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HYPOGLYCEMIC ACTIVITY *OF Stachytarpheta indica* ON STREPTOZOTOCIN INDUCED WISTAR STRAIN RATS

Silambujanaki P*, Chitra V, Soni D, Raju D, Sankari M

Department of Pharmacology,SRM College of pharmacy,SRM University, SRM Nagar, Kattankulathur,Kancheepuram District – 603203,TamilNadu, India.
Corres author: janakidharan@gmail.com
Tel - 9840837813; Fax – 044-27455717

ABSTRACT: The effect of ethanolic extract of *Stachytarpheta indica* on blood glucose level and other biochemical parameters on streptozotocin induced diabetic rats. Diabetes was induced in wistar strain rats by intravenous injection of streptozotocin(50mg/kg). Ethanolic extract of *Stachytarpheta indica* (EESI) (300mg/kg & 600mg/kg) were administered as a single dose per day to the diabetes rats for 15 days. The control group received distilled water for the same duration .Serum glucose level and serum lipid profiles were measured in the diabetic and non diabetic rats. EESI possesses anti hyperglycemic effect .The present investigation of the plant established pharmacological evidence to support the folklore claim that is an antidiabetic agent.

Keywords: Streptozotocin; Hypoglycemia; Stachytarpheta indica.

INTRODUCTION

Stachyarpheta indica belongs to the family, Verbenaceae. S.indica is one of the ancient plants in the world, which is used in the traditional system for diabetes & liver components. The plant is widely used as indigenous plant throughout the Amezon[1]. It is a snake weed which is native to tropical America & Asia and commonly called as Indian snake weed. Leaves are simple, not lobed or divided, opposite, stalked, elliptic or ovate,dentate , apex acute and pinnately veined[2]. It is reported for its antidiarrhoel effect[3] and Cardiovascular effects. The plant contains flavanoids, terpenes & phenol contents[4].

MATERIALS AND METHODS

PLANT MATERIAL

The whole *Stachytarpheta indica* plant was collected from the Government Sidha Medical College, TamilNadu and stored at room temperature in a dry place prior to use. The plant was authenticated as *Stachytarpheta indica* by Professor Chelladurai Research Botanist, Palayamkottai, TamilNadu, India.

ANIMALS

Adult Albino (Wistar strain) rats weighing between 150-200gm (2-3 months) were used for the study. The animals were procured from 'The Animal house' of SRM College of Pharmacy. The use of animals was approved by 'The institutional Ethical Committee'. Throughout the experimental period, the animals were housed in cages under room temperature (20 ± 2^0c); relative humidity (60- 70%) and were exposed to 12:12h light: dark cycle. The food and water were available *ad libitum*.

PREPARATION OF EXTRACT

The dried S.indica plant powder (75g) was extracted in Soxhlet apparatus with 450 ml of 95% ethanol at controlled temperature. The collected extract was concentrated under reduced pressure (< 45^0C) using a vacuum pump for complete removal of the solvent. Pure organic part of the sample thus prepared and was stored at 4-5 ^{0}C until used. The EESI was subjected to qualitative photochemical investigation for the identification of phytoconstituents viz., sterols, alkaloids, glycosides, saponins, tannins, carbohydrates and flavanoids[5,6].

TOXICITY STUDIES

Minimal lethal dose (MLD) in Wistar albino mice in group of 10 each for each dose was calculated for the extract by the method of Litchfield and Wilcoxon[7]. The animals were administered oral graded doses of the extract. MLD for the extract was 3000mg/kg.

HYPOGLYCEMIC ACTIVITY

The animals were divided into five groups of six animals each were used in this experiment.

Group I - Normal untreated Rats
Group II – Diabetic rats
Group III – Diabetic rats given Metformin 500mg/kg(p.o)
Group IV- Diabetic rats given EESI 300mg/kg(p.o)
Group V- Diabetic rats given EESI 600mg/kg(p.o)

Diabetes was induced in the appropriate animals by intravenous injection of Streptozotocin(STZ) (50mg/kg)[8] and monitor the plasma glucose levels at weekly intervals . The rats with fasting glucose value of >250 mg/kg was considered as diabetic.

ESTIMATION OF BIOCHEMICAL PARAMETERS

Blood (1.0ml) was collected from the retro-orbital vein puncture using micro capillary technique, on every day from initial to final day (15^{th} day) of experimentation. The blood was collected in separate test tubes containing Potassium Oxalate and Sodium fluoride. The serum obtained after centrifugation at 3000 rpm was used to estimate the blood glucose levels, serum insulin levels, serum lipid levels like serum cholesterol, serum phospholipids, and serum free fatty acids were measured using standard procedures. Fasting blood glucose level was determined using the Toluidine method[9]. Total cholesterol estimation was done using Erba diagnostic kit[10]

STATISTICAL ANALYSIS

The glucose levels were determined by triplicates and expressed as mean± SEM (Standard error of mean). The statistical analysis was perfomed using one- way (ANOVA) followed by Dunnett's test. P values<0.05 were considered statistically significant.

RESULTS

The changes in the levels of Blood glucose and Serum insulin in diabetic control and rats treated with the extract of SI (p.o) is tabulated in Table:1. The diabetic control rats showed significant increase in Blood glucose level and EESI (300mg/kg and 600mg/kg) and Metformin (p.o) treated animals, showed significant reduction in blood glucose levels (P<0.001).

The changes in the Serum lipid levels treated with the extract of SI (p.o) is tabulated in Table:2.The Serum cholesterol, Serum phospholipids, Serum free fattyacids levels were significantly increased in the diabetic group when compared to normal rats .The EESI (300mg/kg &600mg/kg) has significantly reduced the levels of Serum lipid levels when compared to diabetic control.

DISCUSSION

In the present study the hypoglycemic activity of ethanolic extract of *S.Indica* was evaluated by Streptozotocin induced diabetic rats. The continuous treatment of *S.Indica* extract for a period of 15 days produced a significant reduction in the blood glucose level in diabetic rats indicating that the plant extract may be useful in the management of diabetes.

Several animal experimental models have been in use to evaluate hypoglycemic activity such as the alloxan monohydrate, Streptozotocin, etc. Streptozotocin is a nitrosurea compound produced by Streptomyces achromogenes, which specially induces DNA strand breakage in β-cells causing diabetes mellitus[11]. Therefore the STZ induced diabetic model has been widely employed to induce diabetes in experimental animals. Insulin deficiency leads to various metabolic aberrations in animals viz., increased blood glucose level[12], decreased protein content[13], increased levels of cholesterol and triglyceride[14,15]. The EESI showed potent hypoglycemic activity and the possible mechanism might be by potentiation of pancreatic secretion of insulin from β-cell of islets[16,17] or due to enhanced transport of blood glucose to peripheral tissue[18]. This was clearly evidenced by the increased level of insulin in diabetic rats treated with *S. indica* extract. It is well known that the level of glycemic control is the major determinant of serum level of triglycerides[19]. Several investigations demonstrated that near normalization of blood glucose level resulted in significant reduction in the level of plasma cholesterol and triglyceride level. Similar results were obtained with the ethanol extract of *S.Indica.* Oral administration of EESI resulted in significant reduction in serum lipid levels in the diabetic rats viz.. triglyceride and total cholesterol.

Flavanoids are known for their diverse biological activities including anti diabetic and hypolipidemic activity. Protiens and saponins have been reported to influence plasma cholesterol level[20,21]. EESI showed the presence of flavanoids, Proteins and Saponins which might be responsible for the antidiabetic activity.

CONCLUSION

Our study has shown that the extract of *S.Indica* exhibited significant dose dependent hypoglycaemic activity. The effect of the extract 600mg/kg was almost equal to that of the standard drug used for the control of diabetes.

Table 1: Effect of EESI on Serum glucose and Serum insulin levels in STZ treated diabetic rats

Groups	Blood glucose (mg/dl)	Serum insulin (αU /ml)
Normal	80.26±3.57	16.80±0.25
Diabetic control	322.54±4.54#	4.23±0.55#
Metformin(500mg/kg)	83.46±6.41*	14.55±0.33*
EESI (300mg/kg)	82.55±3.92*	12.84±0.81*
EESI(600mg/kg)	81.82±4.51*	15.56±0.11*

Values are given as mean±SEM for six rats in each group. #P<0.001 Diabetic control group was compared with normal groups. Values are statistically significant at *P<0.001 as compared with Diabetic control.

Table 2: Effect of EESI on Serum Lipids levels in STZ treated diabetic rats.

Group	Serum Cholesterol (mg/dl)	Serum Phospholipids (mg/dl)	Serum FattyAcids (mg/dl)
Normal control	81.52±3.22	101.22±2.22	75.44±1.02
Diabetic control	172.55±5.67#	159.65±4.52#	179.23+2.06#
Metformin(500mg/kg)	103.24±4.58*	112.22±2.22*	89.24±1.15*
EESI (300mg/kg)	98.21±1.88*	109.54±3.55*	93.42±3.09*
EESI (600mg/Kg)	89.88±2.51*	103.65±4.21*	83.54±2.09*

Values are given as mean±SEM for six rats in each group. #P<0.001 Diabetes control group was compared with normal groups. Values are statistically significant at *P<0.001 as compared with Diabetic control.

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