

Effect of *Stereospermum tetragonum* DC. in alloxan induced diabetic rats

Sir,

A larger percentage of the rural population depends on herbal drugs to control diabetes mellitus (DM). More than 100 plants occurring in India are shown to have varying levels of hypoglycemic activity.^[1] Studies from this laboratory have brought to light the anti-DM properties of traditional medicinal plants such as *Artemisia pallens*,^[2] *Cassia*

kleinii,^[3] and *Hemionitis arifolia*.^[4] However, there are only a few satisfactory herbal drugs to treat DM. A search for novel, effective and inexpensive anti-DM drugs from plant resources is likely to be successful. While screening ethno-medical plants for anti-hyperglycemic property, *Stereospermum tetragonum* DC. [family: Bignoniaceae] showed promising activity. *S. tetragonum* root is used in folk medicine to treat DM in certain remote villages in the state of Tamilnadu. This study was undertaken to scientifically validate the traditional claim and to get some insights regarding the utility of this plant to treat DM.

S. tetragonum root was collected from Tirunelveli district of Tamilnadu and identified by taxonomists of Tropical Botanic Garden and Research Institute (TBGRI) and a voucher specimen (TBGRI 8282) has been deposited. The water, alcohol, and *n*-hexane extracts of the plant root powder was prepared as reported.^[4] The yield of the water extract (active extract) was 9% of the root powder.

Inbred Wistar rats (150–200 g weight) reared in TBGRI under standard conditions as per the guidelines of Institute Animal Ethics Committee were used.

The glucose tolerance test (GTT) was used to investigate the glucose lowering effect of the extracts and an active fraction in fed as well as overnight fasted rats.^[4] Blood samples (approximately 100 μ l) were collected by retro-orbital puncture under mild ether anesthesia, just 1 min prior to extract administration, and at 30 and 90 min after glucose loading. Serum was separated under cold conditions, and glucose levels were measured using an assay kit (Monozyme, India Ltd).

The water extract of the plant was precipitated with ethyl alcohol (1:1 v/v), and the alcohol soluble fraction was found to be the active fraction.

Male Wistar rats (190–210 g weight) were made diabetic with alloxan (110 mg/kg, i.p.) injection.^[2] The diabetic rats which showed blood glucose levels in the range of 16.5–19.25 mmol/l were selected for the experiments. The efficacy of the active fraction against these diabetic rats was determined.^[4] Active fraction (25 mg/kg) or insulin was administered daily for 12 days (25 mg/kg active fraction was found to have optimum anti-hyperglycemic activity in GTT). Blood samples were collected 1 h after administration of the active fraction or insulin. On 12th day, animals were killed after blood collection.

To evaluate subacute (short-term) toxicity, four groups of mice, each containing six male mice (20–25 g weight) were used. The active fraction (100, 200, or 400 mg/kg) was administered (p.o.) to the test groups, daily for 28 days and toxicity was evaluated as reported.^[4]

In GTT, the water extract (50 mg/kg) showed significant glucose lowering activity at 30 and 90 min after glucose loading in normal fed rats. The alcohol and hexane extracts of these plants were almost inactive (data not shown). The water extract showed optimum blood glucose level lowering activity at 50 mg/kg in fed rats, not in the fasted rats.

The active fraction (25 mg/kg) obtained by alcohol precipitation of the water extract showed significant anti-hyperglycemic activity in GTT. [Serum glucose levels (mmol/l) 30 min after glucose administration: control 9.6 ± 0.5 ; 25 mg/kg active fraction 6.0 ± 0.1 ($P \leq 0.001$)]. The active fraction was resolved into 10 spots on HPTLC separation using butanol:acetic acid:water (8:2:2 v/v) as the mobile phase [Figure 1]. The presence of coumarins in the active fraction was identified by qualitative tests.^[5]

As given in Table 1, the active fraction (25 mg/kg) showed promising anti-diabetic activity in alloxan diabetic rats. Insulin (5 IU/kg) was found to have almost the same effects. The reduction in body weight and liver glycogen, observed in the diabetic animals, were prevented to a large extent by the drug administration.

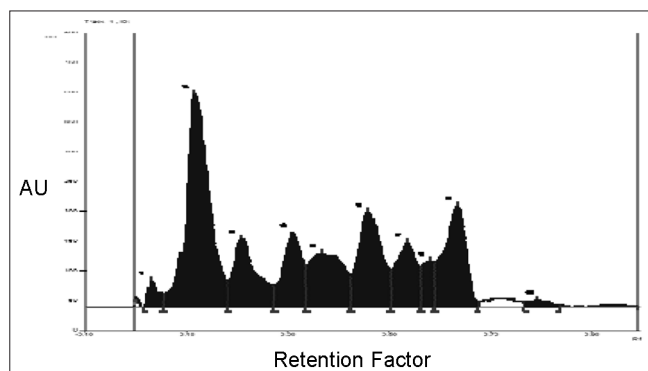


Figure 1: HPTLC profile of the active fraction from *S. tetragonum* root. HPTLC profile of *S. tetragonum* active fraction showing 10 peaks when scanned at 540 nm using CAMAG HPTLC system equipped with sample applicator Linomat V, scanner III, and integration software CATS 4.04. The TLC was done on precoated aluminum plates of silica gel 60 F²⁵⁴ (Merck) using the solvent system, butanol:acetic acid:water (8:2:2)

Table 1: Effect of the active fraction of *S. tetragonum* on blood glucose levels in alloxan-induced diabetic rats

Groups	Serum glucose levels (m mol/l)		
	1 st day	8 th day	12 th day
Normal control rats	5.2 ± 0.2	5.7 ± 0.1	5.4 ± 0.1
Diabetic control rats	16.6 ± 0.3	19.9 ± 0.2	21.0 ± 0.2
Diabetic rats treated with active fraction (25 mg/kg)	16.7 ± 0.4	$12.3 \pm 0.1^*$	$8.8 \pm 0.2^{**}$
Diabetic rats treated with Insulin (5 I.U./kg)	17.1 ± 0.3	$11.9 \pm 0.3^*$	$8.6 \pm 5.8^{**}$

Values are mean $\pm$ SD, $n=6$, $^*P<0.05$, $^{**}P<0.001$ (compared to diabetic control)

In subacute toxicity evaluation, administration of the active fraction (100, 200, or 400 mg/kg) did not show any significant effect on behavior as well as hematological and serum biochemical parameters except a slight reduction in the levels of serum glucose at higher doses.

This study shows for the first time anti-hyperglycemic and anti-DM activity of *S. tetragonum* root in rats and thus the traditional claim is scientifically verified. Unlike insulin, overdose of this herbal drug may not result in hypoglycemia.

Preliminary analysis suggests that the active compounds are two coumarins. Literature survey showed that phytochemical and pharmacological studies were not carried out on this plant.

Severe alloxan-diabetes is, to a large extent, comparable to type one diabetes with near total β -cell destruction and without insulin resistance.^[6] In this study, the blood glucose level was moderate only. Therefore, some residual β -cell function might have been there.

To conclude, *S. tetragonum* root (active fraction) is an attractive material for further studies leading to the development of safe phytomedicine or conventional medicine for diabetes.

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