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Research Article

EFFECT OF ETHANOLIC EXTRACT OF STEM BARK OF *SCHREBERA SWIETENIOIDES* AGAINST STREPTOZOTOCIN INDUCED DIABETES IN RATS

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ABSTRACT

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia. Symptoms include polydypsia, polyphagia, blurred vision, polyurea, weight loss and vascular complications with high rate of mortality. Despite the availability of many synthetic antidiabetic drugs, its treatment has many serious complications. So still remains a challenge for developing a potent antidiabetic drug. The aim of the present study was to evaluate the effect of ethanolic extract of stem bark of *schrebera swietenioides* (EESBSS) against streptozotocin (STZ) (60mg/kg, *i.p*) induced diabetes in rats. Oral glucose tolerance test (OGTT) was carried out to confirm hypoglycemic activity of EESBSS. Albino male Wistar rats (180-200g) were randomly selected after inducing hyperglycemia (blood glucose >250mg/dl) and divided into Normal, Control, Standard (5mg/kg, *p.o*. Glibenclamide), T_1 (250mg/kg, *p.o*. EESBSS), T_2 (500mg/kg, *p.o*. EESBSS). Animals were sacrificed, diffused pancreas were isolated to determine Na^+/K^+ ATPases activity and oxidative stress. EESBSS showed significant reduction in blood glucose and improvement in Na^+/K^+ ATPases activity compared to control group. The activity of antioxidant enzymes were significantly enhanced while LPO levels were significantly reduced. Significant increase in the function of Na^+/K^+ ATPases shows that the plant extract may have offered protection to the cells of pancreas. The results revealed that EESBSS possess significant antidiabetic activity. It also improved the pancreas membrane integrity by significant increase in Na^+/K^+ ATPases activity and antioxidant effect. Further studies are necessary to elucidate the mechanism of action of the plant at cellular and molecular level.

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INTRODUCTION

For centuries, medicinal herbs have been used to treat all types of health maladies. In fact, modern medicine is essentially based on herbal medicine. Even today in the times of advanced technology and medical science still depend on plants for their healing. These medicinal plants consider as a rich source of ingredients which can be used in drug develop-

ment and synthesis. Medicinal plants exhibit phytotherapeutic effects caused by biologically active compounds specific secondary metabolites. *Schrebera Swietenioides* belonging to the family Oleaceae, claimed to be useful as antidiabetic, appetizing, digestive, constipating, anthelmintic, purgative and stomachic.[1,2] It also used in flatulence, skin diseases, leprosy, diarrhoea, anaemia and rectal disease.[3,4] Many of the indigenous plants are still left unexplored for their claimed uses one of such plant is *Schrebera swietenioides* stem bark claimed to be useful as antidiabetic but still the scientific reports are not available for supporting the claim. Hence the present study was undertaken to evaluate the antidiabetic activity for the stem bark of *schrebera swietenioides*.

***Schrebera swietenioides* stem bark**

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and relative insulin deficiency, resistance or both.[5] Till date, it has already affected more than 120 million people worldwide, according to an estimate, 220 million people will be affected by the year 2020.[6] Hence it is imperative to intervene and look for new drugs to manage this metabolic disorder. Use of herbal remedies is gaining higher importance because of synthetic drugs have drawbacks and limitations. The available ethanobotanical information accounts for about 800 plants that may possess anti-diabetic potential.[7] The herbal drugs with antidiabetic activity are extensively formulated commercially because of easy availability, affordability and less side effects as compared to the synthetic antidiabetic drugs. Hence always there is a scope for the development of alternative medicine for diabetes among which herbal medicine is first and foremost.[8] Thus the present study was undertaken to evaluate the effect of ethanolic extract of stem bark of *schrebera swietenioides* (EESBSS) against streptozotosin (STZ) induced diabetes in rats.

MATERIALS AND METHODS

ANIMALS

Male albino rats of Wistar strain weighing 150-200g body weight were obtained from Raghavendra enterprises, Bangalore. Animals were maintained under standard laboratory conditions with normal dark and light cycles and room temperature.

CHEMICALS

Streptozotocin was procured from HI-media chem. Limited, Mumbai. Standard drug Glibenclamide was procured from Aurobindo labs Ltd.; Diagnostic kits used in this study were procured from Span diagnostics Ltd., India. All the other chemicals used were of analytical grade.

INSTRUMENTS

Electronic balance (Shimdazu, model no: DS-852 j), Auto analyzer (Mispa Excell), Cooling Centrifuge (Remi, model no: C-24BL).

COLLECTION OF PLANT MATERIAL

The plant material for the present study was collected from surrounding of Tirumala hills and authenticated by Dr. B. Sitaram, Professor, S.V. Ayurvedic medical college, Tirupati. The *Schrebera swietenioides* was powdered in Wiley mill and extracted with ethanol.

PREPARATION OF EXTRACT

The collected plant material was washed thoroughly in water, chopped, shade dried at room temperature, reduced to a coarse powder in a mechanical grinder and passed through a 40# sieve for desired particle size. In powder, obtained 1 kg of stem bark of *Schrebera Swietenioides* was subjected for hot extraction by using 95% ethanol as a solvent. The extract was concentrated in vaccum. The percentage yield of ethanol extract of *Schrebera swietenioides* was 16 % (w/w).

The obtained extract was stored in a refrigerator at 2-8 ^{o}C until usage.

PRELIMINARY PHYTOCHEMICAL INVESTIGATION

Phytochemical investigation of EESBSS reveals the presence of alkaloids, glycosides (cardiac, anthraquinones), tannins, steroids and triterpenoids, flavanoids in the *Schrebera Swietenioides* plant.

EXPERIMENTAL METHODS

An Institutional Animal Ethics Committee of Sri Padmavathi School of Pharmacy Tiruchanoor, Tirupati (SPSP/CPCSEA/IAEC-1016/a/2014/020), approved the experimental protocol.

Acute toxicity studies

The acute toxicity studies were performed according to the OECD 423 guidelines. The plant extract was administered orally at the four doses of 5, 50, 300 and 2000mg/kg body weight and they were subsequently observed closely for the first 4h for any untoward symptoms such as tremors, convulsions, salivation, diarrhea and lethargy followed by observation for a further 14 days. At the end of the experimental period, the animals were observed for any changes in behavioural pattern and mortality.[9]

Experimental design

Animals were divided into five groups, each group consisting of six (n=6) rats of which each group receiving respective treatments per orally for 14 days as shown in the Table-1.

Oral glucose tolerance test (OGTT)

Hypoglycemic activity was studied in glucose overloaded hyperglycemic rats. The animals were divided into four groups (n=6). Group-I served as normal, group-II as control received vehicle; the remaining three groups were treated orally with 200mg/kg, 400mg/kg and 800mg/kg of EESBSS. Zero hour blood sugar level was determined from overnight fasted animals. After 30 min of the drug treatment (*p.o.*), the animals were fed with glucose (4kg/kg) and blood glucose was determined after 0.5, 1, 2, and 3 hours of the glucose load. Blood glucose concentration was estimated by the glucose oxidase enzymatic method using glucose kits.[10]

Streptozotocin induced diabetes models in rats [11]

In the present study, diabetes was induced by single intra peritoneal injection of streptozotocin (60mg/kg).[12] The streptozotocin was freshly prepared by dissolving 60mg in 1ml citrate buffer (pH 7.4). The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. 72 hrs after injection of streptozotocin, fasting plasma blood glucose was estimated. Animals with plasma glucose of >250g/dl [13] were randomly divided into groups II-IV, (n=6). Group-II includes control and remaining groups were treated with ethanolic extract of *Schrebera swietenioides* at different doses for 14 days.

Estimation of blood glucose levels

Blood glucose was estimated by using Glucose oxidase (GOD) and Peroxidase (POD) method.[14]

Estimation of Na^+/K^+ ATPases

Na^+/K^+ ATPases was assayed by taking 250ml of Tris Hcl (184mM; pH 7.5) buffer followed by the addition of 50 ml of 600mM Nacl, 50ml of 50mM Kcl, along with 50ml of 1Mm Na-EDTA and 50ml of 80Mm ATP. The reaction mixture was pre-incubated at 37^{0}C for 10 min. then 25ml of 10% homogenate was added to test alone and further incubated at 37^{0}C for I h. the reaction was immediately arrested by the addition of 10% trichloro acetic acid (TCA). Control samples were carried out under the same conditions with the addition of 0.1mM ouabain. The precipitate was removed by centrifugation at 3500 rpm for 10min. the supernatant (0.5ml) was used for the estimation of inorganic phosphorous. The specific activity of the enzyme corresponds to the difference between the total ATPases activity and the activity measure in the presence of 1mM ouabain (ouabain-resistant activity).

Table-1: Treatment schedule for evaluation of anti diabetic activity

Group No	Treatment	Purpose
I	No treatment	To serve as normal
II	Streptozotocin (60mg/kg, *i.p*) + Distilled water	To serve as control
III	Streptozotocin (60mg/kg, *i.p*) + Glibenclamide (5mg/kg, *p.o*)	To serve as standard
IV	Streptozotocin (60mg/kg, *i.p*) + EESBSS (250mg/kg, *p.o*)	To study the antidiabetic effect of low dose of *S. swietenioides*
V	Streptozotocin (60mg/kg, *i.p*) + EESBSS (500mg/kg, *p.o*)	To study the antidiabetic effect of high dose of *S. swietenioides*

Table-2: Effect of EESBSS on oral glucose tolerance test

Group	Treatment	Serum glucose (mg/dl) (Mean ± SEM)				
		0 min	30 min	60 min	90 min	120 min
I	Normal	75.53 ± 7.14	110.0± 6.47	120.9 ± 9.94	101.5 ± 6.64	93.3 ± 12.04
II	Glibenclamide (5mg/kg)	58.14 ± 6.87a	85.53 ± 7.05a	75.35 ± 6.55a	69.64 ± 6.38a	62.58 ± 5.47a
III	EESBSS (250mg/kg)	85.89 ± 6.39b	120.84 ± 4.84b	95.97 ± 8.05b	87.48 ± 7.79b	77.43 ± 8.03b
IV	EESBSS (500mg/kg)	80.09 ± 6.34b	130.0 ± 1.02b	91.01 ± 5.18b	82.11 ± 7.33b	71.6 ± 10.24b

a = $p < 0.05$, when compared to normal. b = $p < 0.05$, when compared to normal.

Table-3: Effect of EESBSS on blood glucose levels

Group	Treatment	0^{th} day	7^{th} day	14^{th} day
I	Normal	82.12 ± 0.940	88.52 ± 4.11	86.46 ± 4.89
II	Control	230.41 ± 15.53	239.54 ± 13.34[a]	242.14 ± 15.31[a]
III	Glibenclamide (5mg/kg)	218.09 ± 15.23	100.38 ± 8.86[b]	89.51 ± 5.53[b]
IV	EESBSS (250mg/kg)	201 ± 14.42	120 ± 9.93[b]	99 ± 4.66[b]
V	EESBSS (500mg/kg)	190 ± 13.83	113 ± 10.15[b]	91 ± 5.12[b]

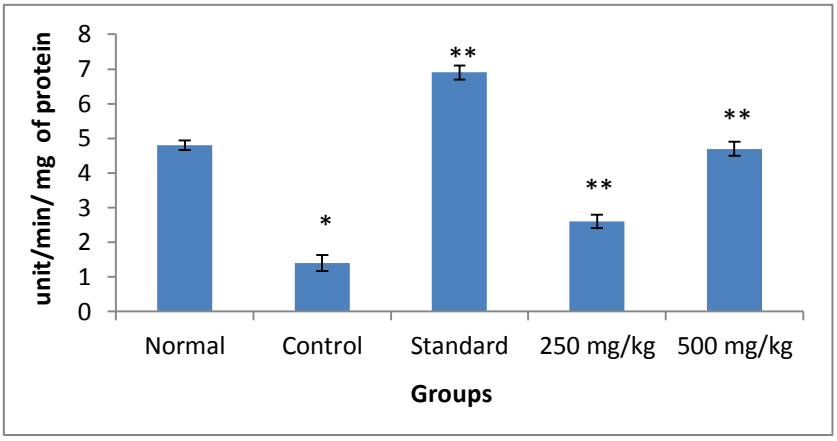
a = $p < 0.05$, when compare to normal. b = $p < 0.05$, when compare to control.

Table-4: Effect of EESBSS on Na^+/K^+ ATPases activty in diabetic rats

Group	Treatment	Na^+/K^+ ATPases activity (µmol Pi/me:/h)
I	Normal	2.000 ± 0.051
II	Control	0.619 ± 0.113[a]
III	Glibenclamide (5mg/kg)	1.013 ± 0.002[b]
IV	EESBSS (250mg/kg)	1.463 ± 0.139[b]
V	EESBSS (500mg/kg)	1.792 ± 0.197[b]

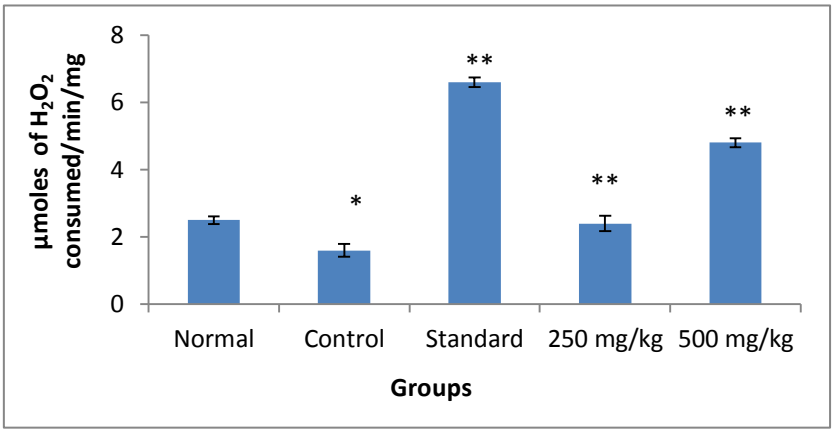
a = $p < 0.0001$, when compared to normal. b = $p < 0.0001$, when compared to control.

Figure-1: Effect of EESBSS on SOD levels in STZ induced diabetes model



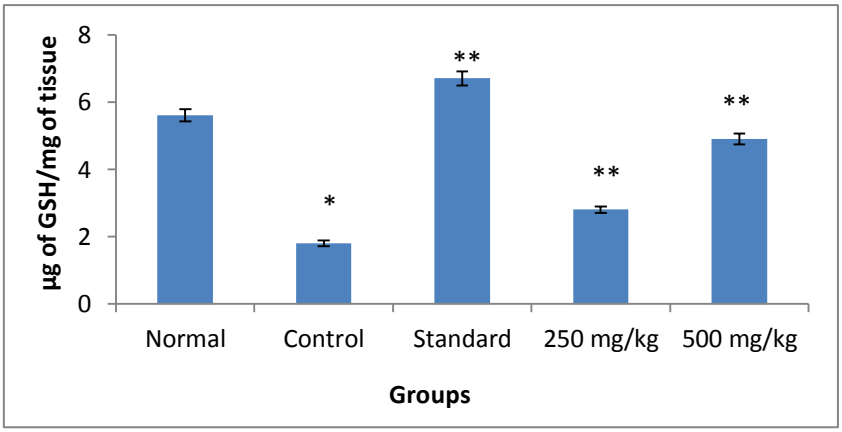
All values are shown as Mean ± SEM and n=6. * indicates $p < 0.05$ when compared with normal. **indicates $p < 0.05$ when compared with control

Figure-2: Effect of EESBSS on CAT levels in STZ induced diabetes model



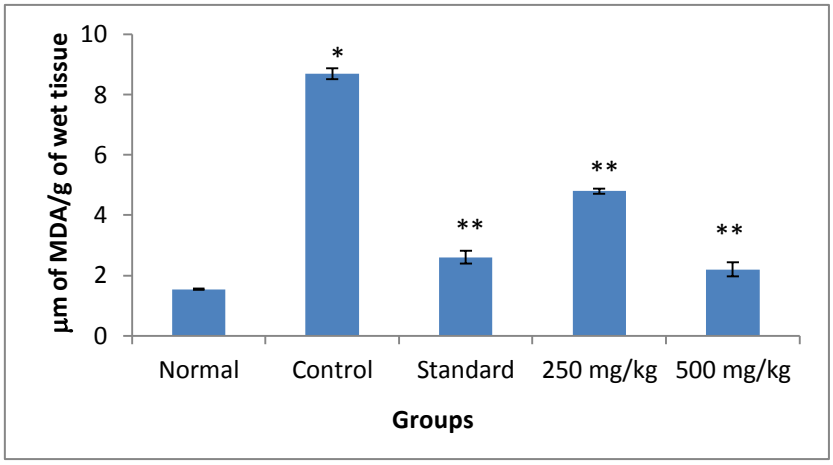
All values are shown as Mean ± SEM and n=6. * indicates $p < 0.05$ when compared with normal. **indicates $p < 0.05$ when compared with control

Figure-3: Effect of EESBSS on reduced GSH in STZ induced diabetes model



All values are shown as Mean ± SEM and n=6. * indicates $p < 0.05$ when compared with normal. **indicates $p < 0.05$ when compared with control

Figure-4: Effect of EESBSS on LPO in STZ induced diabetes model



All values are shown as Mean ± SEM and n=6. * indicates $p < 0.05$ when compared with normal. **indicates $p < 0.05$ when compared with control

Estimation of oxidative stress

Lipid peroxidase was estimated and it was calculated on the basis of the molar extinction coefficient of malondialdehyde (MDA) and expressed in terms nanomolar of MDA/mg protein.

Antioxidant assays

The enzymatic antioxidants such as superoxide dismutase & catalase and non enzymatic antioxidants glutathione were estimated in tissue homogenate.

Statistical analysis

All the data were expressed as the mean ± SEM. Statistical difference between means were determined by one way analysis of variance (ANOVA) followed by Dunnet t-test.

RESULTS

From the acute toxicity studies, the results had shown that ethanolic extract of stem bark of *Schrebera swietenioides* has no toxic effects found at the maximum tested dose level of 2000mg/kg.

DISCUSSION

Diabetes mellitus is a debilitating and often life threatening disorder with increasing incidence throughout the world.[15,16] Hence it was quite interesting to evaluate the potential therapeutic effectiveness of ethanolic extract of *Schrebera swietenioides* against STZ induced diabetes in rats. Streptozotocin induced diabetes mellitus causes the destruction of pancreatic beta cells of the islets of Langerhans which leads to reduced insulin release. An insufficient release of insulin causes high blood glucose, namely hyperglycemia, which results in oxidative damage by generation of reactive oxygen species (ROS) and the development of diabetic complications.[17,18] Antioxidants have been shown to reduce the risk of diabetes onset, improve glucose disposal and improve some of the associated complications.[19] Plants provide a rich source of antioxidants, which include tocopherols, vitamin-C, phenolic compounds, carotenoids, flavanoids, terpenoids, anthraquinones, steroids, strychnine and eugenol alkaloids.[20,21,22] $Na^+ K^+$ ATPases are pumps present in the cell membrane of the cells which help in maintenance of cell membrane integrity.[23] In the present study $Na^+ K^+$ ATPases was significantly increased in the groups treated with test sample showing that the cell membrane integrity of pancreas was retained. This may be helpful for maintenance of the structure of β-cells of pancreas and thereby increasing insulin production.

CONCLUSION

The results of the present study revealed that ethanolic extract of stem bark of *Schrebera swietenioides* possess significant antidiabetic activity against streptozotocin induced diabetes in rats. Further studies are necessary to explore in detail the mechanism of action of EESBSS at both the cellular and molecular levels.

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