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Original Article

PHARMACOLOGICAL ACTION OF THE ACTIVE FRACTION-*STEREOSPERMUM TETRAGONUM* DC

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ABSTRACT

Objective: The objective of the present study was to evaluate the mechanism of action of the active fraction of *Stereospermum tetragonum*.

Methods: An active fraction was isolated from the water extract (active extract by GTT) using ethanol precipitation. To get insight to the mechanism of action of the active fraction, glucose uptake by isolated rat diaphragm was performed. Further the effect of active fraction on glucose tolerance in fed rats was compared with that of fasted rats. Besides, effect of oral administration of the active fraction was compared with that of intra peritonial administration. The effect of active fraction on serum glucose levels in orally glucose loaded rats was compared with that of intra peritoneal glucose loaded rats.

Results: Glucose uptake in the isolated rat hemi-diaphragm, were increased by the active fraction at 40 µg/ml concentrations, the active fraction showed promising anti hyper glycemic activity in fed rats when compared with fasted rats. The active fraction was found to be effective in orally glucose loaded in contrast to intra peritonial route. The result shows that glucose absorbtion from an intestinal tract is inhibited by the active fraction.

Conclusion: Our findings suggest that this plant is promising for further studies leading to the development of valuable medicine for diabetes.

Keywords: *Stereospermum tetragonum,* Active fraction, Glucose tolerance test, Diabetes mellitus.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia arising as a consequence of a relative or absolute deficiency of insulin secretion, resistance to insulin action or both [1]. This is a major health problem throughout the world, with a worldwide prevalence of 171 million people in 2000, and is expected to increase 366 million people by 2030. This is a major and growing public health problem throughout the world, with an estimated worldwide prevalence of 171 million people in 2000, expected to increase to 366 million people by 2030. In particular, the number of people with diabetes in India, currently around 40.9 million, is expected to rise to 69.9 million by 2030 [2]. India leads the world with the largest number of diabetic subjects thus earning the dubious distinction of being termed the "Diabetes Capital of the World" [3]. The basis of abnormalities in carbohydrate, fat and protein metabolism in diabetes is deficient action of insulin on its target tissues, resulting from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action [4]. Although the prevalence of diabetes is consistently increasing, an effective treatment is still lacking. Current pharmacotherapeutics insufficiently reverse hyperglycemia, have limited tolerability, and induce side effects [5]. Hence, the identification of new pharmacological approaches to effectively prevent, treat and cure this metabolic disorder is of crucial importance. In experimental diabetes, enzymes of glucose metabolism are markedly altered and produce hyperglycemia, which leads to pathogenesis of diabetic complications. There is an urgent need to identify indigenous natural resources in order to procure them. In recent times, many traditionally used medicinally important plants have been tested for their antidiabetic potential by various investigations in experimental animals [6-8]. *Stereospermum tetragonum* is a medicinal plant that grows throughout tropical parts of Indian subcontinent, particularly in sandy soils of river beds in Northern India and other parts of Tamil nadu. It is used in folk medical practices to treat DM in certain remote villages of Thirunelveli district of Tamilnadu. In ethno-medical practices, the plant is also used as diuretic, treat anti ulcer, anti-pyritic etc. Phytochemical analysis showed the presence of tannins, phenol, glycosides, terpinoids, coumarins, in the active fraction [9]. Preliminary studies have showed promising anti-hyperglycemic activity of the roots of *Stereospermum tetragonum.* Other studies showed the active fraction showed the presence of anti-diabetes mellitus activity in diabetic rats. Two active principles were isolated and charecterised by spectral data. One of them was identified as an iridoid type glycoside and the other one was a lapachol like compound [10-12]. The present study was established for the first time to determine the mechanism of action of *Stereospermum tetragonum* with an aim to scientifically prove the traditional claim of this plant.

MATERIALS AND METHODS

Collection of plant materials

Root parts of *S. tetragonum* were collected from Tirunelveli district of Tamilnadu and identified, by Dr. Mathew Don, Taxonomist of Tropical Botanic Garden and Research Institute (TBGRI), Palode, Thiruvananthapuram, Kerala State, India. A voucher specimen, TBGRI 8282, was deposited in the herbarium of TBGRI.

Chemicals and reagents

Insulin was purchased from Knoll Pharmaceuticals Ltd., India. Glucose, Sodium chloride, Calcium chloride, Sodium bicarbonate, Sodium di-hydrogen phosphate, Magnesium chloride chemicals used was of analytical grade and purchased from E. Merck Ltd., Mumbai.

Preparation of water extract

The root parts of the plant were collected, cleaned, dried and powdered. To prepare water extract, the powder was extracted with distilled water (5 g/100 ml) by stirring for 4hours and then filtering through filter paper (Whatman No.1). This process was repeated thrice with the residue. The combined filtrate was freeze dried in a lyophilizer [12].

Animals

Inbred Wistar rats (150-200 g weight) reared in TBGRI animal house were used for *in vivo* experimentation. Animals were caged in uniform hygienic conditions and fed with standard pellet diet (Lipton Indian Ltd, Bangalore, India.) and water ad libitum as per the

guidelines of Institute Animal Ethics Committee (IAEC). IAEC is approved by CPCSEA (National Committee for the purpose of control and supervision of experiments on animals). (Approval No: B-31/03/2010/ppd-7 dated 31-03-2010)

Isolation of an active fraction

The water extract of the plant was precipitated with ethanol (1:1) and separated into precipitate fraction and alcohol soluble fraction; both of the fractions were tested for anti-hyperglycemic activity using the glucose tolerance test. Alcohol soluble fraction was found to be active when compared with precipitate fraction. The alcohol soluble fraction is taken as an active fraction. The active fraction (AF) was subjected to mechanism action studies.

Glucose tolerance test

This was done as described elsewhere [13]. Rats were divided into an indicated number of groups. Control group received the vehicle (water, 1 ml, p. o). The experimental groups received indicated doses of the active fraction in an identical manner. The rats of all the groups were loaded with 60% glucose (3 g/kg, p. o) 30 min after herbal drug (active fraction) administration. Blood samples were collected by retro-orbital puncture just 1 min prior to drug administration, and at 30 and 90 after glucose loading. Serum glucose levels were estimated spectrophotometrically using the commercial assay Kit [enzymatic method; Monozyme, India Limited] [12]. Six normal fed animals were used in each group.

Effect of active fraction on glucose uptake by isolated rat hemi-diaphragm

Glucose uptake by isolated rat hemi-diaphragm was estimated as described [14]. Briefly, fifteen over night fasted male wistar rats weighing 150-175g were sacrificed by cervical dislocation. The diaphragms were dissected out, washed in PBS and divided into two equal halves. Weight of each hemi-diaphragm was taken and grouped in to 6 groups of 6 hemi-diaphragms each. To all test tubes in the six groups, 2 ml of Tyrode's solution with 2% (w/v) glucose were added. One set, the control group received no additions. The next sets of test tubes received increasing quantity of the active fraction (10, 20 and 40 µl) each constituting Group 2,3 and 4. To group 5 test tubes were added 20 µl of active fraction and 0.4 units of insulin. The sixth group of test tubes was assigned to insulin control group to which insulin (regular) 0.4 units were added. Final volume in all groups of test tube was made up to 4 ml by adding Tyrodes solution without glucose. The isolated rat hemi-diaphragms were immersed in to these test tubes, making sure that the hemi-diaphragm from the same animal is not used twice in the same group of test tubes. All the test tubes were incubated for 30 min at 37 ^{o}C, with shaking at 140 cycles/min. Glucose uptake per gram of hemi-diaphragm was calculated as the difference between the initial and final glucose content in the incubated medium.

Effect of active fraction on glucose tolerance in fed versus fasted rats

Effect of active fraction on glucose tolerance was done both in fasted and fed normal rats for comparison. Glucose was loaded orally and active fraction was also administered orally.

Glucose tolerance in normal rats: effect of intra-peritoneal versus oral administration of the herbal drug

To compare the efficacy of oral administration of the drug with that of intra-peritoneal administration, male rats (200 to 240 g) were divided into 4 groups of 6 rats in each group. Control groups (groups 1 and 2) received 1 ml of water intra-peritoneal and oral route respectively; and experimental groups (groups 2 and 3) received intra-peritoneal and oral administration respectively of 1 ml active fraction (25 mg/kg). Thirty minutes after, the rats were orally loaded with glucose and glucose tolerance was determined as above.

Glucose tolerance test in intra peritoneal glucose loaded rats

To investigate tolerance to intra-peritoneal glucose loading, rats were divided into 2 groups of 6 each. Control group received 1 ml of water and the experimental group received orally 25 mg/kg active fraction (AF). Thirty min after oral AF administration, rats of both groups were loaded with 60% glucose (3 g/kg, p. o.) by intra peritoneal injection. Blood samples were collected at 0, 60 and 90 min after active fraction administration and glucose levels were measured.

Statistical analysis

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

RESULTS

As shown in Fig.1 under tissue culture conditions, in the isolated rat hemi-diaphragm, glucose uptake was increased by the active fraction treatment at 40 µg/ml concentration, but not at lower concentrations (10µg/ml or 20 µg/ml). Under this *in vitro* condition, insulin (0.1 IU/ml) also stimulated glucose uptake by the hemi-diaphragm. When the herbal drug (20µg/ml) and insulin (0.1 IU/ml) were added together, to a large extent, the effect of insulin was observed on glucose uptake.

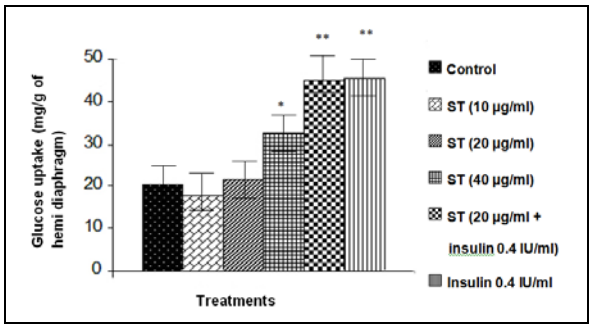

Fig. 1: Effect of active fraction of *S. tetragonum* on glucose uptake in isolated rat hemi-diaphragm

As shown in table 1, in fasted rats, in contrast to fed rats, the herbal drug did not influence significantly serum glucose levels in response to oral glucose loading.

Table 1: Effect of active fraction on glucose tolerance in fed versus fasted rats

Time (min) after glucose administration

Treatment	0 (initial)	30	90
Fed rats			
Control	5.32±0.30	8.22±0.56	6.53±0.31
Active fraction (25 mg/kg) (Oral)	5.37±0.29	6.78±0.49**	5.7±0.34*
Fasted rats			
Control	3.14±0.29	6.44±0.36	4.05±0.29
Active fraction (25 mg/kg) (oral)	3.16±0.3	6.21±0.4	4.10±0.2

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

As shown in table 2, in glucose tolerance test, intera-peritonial as well as oral route of AF administrations showed almost the same level of anti-hyperglycemic activity in orally glucose loaded rats.

Table 2: Effect of different routes of administration of active fraction of *S. tetragonum* on glucose tolerance in fed and glucose loaded normal rats

Time (min) after glucose administration

Treatment	0 (initial)	30	90
Control (oral)	5.31±0.30	9.29±0.50	7.17±0.22
Control (i. p.)	5.36±0.20	9.26±0.46	7.04±0.26
Active fraction (25 mg/kg) (Oral)	5.49±0.26	7.36±0.8**	6.05±0.5*
Active fraction (25 mg/kg) (i. p)	5.43±0.25	77.29±0.47**	5.86±0.26**

Values are mean±SD; n=6; **, P<0.001, *, P<0.05 (compared to respective control values, Student's *t* test)

As shown in table 3, AF did not influence significantly the levels of serum glucose in intra-peritoneal glucose loaded rats, in contrast to oral administration.

Table 3: Effect of active fraction on serum glucose levels in intra-peritoneal glucose loaded, fed normal rats

Time (min) after glucose administration

Treatment	0 (initial)	30	90
Control	5.30±0.31	9.22±0.52	6.97±0.29
Active fraction (25 mg/kg) (Oral)	5.28±0.28	8.42±0.59	6.55±0.39

Values are mean±SD; n=6; Values are not significantly different from respective control values (Student's *t* test)

DISCUSSION

The active fraction increased glucose uptake by isolated hemi-diaphragm at a relatively higher concentration (40µg/ml). These results suggest a direct stimulatory effect of the fraction on glucose uptake without the involvement of insulin [15]. This may be the major mechanism of action of this drug. In contrast to oral administration of glucose, active fraction did not substantially influence serum glucose levels, when glucose was loaded through an intra-peritoneal route to fed rats. Thus, it appears that glucose absorption from the intestinal tract is inhibited by the active fraction. It can be assumed that the herbal drug directly or indirectly inhibits to a large extent glucose absorption/transport from the intestine [16]. In the fasted rats, the active fraction did not significantly influence serum glucose levels even in orally glucose loaded rats. Thus, interestingly, the herbal drug showed anti-hyperglycemic effect, not hypoglycemic activity. Since the active fraction was found to be effective in orally glucose loaded, fed rats, not fasted rats, in glucose tolerance test, in fed rats the drug may be stimulating the release of one or more known or unknown factors from the gastro-intestinal tract which may block absorption/transport of glucose directly or indirectly from intestinal tract [17].

CONCLUSION

Glucose uptake in the isolated rat hemi-diaphragm, was increased by the active fraction treatment at 40 µg/ml concentration, The active fraction was found to be effective in orally glucose loaded in contrast to intraperitonial route glucose tolerance test this suggest that the drug may be stimulating the release from the gastro-intestinal tract which may block absorption of glucose directly or indirectly from intestinal tract. Active fraction did not substantially influence serum glucose levels, when glucose was loaded intra-peritoneal route. Thus it appears glucose absorption from the intestinal tract is inhibited by the active fraction.

Finally, we suggest that the active fraction of *S. tetragonum* root is an attractive material for further studies leading to the likely development of safe phytomedicine or conventional medicine for diabetes.

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CONFLICT OF INTERESTS

All authors have none to declare

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