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ANTIHYPERGLYCEMIC ACTIVITY OF BARK OF *ELAEODENDRON GLAUCUM PERS.* EXTRACTS ON BLOOD GLUCOSE LEVELS OF STREPTOZOCIN INDUCED DIABETIC RATS

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ABSTRACT

The prevalence of diabetes mellitus continues to rise worldwide and treatment with oral hypoglycemic drugs leads to numerous side effects and huge monetary expenditure. Therefore, active research on identification of new anti diabetic drugs with minimal side effects from medicinal plants is a challenge according to WHO recommendations. In this aspect, the present study was undertaken to evaluate the antidiabetic potential of *Elaeodendron glaucum Pers.* bark in streptozocin (STZ) induced diabetic rats. Diabetes was induced in albino wister rats by intraperitoneal administration of STZ (45 mg/kg b.wt). Fasting blood glucose (FBG) levels were measured by glucose-oxidase & peroxidase method. The statistical analysis of results was carried out using and one-way analysis (ANOVA) followed by Student's t-test. Antidiabetic potentials of bark of *E. glaucum* Pers. extract has been investigated at the doses of 150, 300 and 600 mg/kg b.wt orally administered against streptozocin induced diabetes male wistar rats. Treatment of streptozocin diabetic male wistar rats with the extracts caused a significant ($P<0.01$) reduction in the blood glucose levels. The highest activity resides at the dose of 600 mg/kg b.wt with mean percentage change of 57.79% after 28 days of extract administration while the other two doses 150 and 300 mg/kg have changes of 41.88% and 43.12% respectively. This result suggests that the bark of *E. glaucum* Pers. extracts possess antidiabetic effect on streptozocin induced diabetic male wistar rats.

KEYWORDS*:* Antihyperglycemic activity, Streptozocin, *Elaeodendron glaucum,* Diabetes mellitus

INTRODUCTION

Diabetes mellitus is an endocrine metabolic disorder characterized by hyperglycemia, altered lipids, carbohydrates, proteins metabolism and it increases risk of cardiovascular diseases complications. [1] The two forms of diabetes, type 1 and 2, differ in their basic mechanisms of development and in physiologic characteristics such as associations with obesity, age, and insulin. But, both types of the diabetes share the common characteristics of hyperglycemia, micro vascular and macro vascular complications. Moreover, the alterations of lipoproteins metabolism are involved to the pathogenesis of the cardiovascular disease in both forms of diabetes in a similar way. [2] Also, diabetes is usually accompanied by increased generation of free radicals or impaired antioxidant defenses. Oxidative stress is also responsible for the development and progression of diabetes and its complications.[3] Diabetes has a considerable impact on the health, life style, life expectancy of patients and its related complications are major healthcare problems. Currently, diabetes is controlled by handful of available drugs such as oral hypoglycemic agents and insulin, but they have their own limitations. Traditionally, many herbal medicines and medicinal plants have been used for the treatment of diabetes as an alternative medicine.[4] Presence of various phytoconstituents in medicinal plants is thought to act on a different series of targets by multiple modes and mechanisms. Hence, plants have the potential to impart therapeutic effect in complicated disorders like diabetes and its complications.[5] Screening of medicinal plants is one of the alternative and valid approaches in the drug development process because they contain diverse phytoconstituents which may give new drug leads and may be effective and safe in diabetes .[6] In India, traditionally numbers of plants are used to manage the diabetic conditions and their active principles were isolated but few plants have been scientifically studied.[7] *E. glaucum* Pers. (Celastraceae) an Indian plant known as Jarmrassi. Leaves, inflorescence, bark and fruits of

this plant are traditionally employed in several regions for medicinal purposes .[8] The present study was designed to test the antihyperglycemic activity of bark of *E. glaucum* Pers. extract on streptozocin- induced diabetes.

MATERIAL AND METHODS

Plant material

The plant of *E. glaucum* Pers. has been collected from campus of the Dr. H. S. Gour Vishwavidyalaya Sagar, Madya Pradesh, in the month April-June. The plants of *E. glaucum* Pers. have been authenticated by Prof. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Pharmacognosy Institute, and Chennai. A voucher specimen (No. PARC/69-70) were deposited in the herbarium of department of Pharmacognosy.

Preparations of extracts

The dried whole plant was subjected to size reduction to a coarse powder by using dry grinder and passed through sieve. About 150 g of this powder was packed into Soxhlet apparatus and extracted successively with petroleum ether, chloroform, and ethanol (yield 1.28 %, 1.74 %, 2.70 %, respectively). The solvent was recovered by distillation in vacuum and extracts were stored in desiccators and used for subsequent experiments.

Experimental animals

Adult albino wistar rats (200-225 g) were used to assess antihyperglycemic activity. All animals were housed in standard laboratory conditions temperature (25^0C ± 2) and humidity (45-50 %) with 12 h: 12 h light-dark cycle. The animals were fed with standard pellet diet (Hafed, Rohtak) and water was given *ad libitum.* For experimental purpose the animals were kept fasting overnight but allowed free access to water. A prior permission was taken from Institutional Animal Ethics Committee to carry out the activity (Approval no. LSCP/IAEC/07/01 and date 23rd Feb. 2007.

Chemicals

The estimation of biochemical parameters was carried out using commercially available kits (Primal Healthcare Limited, Lab Diagnostic Division, and Mumbai, India). STZ and other chemicals were procured from Himedia Laboratories, Mumbai, India.

Acute toxicity study

Acute oral toxicity study was performed as per Organization for Economic Cooperation and Development guidelines 423 (acute toxic classic method) .[9] After the oral administration of bark of *E. glaucum* Pers. (2,000 mg/kg), animals were observed individually at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, they were observed for a total of 14 days for toxicity determination.

Induction of experimental diabetes in rats: STZ was dissolved in freshly prepared 0.1 M cold citrate buffer (pH 4.5) and administered by intraperitoneal route (45mg/kg b.wt) to the overnight fasted rats .[10] After 6 h of STZ injection, rats were received 5% dextrose solution for the next 24h to prevent STZ induced fatal hypoglycemia as a result of massive pancreatic insulin release after its administration. Diabetes was confirmed 72 h after induction by measurement of tail vein blood glucose levels using glucose meter (Glucocard™ 01-mini, Arkray Factory, Inc., Japan) by glucose oxidase-peroxidase method using strips. Diabetic rats were kept 7 days under standard laboratory condition for the stabilization of blood glucose levels .[11] After 7 days induction of diabetes, blood glucose was again determined and animals with a blood glucose level greater than 250 mg/dl were selected for the study.

Phytochemical screening:

The preliminary phytochemical screening of the crude extract of *E. glaucum* Pers. was carried out in order to ascertain the presence of its constituents utilizing standard conventional protocols.[12]

Experimental design: The Streptozocin-induced diabetic wistar rats were randomly assigned into six groups (1-6) of six rats (n=6) each as follows, namely

Group 1	**Normal control (NC)**
Group 2	Diabetic control (DC)
Group 3	Glibenclamide 10 mg/kg b. wt., per orally (Standard drug 10)
Group 4	Ethanolic extract of *E. glaucum* 150 mg/kg b. wt., per orally (EEEG 150)
Group 5	Ethanolic extract of *E. glaucum* 300 mg/kg b. wt., per orally (EEEG 300)
Group 6	Ethanolic extract of *E. glaucum* 600 mg/kg b. wt., per orally (EEEG 600)

Determination of blood glucose levels

Blood samples were collected by cutting the tail-tip of the rats, for blood glucose determination at intervals of 7, 14, 21 and 28 days by the glucose-oxidase principle [13] using the one touch basic instrument and results were reported as mg/dl.[14-15]

Statistical analysis

Blood glucose levels were expressed in mg/dl as mean ± SEM. The data were statistically analyzed using ANOVA with multiple comparisons versus control group. The values of $p<0.01$ were considered as significant .[16] The criterion for statistical significance was considered as P value <0.001. The difference between test and controls were evaluated by student's t-test.

RESULTS

Phytochemical analysis

Freshly prepared extracts were subjected to preliminary phytochemical screening test for various constituents. This revealed the presence of alkailoid, tannins, carbohydrate, terpenes, saponins, and flavonoids.

Acute toxicity study (LD50)

The sign of toxicity were first noticed after 10-12 hours of extract administration. There was decreased locomotor activity and decreased in sensitivity to touch. Also there was decreased feed intake, and prostration after 18 hours of extract administration. The median lethal dose (LD50) in rats was calculated to be 2,000 mg/kg body weight.

Anti hyperglycemic study

Table-1 Effect on reduction of blood glucose levels (mg/dl) of *E. glaucum* Pers. in streptozocin-induced diabetic rats treated by various doses of ethanolic extracts (Sub-acute study).

S. no.	Groups	Initial 0 day	7 days	14 days	21 days	28 days
1	NC	68.26±3.44	68.96±3.62	70.16±3.74	71.83±4.44	72.13±3.44
2	DC	379.83±8.04	384.66±9.61	389.66±11.52	393.16±10.87	402.83±10.30
3	Standard 10	395.00±8.63	266.16±6.40* (32.61%)	194.66±8.95* (50.71%)	183.83±6.94** (53.46%)	145.00±5.65** (63.29%)
4	EEEG 150	401.50±9.33	320.00±11.89 (20.29%)	311.33±10.51 (22.45%)	293.00±7.22 (27.02%)	233.33±7.45* (41.88%)
5	EEEG 300	392.00±10.68	300.66±9.89 (23.36%)	298.00±7.94 (23.97%)	273.50±8.08 (30.22%)	222.16±5.67** (43.32%)
6	EEEG 600	392.16±11.16	295.33±8.36 (24.69%)	224.50±5.96* (42.75%)	218.33±5.39** (44.32%)	165.66±5.31** (57.79%)

*Values are given as mean ± S.E.M. for groups of six rats of each. Values are statistically significant at *P<0.05., **P<0.01, non significant = ns>0.05.*

NC-Normal Control, DC-Diabetic Control, Standard 10-Glibenclamide 10 mg/kg b.wt., EEEG 150- Ethanolic Extract of E. glaucum Pers. 150 mg/kg b.wt., EEEG 300- Ethanolic Extract of E. glaucum Pers. 300 mg/kg b.wt., EEEG 600- Ethanolic Extract of E. glaucum Pers. 600 mg/kg b.wt.,

Tables-1 showed the results of effects of ethanolic extract *E. glaucum* Pers., glibenclamide and control groups in streptozocin-induced diabetic albino wistar rats. Blood samples were collected before and at 0, 7, 14, 21, and 28 days after glucose administration. Oral glucose tolerance test (OGTT) of rats was found to be glucose intolerance. Sub-acute studies were carried out on STZ-induced diabetes rats. The ethanolic extract *E. glaucum* Pers. (150, 300 and 600 mg/kg, b.wt.) has shown a significant (P<0.01) reduction in blood glucose levels of about 41.88%, 43.32% and 57.79%, respectively, after treatment. At the same time, glibenclamide caused a significant (P<0.01) reduction of blood glucose levels of 63.29%.

DISCUSSION

Medicinal plants are widely used by the populations of underdeveloped countries as alternative therapy. In India, hundreds of plants are used traditionally for the management and/or control of diabetes mellitus. Unfortunately only a few of such Indian medicinal plants have received scientific scrutiny. The present work was therefore designed to study the anti-hyperglycemic activity of bark *of E. glaucum* Pers. extract in Streptozocin-diabetic rats. Streptozocin-induced hyperglycemia has been described as a useful experimental model to study the activity of hypoglycemic agents.[17] Streptozocin selectively destroyed the pancreatic insulin secreting β –cells, leaving less active cell resulting in a diabetic state.[18] Many secondary metabolites participate in a variety of anti-diabetic functions in vivo .[19] The glycemic change in blood glucose levels of diabetic rat at different time intervals after oral administration *E. glaucum* Pers. extract of at the doses of 150, 300, and 600 mg/kg b.wt as showed in Table 1.

In relation to the diabetes rats that received 150, 300, and 600mg/kg b.wt of *E. glaucum* Pers. extract there was a significant (p<0.01)reduction in the blood glucose levels when compared to the control group after different time hours of extract administration as regard to the dose of 600 mg/kg b. wt and the reference drug . In relation to the dose of 150 and 300 mg/kg b.wt of the *E. glaucum Pers.* there was a less significant change in the blood glucose levels after different time interval of extract administration. In relation to the reference drug glibenclamide 10 mg/kg b.wt given orally. The dose of 600 mg/kg b.wt was found to be more effective in the blood glucose level after 28 days of extract administration than the other two doses of the extract 150 and 300 mg/kg b.wt. The extract might possess glibenclamide like effect on peripheral tissues either by promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis. The phytochemical studies of *E. glaucum* Pers. extract revealed the presence of tannins, carbohydrate, terpenes, saponins, flavonoids and alkaloids .[20] Effect of the flavonoids, quercetin and ferulic acid on pancreatic β-cells leading to their proliferation and secretion of more insulin have been proposed.[21-22] The presence of these constituents leads to antidiabetic activity caused by streptozocin in diabetic rats. The flavonoids present in *E. glaucum* Pers. may also be acting similarly thereby decreasing the high blood glucose levels of streptozocin-diabetic rats.

CONCLUSION

The experiment evidence obtained in the present laboratory animal study indicate that bark of *E. glaucum*

Pers. extract possess anti-hyperglycemic activity which suggest the presence of biologically active components which may be worth further investigation and elucidation.

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