obtained in the fractions of chloroform: methanol (25:75, v/v). HPTLC profile of the active fraction and isolated compounds was done using CAMAG HPTLC system equipped with sample applicator LINOMAT- V, Scanner III and integration software CATS 4.04.

The sub-fraction-3 (2.4 g) was subjected to column chromatography [50 cm × 25 mm] using 70 g Merck silica gel (60–120 mesh size) and eluting with increasing polarity of solvents. The eluting solvents were 100% chloroform [100 mL × 5], chloroform:methanol (95:5 v/v) [100 mL × 5], chloroform:methanol (90:10 v/v) [100 mL × 5] and chloroform:methanol (85:15 v/v) [100 mL × 5]. About 100 mL fractions were collected. The fractions collected were monitored by silica gel 60 F_{254} TLC. Silica gel plates were developed using butanol:acetic acid:water (8:2:2 v/v) as the mobile phase.

1.7 g of fractions eluted in chloroform:methanol (85:15 v/v) were again loaded into a packed silica gel (60 g; 60–120 mesh size) column (75 cm × 12.5 cm). The eluting solvents or solvent mixtures (increasing polarity) were (100%) chloroform [25 mL × 5], chloroform:methanol (95:5 v/v) [25 mL × 5], and chloroform:methanol (93:7 v/v) [25 mL × 33]. About 25 mL fractions were collected. The eluted fractions were monitored by silica gel 60 F_{254} TLC as above. Similar fractions were combined together, allowed to dry and weighed.

1.2 g of fractions eluted in chloroform:methanol (93:7 v/v) were again loaded to a packed silica gel (40 g; 60–120 mesh size) column (75 cm × 12.5 cm). The eluting solvents were 100% chloroform

[25 mL × 3], chloroform:methanol (98:2 v/v) [10 mL × 5], chloroform:methanol (90:10 v/v) [10 mL × 5] and chloroform:methanol (80:20, v/v) [25 mL × 10]. About the same volume of solvents as poured was collected as fractions. The fractions collected were monitored by silica gel 60 F_{254} TLC. A pure compound was obtained in chloroform:methanol (80:20, v/v) fractions.

2.8.3. Characterization of the active principles

All the IR spectra were measured using a Nicolet 170SX. Liquid samples were measured with liquid film method, and solid samples were measured by using KBr disc and Nujol paste methods.

^{1}H NMR was measured with a JEOL AL-400 (399.65 MHz). The measuring conditions for the most of the spectra were as follows: flip angle of 22.5–30.0°, pulse repetition time of 30 s. The long pulse repetition time and small flip angle were used to ensure precise relative intensities. The ^{1}H NMR chemical shifts were referred to TMS in organic solvents and TSP in D_2O.

^{13}C NMR was measured with a Bruker AC-200 (50.323 MHz). The measuring conditions for the most of the spectra were as follows: a pulse flips angle of 22.5–45°, a pulse repetition time of 4–7 s, and a resolution of 0.025–0.045 ppm. The spectra whose spectral codes started with "CDS" were reconstructed from peak positions, intensities and line widths by assuming all resonance peaks were Lorenz lines. The chemical shift was referred to a TMS for all solvents.

Measurements were performed by LC-MS with ESI probe. The isolated compound was injected into an end capped C_{18} reverse phase column (250 mm × 4.0 mm, 5 μm) [E. Merck] and eluted at a flow rate of 0.5 mL/min in isocratic mode with a mobile phase consisted of methanol and water (90:10 v/v) by keeping MS scan range 50–850 AMU. The mass peaks were observed respective to their m/z values. Drying gas flow was adjusted at 10 L/h and temperature was fixed at 250 °C.

2.9. Statistical analysis

Statistical comparisons were done using one-way analysis of variance (ANOVA) followed by Dunnett's test. P values <0.05 were considered significant.

3. Results

3.1. Effect of AF on streptozotocin-induced type-2 diabetic rats

As given in Fig. 1, the active fraction from *S. tetragonum* (AF) showed significant anti-DM activity in streptozotocin-induced type-2 diabetic rats. The daily drug administration at a dose of 25 mg/kg gradually decreased the serum glucose levels from 11.7 to 8.2 mmol/L in 21 days. In untreated diabetic control rats, the glucose levels increased from 11.9 to 14.6 mmol/L during the same period. The effect was almost comparable to that of 0.5 mg/kg glibenclamide.

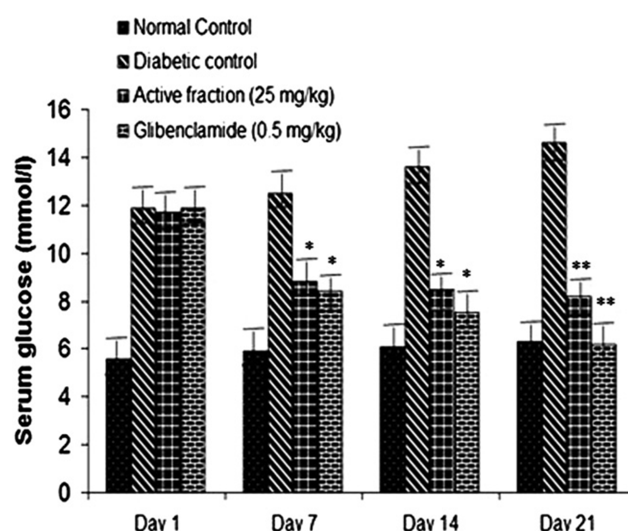


Fig. 1. Effect of active fraction of *S. tetragonum* on the levels of glucose in streptozotocin-induced diabetic rats. Values are mean ± S.D.; $n = 6$, *$P < 0.05$, **$P < 0.001$ (compared to diabetic control).

The body weight loss and drastic reduction in liver glycogen observed in the diabetic animals were restored, to a large extent, by the herbal drug administration (Table 1). AF (25 mg/kg) administration restored the altered levels of serum biochemical parameters of diabetic animals, almost to the normal levels seen in normal control rats. The serum cholesterol and triglyceride levels were substantially improved in AF treated diabetic animals. Vitamin C and glutathione levels were markedly decreased in the diabetic rats. AF treatment resulted in substantial improvement in the levels of vitamin C and glutathione (Table 1). The elevated levels of serum lipid peroxides seen in the diabetic animals were reduced to marginally below normal levels by AF treatment (Table 1). It is of interest to note that the drug decreased both oxidative stress and hyperlipidemic conditions seen in diabetic rats. These conditions are responsible to a large extent for the cardiovascular complications associated with diabetes.

This study reports for the first time the anti-DM (type-2) activity of the active fraction (AF) isolated from *S. tetragonum* root. The anti-DM activity of the active fraction (25 mg/kg) was almost comparable to that of glibenglamide (500 μg/kg). We have already shown that the active fraction has activity against alloxan-induced diabetes in rats.[7] Thus, the AF is effective in both alloxan-induced DM and streptozotocin-induced type-2 DM.

Table 1
Effect of active fraction from *Stereospermum tetragonum* on body weight, liver weight, liver glycogen and serum biochemical parameters in streptozotocin-induced diabetic rats.

Parameters	Normal control	Diabetic control	Diabetic treated with active fraction (25 mg/kg)	Diabetic treated with glibenclamide (0.5 mg/kg)
Final body weight (g)[a]	227.3 ± 7.8	139.3 ± 9.1	213.7 ± 7.1**	203.3 ± 10.1**
Liver weight (g)	4.94 ± 0.95	6.90 ± 0.18	6.09 ± 0.12*	5.69 ± 0.22**
Liver glycogen (mg/g wet tissue)	11.98 ± 1.46	5.19 ± 0.13	10.74 ± 0.15**	11.09 ± 0.22**
Cholesterol (mmol/L)	2.50 ± 0.4	3.57 ± 0.04	2.96 ± 0.05*	2.69 ± 0.04**
Triglycerides (mmol/L)	6.10 ± 0.19	17.35 ± 0.17	9.57 ± 0.22**	9.58 ± 0.17**
Lipid peroxides (μmol/L)	4.28 ± 0.32	5.91 ± 0. 33	3.85 ± 0.20*	4.05 ± 0.19*
Glutathione (mmol/L)	0.91 ± 0.05	0.56 ± 0.04	0.79 ± 0.03*	0.82 ± 0.04*
Vitamin C (μmol/L)	128.1 ± 10.0	45.1 ± 2.5	101.0 ± 6.3**	106.3 ± 3.5**

Values are mean ± S.D.; $n = 6$; *$P < 0.05$, **$P < 0.001$ (compared to diabetic control) [One-way ANOVA followed by Dunnett's post hoc comparison was done].
[a] Initial body weight was not significantly different among different groups.

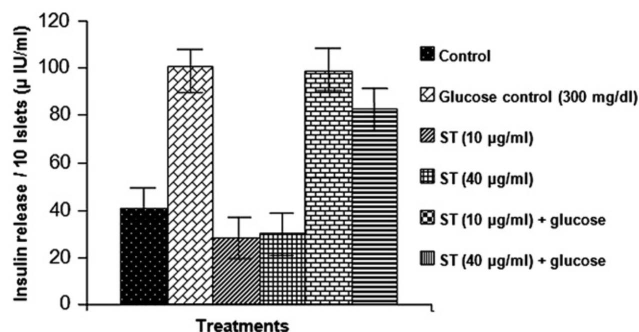


Fig. 2. Effect of active fraction of *S. tetragonum* on insulin release from isolated and cultured pancreatic β-cell islets. Values are mean ± S.D.; $n = 3$; insulin values in glucose added groups are significantly different compared to control, $P < 0.001$.

Table 2
Effect of different routes of administration of the active fraction as well as glucose on glucose tolerance in fed normal rats.

Serum glucose levels (mmol/L)			
Treatment	Time (min) after glucose administration.		
	0 (initial)	30	90
Control (oral glucose)	5.31 ± 0.30	7.81 ± 0.50	6.27 ± 0.22
Active fraction (25 mg/kg; oral) + oral glucose	5.49 ± 0.26	6.26 ± 0.8**	5.65 ± 0.33*
Active fraction (25 mg/kg; I.P.) + oral glucose	5.43 ± 0.25	6.19 ± 0.47**	5.76 ± 0.26*
Control (I.P. glucose)	5.30 ± 0.31	7.86 ± 0.52	6.18 ± 0.29
Active fraction (25 mg/kg; oral) + I.P. glucose	5.28 ± 0.28	7.42 ± 0.59	6.25 ± 0.39

Values are mean ± S.D.; $n = 6$; **, $P < 0.001$, *, $P < 0.05$ (compared to respective control values, Student's *t* test); I.P., intraperitoneal.

3.2. Effect of AF on insulin release from cultured pancreatic β-cell islets

As shown in Fig. 2, glucose markedly stimulated insulin release into the medium from cultured pancreatic β-cell islets. AF did not influence the insulin release at the concentrations (10–40 μg/mL) studied both in the presence or absence of high level of glucose (16.5 mmol/L).

3.3. Effect of chemical fractions and principles on glucose tolerance in glucose loaded normal rats

As shown in Table 2, AF was found to be effective in glucose tolerance test (GTT) when administered orally as well as intra-peritoneally. But, it did not influence substantially the levels of serum glucose in intra-peritoneally glucose loaded rats, in contrast to oral glucose administration to fed rats. Thus, it appears that glucose absorption/transport from the intestinal tract is inhibited by the AF. It can be assumed that the herbal drug directly or indirectly inhibits, to a large extent, glucose absorption or transport from the intestine.

The AF from *S. tetragonum* root was resolved into 10 components on TLC and HPTLC (Fig. 3 A and B). Using large scale preparative silica gel-G TLC, AF was separated in to three sub-fractions (Fig. 3 A) and the percentage of yield of sub-fraction-1 (SF-1), SF-2 and SF-3 were about 29, 39, 19.3% respectively of AF. When all these three sub-fractions were tested for glucose lowering activity in fed and orally glucose loaded normal rats, at the dose of 25 mg/kg body weight each, SF-1 and SF-3 showed significant glucose lowering activity. SF-2 did not show any reduction in serum glucose levels (Table 3).

The major active compounds in SF-1 and SF-3 were isolated by column chromatography in pure forms (Fig. 3 A and B). The compound isolated from SF-1 (C1) and SF-3 (C2) showed significant anti-hyperglycemic activity at a relatively low concentration (2 mg/kg body weight) in GTT on fed and glucose loaded normal rats (Table 3).

3.4. Spectral analysis and elucidation of structures of the compounds

Compound 1 (C1): IR (KBr): 3400 (O–H str.), 2900 (C–H aliphatic str.), 1000 (C–O str.) cm^{-1}. MS: m/z 437 (M^{+}. 432 + 5) of $C_{19}H_{28}O_{11}$ ^{1}H NMR (CD_3OD 400 MHz). δ 8.52 (s, 1 H, C_4–COOH), 5.169 (s, 1 H, C_3–H), 5.162 (s, 1H, C_6–H), 5.14 (d, 1H,C_1–H), 4.70 (s, 1H, C'_2–H) 4.68 (brd.1H, C_{7a}–H), 4.55 (t, 1H, C'_3–H), 4.14 (s, 1H, C'_6–CH_2–OH), 4.12 (s, 1H, C_7–CH_2–OH) 4.08–4.03 (m, 3H, $C'_{45\&6}$–H), 3.95 (s, 1H, C_5–OH) 3.79 (s, 3H, C'_3–OCH3), 3.78 (s, 3H, C'_4–

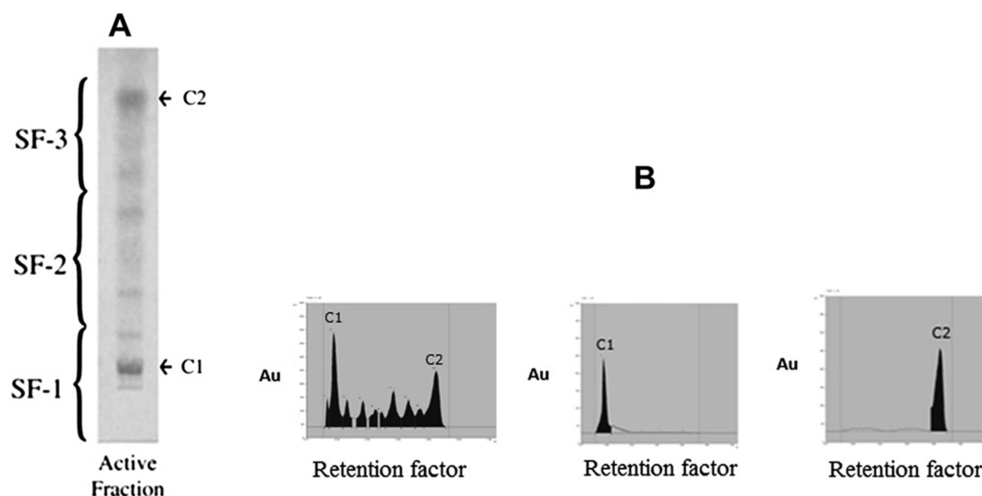


Fig. 3. Thin layer chromatographic separation of active fraction (AF) and isolated compounds from *S. tetragonum* root. A. TLC separation of AF from the water extract of *S. tetragonum* using silica gel 60 F_{254} and the solvent system, butanol:aceticacid:water (8:2:2). Visualization done by spraying with anisaldehyde sulfuric acid reagent. AF, active fraction; SF-1, SF-2, SR-3, sub-fractions; C1, compound 1 (lower compound); C2, compound 2 (upper compound). B. HPTLC profile of active fraction showing 10 peaks and compound 1 and 2 from the AF at 580 nm. The HPTLC was done on precoated aluminum plates of silica gel 60 F_{254} (Merck). The Rf value of compound 1 was 0.09 and that of compound 2 was 0.69. AU, arbitrary units.

Table 3
Effect of chemical fractions and principles isolated from the active fraction on glucose tolerance in fed and glucose loaded normal rats.

Serum glucose levels (mmol/L)			
Treatment	Time (min) after glucose administration		
	0 (initial)	30	90
Control	5.80 ± 0.24	7.81 ± 0.59	6.77 ± 0.30
Active fraction (25 mg/kg)	5.62 ± 0.31	6.47 ± 0.56**	5.85 ± 0.36*
Sub-fraction-1 (25 mg/kg)	5.96 ± 0.24	6.07 ± 0.46**	5.44 ± 0.26*
Sub-fraction-2 (25 mg/kg)	5.85 ± 0.29	8.16 ± 0.25	6.95 ± 0.25
Sub-fraction-3 (25 mg/kg)	5.99 ± 0.26	6.13 ± 0.27**	5.39 ± 0.19*
Compound 1(2 mg/kg)	5.64 ± 0.29	6.21 ± 0.26**	5.63 ± 0.34*
Compound 1 (5 mg/kg)	5.63 ± 0.24	6.04 ± 0.37**	5.50 ± 0.26*
Compound 2 (2 mg/kg)	5.66 ± 0.34	6.40 ± 0.40*	5.88 ± 0.29
Compound 2 (5 mg/kg)	5.74 ± 0.29	6.18 ± 0.05**	5.58 ± 0.24*

Values are mean ± SD, $n = 6$ in all groups except in control where $n = 12$; $^*P < 0.05$, $^{**}P < 0.001$(Compared to control); 0 min, initial glucose level just before drug administration; 30 min and 90 min, time after glucose loading [GTT for sub-fractions and the isolated compound were done separately. Since the control values for the 2 sets of experiments did not differ significantly they were combined].

OCH3), 3.73 (s, 3H,C'_5—OCH3), 3.67 (s, 2H, C_7—CH_2—OH) and 3.64 (d, 2H, C'_6—CH_2—OH) ppm.

^{13}C NMR (CD_3OD 400 MHz): δ 151.90 (C, C_4—COOH) 138.56 (C,C—3), 112.37(C,C—1), 98.67 (C,C—6),97.87(C,C—7), 97.00 (C,C—2′), 81.87 (C,C—7), 76.83(C,C—5), 74.70 (C, C—4′), 70.99(C,C—5′),70.43 (C, C—4a), 69.80(C, C—3′) 69.01(C,C—6′) 68.81(C,C—7a) 64.43(C,C'_3—OCH_3),63.13(C,C'_4—OCH_3), 61.25 (C,C'_5—OCH_3), 29.34(C_7—CH_2—OH) and 22.69 (C,C'_6 —CH_2—OH) ppm.

^{1}H NMR (D_20, 400 MHz): δ 4.14 (s, 1H, C'_6—CH_2—OH), 4.11 (s, 1H, C_7—CH_2—OH), 3.97—3.96 (d, 1H, C_5—OH), 3.93 (s, 1H, C'_3—H) and 3.90—3.88 (brd.1H, C'_5—H).

The molecular formula of C1 was deduced as $C_{19}H_{28}O_{11}$ by the analysis of the spectra. C1 was an iridoid glycoside, 1,4a,5,7a-tetrahydro-5-hydroxy-7-(hydroxymethyl)-1-(tetrahydro-6-(hydroxymethyl)-3,4,5-trimethoxy-*2H*-pyran-2-yloxy)cyclopenta[c]pyran-4-carboxylic acid. The structure of this active principle (C1) is shown in Fig. 4.

Compound 2 (C2): IR (KBr): 3400 (O—H str.), 2380 (C—H of—CH_2— str.), 1600 (C—H $_{(aromatic)}$ str.) and 1250—1300 (C—O—C str.) cm^{-1}. MS: m/z 371. (M^+. 368 + 3) of $C_{19}H_{28}O_7$.

^{1}H NMR (DMSO—d_6 400 MHz). δ 7.46 (s, 1 H, C_1—OH), 7.40 (s, 1 H, C_4—OH), 7.35—7.32 (d, 1H, C_6—OH), 6.89—6.81 (m, 1H,C_5—H), 6.75—6.57 (m, 1H, C_8—H) 3.73 (s, 6H, C_2 —& C_3—OCH_3), 3.69 (s, 3H, C_5—OCH_3) 3.67 (s, 3H, C_8—OCH3), 3.54—3.29 (m, 5H, $C_{11,12\&13}$—H) and 15.17 (d, 6H, C_{13}—CH_3)ppm.

^{13}C NMR (DMSO—d_6 400 MHz): δ 129.60 (C,C—1&4) 125 (C,C—2&3), 115 (C,C—9&10), 79.25 (C,C—6), 73.13 (C,C—7), 69.93 (C,C—5&8), 64.87(C,$C_{2\&3}$—OCH_3), 58.69 (C,C_5—OCH_3), 56.58(C,C_8—OCH_3), 56.29 (C,C—13), 50.40 (C,C—12), and 15.17(C,C—14&15) ppm.

Based on the spectra the second active principle, compound 2 (C2) was a naphthoquinone derivative, 5,8-dihydro-7-isopentyl-2,3,5,8-tetramethoxynaphthalene-1,4,6-triol. The structure of this active principle (C2) is shown in Fig. 4.

The scanned spectra of compound 1 and 2 are given as Supplementary information (Fig. 5 and 6).

4. Discussion

The iridoid glycoside isolated in the present study is a new molecule, not known previously. The other active compound was a derivative of naphthoquinone, 5,8-dihydro-7-isopentyl-2,3,5,8-tetramethoxynaphthalene-1,4,6-triol. This molecule is also a new molecule, not reported previously. The existence of other minor active principles in the AF cannot be ruled out. It is of interest to note that the novel isolated compounds showed *in vivo* anti-hyperglycemic activity at a very low concentration (2 mg/kg).

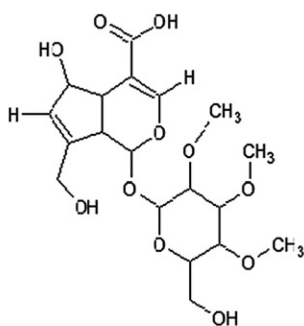
The herbal drug is effective in orally glucose loaded, fed rats, but not in fasted rats.[7] The herbal drug showed anti-hyperglycemic effect, not hypoglycemic activity.

Therefore, in fed rats the herbal drug (AF) may be stimulating the release of one or more known or unknown factors from the gastro-intestinal tract which may block absorption of glucose directly or indirectly from intestinal tract. This likely mechanism of action remains to be studied.

The herbal drug is likely to be useful as an anti-DM medicine. It appears to be non-toxic. In the traditional medicine, there is no reported or known toxicity to the herbal drug. Further, in the preliminary toxicity evaluation in rats, the active fraction did not exhibit any toxicity.[7] However, toxicity to long time treatment of the active fraction remains to be studied. Although there are many drugs to treat type-2 diabetes in the market, in the long term treatment, immune response dependent or other toxicity develops; the drug becomes ineffective in some cases. In this context, this herbal drug is likely to be useful as an alternative medicine or as a combination drug. There is a need to carry on further studies leading to likely development of the active fraction as a standardized, safe and effective phytomedicine. The active compounds are promising molecules for the development of conventional chemical entity drugs.

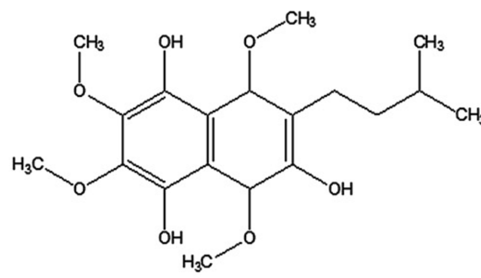
In conclusion, the active fraction (AF) from *S. tetragonum* DC. showed anti-diabetes activity against streptozotocin-induced type-

Structure of compound 1



1,4a,5,7a-tetrahydro-5-hydroxy-7-(hydroxymethyl)-1-(tetrahydro-6-(hydroxymethyl)-3,4,5-trimethoxy-2*H*-pyran-2-yloxy)cyclopenta[*c*]pyran-4-carboxylic acid

Structure of compound 2



5,8-dihydro-7-isopentyl-2,3,5,8-tetramethoxynaphthalene-1,4,6-triol

Fig. 4. Structures of compounds 1 and 2.

2 diabetic rats. In contrast to oral glucose administration, when glucose was loaded intra-peritoneally to normal rats, the AF did not exhibit anti-hyperglycemic activity in glucose tolerance test. The AF did not significantly influence insulin release from cultured rat pancreatic islets. The major mechanism of action could be indirect inhibition of glucose absorption or transport from intestine. Two active principles (active at 2 mg/kg body weight) were isolated and characterized with spectral data. These active molecules are very promising for further studies leading to the development of a valuable medicine for mono-therapy and/or combination therapy for diabetes.

Conflicts of interest

All authos have none to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jyp.2012.09.002.

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