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EVALUATION OF ANTIDIABETIC ACTIVITY OF *SOLANUM TRILOBATUM* IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

The present study was conducted to evaluate the anti-hyperglycemic activity of the ethanolic extracts of *Solanum trilobatum* (STEt) leaves on blood glucose of albino rats. STEt was administered at doses of 300 mg/kg and 400mg/kg body weight on Streptozotocin (STZ) induced diabetic rats for 3 weeks. Diabetic rats had much reduced body weight than normal rats. Administration of the extracts at the dose of 400 mg/kg body wt. /day resulted in a marked decrease in the levels of fasting blood glucose with a concomitant increase in body weight. *Solanum trilobatum* extract at 400 mg/kg was found to be comparable to glibenclamide. STZ-diabetic rats treated with STEt (400mg/kg) significantly reversed all these changes to near normal. These results suggest that STEt induce antihyperglycemic as well as antihyperlipidemic activities in STZ-diabetic rats.

INTRODUCTION

Diabetes mellitus is a systemic metabolic disease characterized by hyperglycemia, hyperlipidemia, hyperaminoacidemia, and hypoinsulinaemia it leads to decrease in both insulin secretion and insulin action. It is a non-communicable disease considered to be one of the five leading causes of death world-wide[1]. It is a syndrome characterized by hyperglycemia, polydipsia and polyuria and causes complications to the eyes, kidneys and nerves. It is also associated with an increased incidence of cardiovascular disease[2]. The clinical manifestations and development of diabetes often differ significantly between countries and also between racial groups within a country. Diabetes mellitus is a metabolic disorder which is emerging as a severe problem and is currently affecting around 143 million people[3] and by 2030 it is predicted to reach 366 million population worldwide[4].

Many traditional plant treatments for diabetes mellitus are used throughout the world[5]. Few of the traditional plant treatments for diabetes have received scientific scrutiny, and the World Health Organization has recommended that this area warrants attention[6]. *Solanum trilobatum*, a thorny creeper with bluish violet flowers, most commonly available in Southern India has been used traditionally in Siddha system of medicines to treat various diseases. It possesses antioxidant activity[7], hepatoprotective activity[8], antibacterial[9], anti-ulcerogenic activity[10], tumor reduction[11], protection of UV induced damage and radiation induced toxicity in mice[12] and has been widely used to treat bronchial asthma[13]. This study was thus initiated with the aim of evaluating the effects of an ethanolic extract of *Solanum trilobatum* leaves on the blood glucose level, serum lipids in streptozotocin diabetic rats.

MATERIALS AND METHODS:

Animals

Male albino rats weighing 150-200g were used for this study. These animals were treated with robust health by providing pellet diet and water *ad libitum* in the animal house, which is well ventilated and lighted. A total of 30 healthy male albino rats selected were acclimatized to the lab conditions for 15 days and then randomly divided into five groups of six each. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC) and were in accordance with the guidelines of the IAEC.

Plant Material

The healthy plant of *Solanum trilobatum* were collected from the from the Western Ghat biosphere region and used for study. The plants were identified and the voucher specimens were deposited in the herbarium cabinet facility sponsored by St. Joseph's College, Tiruchirappalli. After authentification, the plants were cleaned and shade dried and milled into coarse powder by a mechanical grinder.

Preparation of Extract

The plant was cut into small pieces, shade dried and made into powder. 50g of plant powder was suspended in 200ml of ethanol and the mixture was filtered and air dried by low pressure using soxhlet apparatus. The residue was collected and dissolved in water in a fixed dose and used for the treatment.

Induction of experimental diabetes

A freshly prepared solution of Streptozotocin (45 mg/kg i.p) in 0.1 M citrate buffer, pH 4.5 was injected intraperitoneally in a volume of 1 ml/kg. After 48 hours of streptozotocin administration, rats with moderate diabetes having hyperglycaemia (i.e. with blood glucose of 200-300 mg/dl) were taken for the experiment[14].

Experimental procedure

In the experiment, a total of 30 rats (24 diabetic surviving rats, six normal rats) were used. The rats were divided into six groups of six rats each. Group 1: Normal untreated rats. Group 2: Diabetic control rats given 1 ml of aqueous solution daily using an intragastric tube for 30 days. Group 3: Diabetic rats given STEt (0.30 g/kg body weight) in 1 ml of aqueous solution daily using an intragastric tube for 30 days. Group 4: Diabetic rats given STEt (0.40 g/kg body weight) in 1 ml of aqueous solution daily using an intragastric tube for 30 days. Group 5: Diabetic rats given glibenclamide (0.6mg/kg body weight)[15] in 1 ml of aqueous solution daily using an intragastric tube for 30 days.

At the end of 30 days, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in two different tubes (i.e.,) one with anticoagulant- potassium oxalate and sodium fluoride for plasma and another without anticoagulant for serum separation. Plasma and serum were separated by centrifugation.

Analytical Procedure

Fasting blood glucose was estimated by O-toluidine method[16]. Plasma insulin level was assayed by Enzyme Linked Immunosorbent Assay (ELISA) kit, using human insulin as standard. Haemoglobin was estimated by the method of Drabkin and Austin[17] and glycosylated haemoglobin by the method of Sudhakar Nayak and Pattabiraman[18]. Lipids were separated from serum by the method of Folch[19]. Total cholesterol and triglycerides were estimated by the method of Zlatkis[20] and Foster and Dunn[21] respectively. Free fatty acids were analyzed by the method of Falholt[22].

Statistical Analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's 't' – test[23]. P values <0.05 were considered significant.

RESULTS

The results of antidiabetic activity of ethanolic extract of *Solanum trilobatum* on STZ treated rats are shown in Table 1. The levels of blood glucose, plasma insulin, total haemoglobin, glycosylated haemoglobin and changes in body weight of normal and experimental rats. There was a significant elevation in blood glucose and glycosylated haemoglobin levels, while the plasma insulin and total haemoglobin levels decreased significantly in streptozotocin diabetic rats when compared with normal rats. Administration of STEt and glibenclamide tends to bring the parameters significantly towards the normal. The effect of STEt at a dose of 400 mg/kg body weight was more highly significant than 300mg/kg body weight and therefore the dose was used for further biochemical studies. These effects were compared with glibenclamide.

Table 1. Blood glucose, plasma insulin, total haemoglobin, glycosylated haemoglobin and changes in body weight of normal and experimental animals.

Treatment	Body Weight (g)		Fasting Blood Glucose (mg/dl)	Haemoglobin (g/dl)	Glycosylated haemoglobin (mg/g-Hb)	Plasma Insulin (µU/ml)
	Initial	Final				
Control	120 ± 1.63	160 ± 5.88	92.46±4.51^{-}	13.41±1.67^{-}	0.42±0.04^{-}	14.20±1.26^{-}
Diabetic control	120 ± 5.09	140 ± 7.30**	292.65±5.64^{a}	04.00±0.87^{a}	1.53±0.50^{a}	04.09±0.69^{a}
Diabetic + STEt 300 mg/kg	125 ± 5.09	155 ± 3.20**	126.61±5.21^{b}	08.06±0.43^{b}	0.73±0.30^{b}	13.05±0.24^{b}
Diabetic + STEt 400 mg/kg	130±10.03	160 ± 7.70**	98.25±4.32^{b}	10.34±0.89^{b}	0.53±0.29^{b}	14.06±0.36^{ab}
Diabetic + glibenclamide 0.6mg/kg	140 ± 5.22	160 ± 9.93**	143.52±6.31^{ab}	10.05±0.96^{b}	0.57±0.04^{ab}	12.69±0.53^{ab}

Values are given as mean ± S.D for six rats in each group.
Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).
Experimental groups were compared with diabetic control, ** $p<0.001$.

Table 2: Effect of STEt (400 mg/kg) on serum cholesterol, triglyceride and free fatty acid levels in control and STZ-diabetic rats

Treatment	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	Free fatty acids (mg/dl)
Control	73.25±4.5	22.34±1.31	69.86±2.47
Diabetic control	253.9±3.31^{a}	61.25±6.28^{a}	123.40±2.02^{a}
Diabetic + STEt 400 mg/kg	96.47±5.71^{b}	33.00±2.74^{ab}	71.75±4.19^{ab}
Diabetic + glibenclamide 0.6mg/kg	134.53±2.46^{ab}	35.76±2.34^{ab}	74.00±5.36^{ab}

Each value is mean ± S.E.M for 6 rats in each group
a: $p<0.05$ by comparison with normal rats
b: $p< 0.05$ by comparison with streptozotocin induced diabetic rats

The effect of STEt on serum lipids of normal and experimental rats is summarised in Table 2. A marked increase in the frequency of cholesterol, free fatty acids and triglycerides were observed in diabetic control rats. Treatment with STEt significantly reduced the lipid levels.

DISCUSSION

Streptozotocin is well known for its selective pancreatic islet β-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals. It interferes with cellular metabolic oxidative mechanisms[24]. Intraperitoneal administration of streptozotocin (45mg/kg) effectively induced diabetes in normal rats as reflected by glycosuria, hyperglycaemia, polyphagia, polydipsia and body weight loss when compared with normal rats[25]. In our present study we have observed that STEt can reverse these effects. The prolonged treatment with *S. trilobatum* extracts on STZ-induced diabetes rats produced consistent reduction in the blood glucose levels. It is possible that the drug may be acting by potential activation of pancreatic secretion or increasing the glucose uptake. This was clearly evidenced by the increased level of insulin in diabetic rats treated with STEt. In this context a number of other plants have also been reported to have antihyperglycemic and insulin-release stimulatory effect[26,27].

We have observed a decrease in total haemoglobin during diabetes and this may be due to the formation of glycosylated haemoglobin. Increase in the level of haemoglobin in animals given STEt may be due to decreased level of blood glucose and glycosylated haemoglobin. STEt administration to streptozotocin dosed animals reversed the weight loss. The ability of STEt to recover body weight loss seems to be due to its antihyperglycemic effect. Excess of fatty acids in serum produced by the streptozotocin-induced diabetes promotes conversion of excess fatty acids into phospholipids and cholesterol in liver. These two substances along with excess triglycerides formed at the same time in liver may be discharged into blood in the form of lipoproteins[28]. The abnormal high concentration of serum lipids in the diabetic subject is due, mainly to increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in streptozotocin diabetic rats[29,30] and significant increase observed in our experiment was in accordance to these studies. The marked hyperlipidaemia that characterize the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots[31].

CONCLUSION

The results of the present investigation indicate that the ethanol extract of *S. trilobatum* possess good anti-hyperglycemic activity. Further investigations are required to characterize the active anti-hyperglycemic principle and its mechanism of action.

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