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Ameliorate the effect of *Solanum trilobatum* L. on hepatic enzymes in experimental diabetes

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ABSTRACT

This paper deals with oral administration of leaves extract of *Solanum trilobatum* L. on blood glucose and plasma antioxidant status in streptozotocin (STZ) diabetic rats. The study was undertaken to evaluate hepatic enzymes in experimental diabetes. Oral administration of aqueous extract (100, 200mg/kg) in STZ diabetic rats increased hepatic hexokinase activity and decreased hepatic glucose-6-phosphatase, serum acid phosphatase (ACP), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). The *Solanum trilobatum* leaves extract is hypoglycemic, hepatoprotective and is able to ameliorate biochemical damages in STZ induced diabetic rats.

KEYWORDS

Solanum trilobatum;
STZ diabetes;
Hepatic enzymes;
Liver protection.

INTRODUCTION

The increasing worldwide incidence of diabetes mellitus in adults constitutes a global public health burden. It is predicted that by 2030, India, China and the United States will have the largest number of people with diabetes[1]. The vast majority of cases of diabetes fall into two broad etiopathogenetic categories. In one category, type I diabetes, the cause is an absolute deficiency of insulin secretion. In the other, much more prevalent category, type II diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin-secretory response[2,3]. To date there are different groups of oral hypoglycemic agents for clinical use, having characteristic profiles of side effects[4,5].

Despite the great interest in the development of new drugs to prevent the burden of complications associated with diabetes and the raised interest in the scientific community to evaluate either raw or isolated natural products in experimental studies, few of them were tested in humans[6,7]. Nowadays, natural supplements are widely used around the world to treat diabetes.

The *Solanum trilobatum* (Solanaceae) is a common shrub, called as 'Tuduvelai', used in various diseases distributed over Gujarat, Deccan, Ceylon, North Circars, Carnatic and Malay Peninsula[8]. In Indian Ayurveda and Siddha medicinal system, the roots and leaves are bitter and prescribed in consumptive cases of acute and chronic bronchitis[8,9], asthma[10,11], cough[12], and analgesic action[13]. The herbs are useful in treating indigestion, spermatorrhoea, tuberculosis and disease of ear[14].

Pharmacological investigations have demonstrated

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that *S. trilobatum* possess an antibacterial, antifungal & anticancer activity[15-22]; antioxidant activity[23]; hepatoprotective activity[19]; anti-ulcerogenic activity[24] and anti-inflammatory activity[25,26]. The leaves of the plant possess calcium, iron, phosphorus, fat, carbohydrates, crude fibre and minerals[27]. The whole plant contains alkaloids, phenolics, flavanoides, sterols, saponins and their glycosides[24]; solasodine and β-solamarine[28]. The present paper reports the hypoglycemic effect of *Solanum trilobatum* leaves extract and the hepatic and serum enzymes on STZ induced diabetic rats.

MATERIALS AND METHODS

Plant material

The leaves of *Solanum trilobatum* L. was collected during Jan-Feb 2003 from Palayapalayam, Namakkal district, Tamilnadu. The plant was authenticated by Fr. K. M. Matthew, Taxonomist and comparison with reference specimens preserved at the Rapinat Herbarium, St. Joseph's College, Tiruchirapalli. Voucher Herbarium specimens are kept in the Herbarium for future references.

Preparation of plant extract

Fresh leaves of *Solanum trilobatum* (500g) were washed and homogenized in a waring blender with 2 litres of distilled water. The extraction was carried out in a cold room ($20^{o} \pm 1^{o}$C) with constant stirring over night. The homogenate was then squeezed through cheese cloth and centrifuged at 1200g for 10 min at 4^{o}C. The supernatant being the *S. trilobatum* leaves extract (yield 210 w/w) was decanted and kept at 4^{o}C until used.

Chemicals

Streptozotocin (STZ) was obtained from Sigma Chemical Co, (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Animals

Albino rats of Wister strain weighing about 150-200gms were used in the study. Animals were obtained from Animal house, Bharathidasan University, Tamilnadu and kept under standard laboratory conditions in 12h light/dark cycles at 25-28^{0}C and 60-80% relative humidity. Animals were reared with robust health by providing pellet diet (Lipton, India) and water ad libitum. Six rats were housed per cage, to provide them sufficient space and to avoid unnecessary morbidity and mortality. All studies were conducted in accordance with the National Institute of Health guide[29]. The study was approved by the ethics committee CPCSEA and ethical norms were strictly followed during all experimental procedures.

Preparation of diabetic rats

STZ (Sixty mg/kg) dissolved in saline was injected to tail vein of animals intraperitoneally. After a fortnight rats with moderate diabetes having glycosuria (indicated by Benedict's test for urine) and hyperglycemia, i.e. with blood glucose levels of 200-280 mg per 100ml were used for the investigation. Blood was collected from eyes (venous pool).

Experimental design

Diabetes was induced in animals 2 weeks before starting the treatment. After the induction, diabetic rats were divided in to 5 groups of 6 animals each. Group I received vehicle alone, served as control. Group II received STZ (60mg/kg/i.p) dissolved in saline. Group III & Group IV received the levaes extract of *S. trilobatum* (100mg, 200mg/kg/p.o) daily once for 42 days. Group V received Tolbutamide (100mg/kg/p.o) daily once for 42 days. During the second, fourth and sixth week of treatment, the urine sugar and blood glucose of all the rats were determined. Animals described as fasted were deprived of food for 12hours but allowed free access to drinking water. After 42 days of treatment, the animals were killed by cervical dislocation.

Collection of blood

Blood was collected in two separate tubes. One tube containing heparinized blood used for estimation of glucose, and other tube containing the blood was allowed to clot at room temperature and the serum obtained after centrifugation was used for enzymes assays. Liver was excised and kept in ice-cold containers for enzyme assay.

Estimation of biochemical parameters

Blood glucose level was measured by glucose oxi-

dase method[30]. The activity of hexokinase in liver was determined by the method of Brandstrup et al.[31]. Liver glucose-6-phosphatase, Acid phosphatase (ACP), alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) were determined following the methods[32,33].

Statistical analysis

All experimental data were expressed as Mean ± S.D, and statistically assessed by one-way analysis of variance (ANOVA). The difference between test animals and controls were evaluated by Student's t- test[34].

RESULTS

Changes in blood and urine glucose on treatment of diabetic rats with leaves extract and Tolbutamide are presented in TABLE 1. The blood and urine glucose increased in STZ diabetic rats as compared to controls. Administration of leaves extract (100mg, 200mg/kg/p.o) and Tolbutamide (100mg/kg/p.o) decreased blood and urine glucose levels.

TABLE 1 : Effect of aqueous extract of leaves of *Solanum trilobatum* on blood glucose and urine sugar in STZ-diabetic rats

Group	Blood glucose (mg per 100 ml)		Urine sugar
	Initial	Final	
Control	70.2 ± 5.6	79.6 ± 6.2	Nil
Diabetic control	259.4 ± 10.6	306.4 ± 12.8**	+++
S. trilobatum (100mg/kg/p.o)	255.5 ± 11.3	142.7 ± 10.3*	++
S. trilobatum (200mg/kg/p.o)	251.5 ± 9.2	127.6 ± 9.3*	+
Tolbutamide (250mg/kg/p.o)	239.6 ± 9.6	109.8 ± 6.9*	+

Values are mean ± S.D from 6 rats in each group; Diabetic control was compared with normal; experimental groups are compared with Diabetic control; values are statistically significant at **P<0.001 as compared with the normal; * P< 0.001 as compared with the Diabetic control; + indicates 0.25% sugar; +++ indicates 2% sugar.

Effect of the administration of leaves extract and Tolbutamide on hepatic hexokinase and glucose-6-phosphatase are presented in TABLE 2. The activity of hepatic hexokinase decreased while the activity of hepatic glucose-6- phosphatase increased in STZ treated diabetic rats as compared to controls. Administration of leaves extract (100mg, 200mg/kg/p.o), Tolbutamide (100mg/kg/p.o) increased the activity of hexokinase and decreased the activity of glucose-6-phosphatase as compared. Effect of leaves extract and Tolbutamide on serum Acid phosphatase, alkaline phosphatase and serum lactate dehydrogenase are shown in TABLE 3. Administration of leaves extract (100mg, 200mg/kg/p.o), Tolbutamide (100mg/kg/p.o) decreased enzymes as compared to diabetic rats.

TABLE 2 : Effect of aqueous extract of leaves of *S. trilobatum* on hepatic hexokinase and glucose-6-phosphatase in STZ diabetic rats

Group	Hexokinase (µmol glucose phosphorylated / mg protein/ h)	Glucose-6-phosphatase (µmol phosphate / mg protein / min)
Control	0.160 ± 0.49	0.192 ± 0.030
Diabetic control	0.079 ± 0.14*	0.415 ± 0.045*
S. trilobatum (100mg/kg/p.o)	0.136 ± 0.029**	0.229 ± 0.026**
S. trilobatum (200mg/kg/p.o)	0.160 ± 0.022**	0.203 ± 0.029**
Tolbutamide (250mg/kg/p.o)	0.164 ± 0.020**	0.190 ± 0.015**

Values are mean ± S.D from 6 rats in each group; Diabetic control was compared with normal; experimental groups are compared with Diabetic control; values are statistically significant at *P<0.001 as compared with the normal; ** P< 0.001 as compared with the Diabetic control.

TABLE 3 : Effect of aqueous extract of leaves of *S. trilobatum* on serum acid phosphatase, alkaline phosphatase, Lactate dehydrogenase in STZ diabetic rats

Group	Acid phosphatase (K.A unit / dl)	Alkaline phosphatase (K.A unit / dl)	Lactate dehydrogenase (µmol pyruvate / g protein / min)
Control	2.8 ± 0.5	12.4 ± 2.6	122.3 ± 19.8
Diabetic control	8.4 ± 1.6*	24.6 ± 4.2*	186.6 ± 16.2*
S. trilobatum (100mg/kg/p.o)	4.3 ± 0.9**	16.1 ± 3.6**	144.9 ± 12.2**
S. trilobatum (200mg/kg/p.o)	3.9 ± 1.1**	13.2 ± 3.2**	132.8 ± 9.8**
Tolbutamide (250mg/kg/p.o)	3.2 ± 1.2**	12.8 ± 3.5**	126.8 ± 12.9**

Values are mean ± S.D from 6 rats in each group; Diabetic control was compared with normal; experimental groups are compared with Diabetic control; values are statistically significant at *P<0.001 as compared with the normal; ** P< 0.001 as compared with the Diabetic control.

DISCUSSION

The STZ-induction in adult animals produced Type II diabetes mellitus model. STZ selectively destroys the

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pancreatic insulin secreting beta cells; pancreatic cells became less active resembling condition of diabetes mellitus[35]. In the present study the leaves extract of *Solanum trilobatum* decreased blood glucose in STZ diabetic rats.

Clinically used tolbutamide (A sulphonylurea drug) lowered the blood glucose level by stimulating β-cells to release insulin. STZ induced diabetes destroyed β-cells, impairing renal function[36]. Results in the present study using aqueous extract of *S.trilobatum* showed marked hypoglycemic effect.

The plasma glucose lowering effect suggested that the *S. trilobatum* treatment revealed insulin-independent-mechanism. The extract perhaps produced hypoglycaemic effect by extra-pancreatic action[37], possibly by stimulating glucose utilization in peripheral tissues[38,39] or due to an increase in glycolytic[40] and /or glycogenic enzymes activity in peripheral tissues[38]. The extract may have decreased the secretion of the counter-regulatory hormones (glucagons, cortisol and growth hormones)[41] or reduced absorption of glucose from gut[42,43]. Further studies are required to confirm this.

The activity of hexokinase enzymes decreased in the liver[44,45]. Administration of aqueous extract of *S.trilobatum* leaves increased the activity of hexokinase in liver. The increased activity of hexokinase could increase glycolysis and utilization of glucose for energy production. The activity of hepatic glucose-6-phosphatase increased in alloxan treated diabetic rats[44,45]. Administration of aqueous extract of leaves of *S. trilobatum* reduced the activity of glucose-6-phosphatase in liver. The reduction in glucose-6-phosphatase can result in decreased concentration of blood.

Increased activity of serum alkaline phosphatase, Acid phosphatase, and Lactate dehydrogenase were also observed in diabetic rats[46]. The increase in the levels of these enzymes in diabetes may be as a result of the leaking out from the tissue, joining the blood stream. The extract did not produce any lethality or any changes in general behavior of rats[14]. However, the full potential of hypoglycaemic constituents can only be realized after further comprehensive pharmacological and toxicological investigations. Thus, it may be concluded that aqueous leaves extract of *S.trilobatum* ameliorates STZ induced toxicity due to its combined antioxidant potential as well as hepatoprotective action.

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