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Research Article

Hypoglycemic Effects of *Elephantopus Scaber* in Alloxan-Induced Diabetic Rats

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ABSTRACT

Oral administration of aqueous extract of *Elephantopus scaber* leaves (300 mg/kg body weight) and roots (300 mg/kg body weight) for 84 days significantly reduced serum glucose, glycosylated hemoglobin and the activity of gluconeogenic enzyme glucose-6-phosphatase, but increased serum insulin, liver and skeletal muscle glycogen content and the activity of glycolytic enzyme glucokinase. The light microscopic studies of the pancreatic section of rats administered with the aqueous extract of of *Elephantopus scaber* leaves and roots showed an improvement in the histoarchitechture. For all the biochemical and histological studies performed, *Elephantopus scaber* roots treated rat showed a little better activity than *Elephantopus scaber* leaves treated diabetic rats. The present investigation suggests that *Elephantopus scaber* leaves and roots extract exhibit antihyperglycaemic effects and consequently may alleviate damage of pancreas and liver associated with alloxan-induced diabetes mellitus in rats.

INTRODUCTION

Diabetes mellitus is a metabolic syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion or insulin action [1, 2]. Insulin therapy and oral hypoglycemic agents offer effective glycemic control, but, Insulin therapy has shortcomings such as ineffectiveness on oral administration, short shelf life, and requirement of constant refrigeration and, in the event of excess dosage fatal hypoglycemia limit its usage [3]. Due to the ineffectiveness of insulin through oral route in the treatment of diabetes, search was made for compounds, which would prove effective if taken orally; the oral hypoglycemic agents that are capable of reducing blood sugar level belong to two chemical classes, sulfonylureas and biguanides [4]. The use of oral drugs is limited due to adverse side effects including hematological, cutaneous and gastrointestinal reactions, hypoglycemic coma and disturbances of liver and kidney functions, in addition, they are not suitable for use during pregnancy [5].

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Plants are reputed in the indigenous systems of medicine for their hypoglycemic activities; the available literature shows that there are more than 800 plant species showing hypoglycemic activity [6, 7]. The world Health organization has also recommended the evaluation of the effectiveness of plants in conditions where we lack safe modern drugs [8]. Studies have shown that phytochemical isolated from plant sources have been used for the prevention and treatment of cancer, heart disease, diabetes mellitus, and high blood pressure [9].

Elephantopus scaber L. belongs to the family Asteraceae is found throughout the dry or semi arid regions including central India where it is an important part of ground cover in forests and open places. The plant has been extensively used in different systems of medicine, for the treatment of various types of diseases [10]. The infusion and decoction of the whole plant, roots and leaves are used in folk medicine for the treatment of fever and to eliminate bladder stones [11]. The plant extract is bitter, acrid, astringent, antipyretic, antidiabetic, diuretic and tonic. A decoction of the roots and leaves are given in dysuria, intermittent fevers, diarrhoea, and bronchitis and especially for haemorrhoids and various other disease conditions [12]. The

plant extract shows anticancer [13] and antibiotic activity [14]. *Elephatopus scaber* plant is less known in the indigenous systems of medicine for diabetes mellitus, therefore, the present study was undertaken to evaluate the effectiveness of *E. scaber* (leaf/root) in alloxan-induced diabetic rats.

Materials and Methods

Alloxan monohydrate was obtained from Sigma Chemical Company, St. Louis, Mo, USA. All the other chemicals used were of analytical grade and were purchased from commercial sources. Leaves and roots of *Elephantopus scaber* were collected from Kerala, India. They were carefully identified and authenticated by Dr. Annie Xavier, Professor of Botany, Holy Cross College, Tiruchirappali, 620 002. Shade dried leaves and roots of *Elephantopus scaber* were powdered and boiled in water (100 g/L distilled water). The decoction was filtered through nitrocellulose filter and the filtrates were evaporated to dryness under vacuum and 50□C temperature in a rotary evaporator. The dried residues were stored in airtight containers for further use.

Animal Studies

Male adult Wistar strain albino rats (100-150 g) were used for the studies. Ethical approval was obtained from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, approval no.585/05/A/ CPCSEA), the institutional ethical review committee. The animals were obtained from Tamilnadu Veterinary and Animal Science University, Chennai, India, and fed on a standard feed (Sai Durga Feeds and Foods, Bangalore, India) and water ad libitum. The animals described as fasted were deprived of food for 16 h but were allowed free access to water. After randomization into groups, the rats were acclimatised to the laboratory conditions of temperature and photoperiod for a period of 1-2 weeks prior to commencement of the experiment. Diabetes mellitus was induced in a batch of normoglycemic albino rats starved for 16 h, by injecting intraperitoneally 150 mg/kg body weight of alloxan monohydrate dissolved in physiological saline. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20 % glucose solution intraperitoneally after 6 h. For the next 24 h, the rats were kept on 5 % glucose solution in their cages to prevent hypoglycemia. . Seven days after alloxan injection, rats with blood glucose > 300 mg/dl were considered as diabetic and included in the study. They were divided into different groups, with five rats in each group. Aqueous extracts of *Elephantopus scaber* leaves (ESL) and *Elephantopus scaber* roots (ESR) in doses ranging from 50 mg/kg body weight to 500 mg/kg body weight, at incremental doses of 50 mg/kg body weight, were administered by oral intubation to the animals, and blood glucose was estimated 5 h after. The lowest dose that brought about the maximum antihyperglycemic effect for each extract (300 mg/kg body weight for both ESL and ESR) was selected for further studies. In the further studies that followed, rats in which diabetes was induced as described above were used. They were divided into three groups with ten rats in each group. One group received only distilled water while two other groups received 300 mg/kg of ESL and ESR, respectively. A fourth group (control) consisted of normal rats that received distilled water only. The treatments were continued daily for 84 days while normal control and diabetic control groups were given distilled water every day for 84 days. All the treatments were via oral intubation.At the end of the experiment, the animals were sacrificed by cervical dislocation. Blood was collected from the heart using a syringe and transferred to sodium fluoride containing bottles and allowed to clot and the serum was separated by centrifugation at 3500 r.p.m for 10 min. The serum was assayed either immediately or stored at 20 □C pending assay. Commercial diagnostic kits were used to assay serum glucose (glucose oxidase method, kit supplied by Reddy.s Laboratories, Hyderabad, India), glycosylated hemoglobin (kit obtained from Bio Systems, Costa Brava, Spain), and insulin (Radioimmunoassay kit from Diasorin, Italy). Tissues from the liver and skeletal muscle were collected. The glycogen contents of both the liver and skeletal muscle were estimated by the method described by Plummer, 1987[15]. All the group data were statistically evaluated using the Statistical Package for Social Sciences (SPSS) version 7.5. Hypothesis testing was by one-way analysis of variance (ANOVA) followed by least significant difference test. P-values of less than 0.05 were considered statistically significant. All the results were expressed as mean ±SD (n = 10).

Light Microscopic Studies

Small slices of the pancreas were fixed in Bouin's fluid [16] dehydrated in graded alcohol and embedded in paraffin wax. Sections (4-5 μm thick) were cut and stained with hematoxylin and eosin and were studied under a Leitz microscope.

Table 1: Effect of treatment with ESR (300 mg/kg) and ESL (300 mg/kg) on serum parameters of control and alloxan diabetic rats

Parameters	Control	Diabetic control	Diabetic±ESR	Diabetic±ESL
Glucose (mg/dl)	74.8±3.1	361.0±10.4	85.6 ±2.4*	87.6 ±5.7 *
Glycosylated hemoglobin(%)	2.44±0.29	4.86±0.68	2.58 ±0.10 *	2.69 ±0.45 *
Insulin (µU/ml)	38.6±4.5	8.2±1.3	34.8±2.4 *	32.0±2.5 *

Values are given as mean±SD for groups of ten animals each. Values are statistically significant at *P< 0.05. ESR and ESL treated diabetic rats were compared with diabetic rats.

Table 2: Effect of treatment with ESR (300 mg/kg) and ESL (300 mg/kg) treated diabetic rats on liver glycogen and skeletal muscle glycogen of control and alloxan- induced diabetic rats

Parameters	Control	Diabetic control	Diabetic±ESR	Diabetic±ESL
Liver glycogen (mg/g)	49.0±1.3	9.0±2.5	42.0 ±1.1*	41.0 ±0.8 *
Skeletal muscle glycogen (mg/g)	9.8±1.8	1.8±0.6	8.2 ±0.8 *	7.8±0.5 *

Values are given as mean±SD for groups of ten animals each. Values are statistically significant at *P< 0.05. ESR and ESL treated diabetic rats were compared with diabetic rats

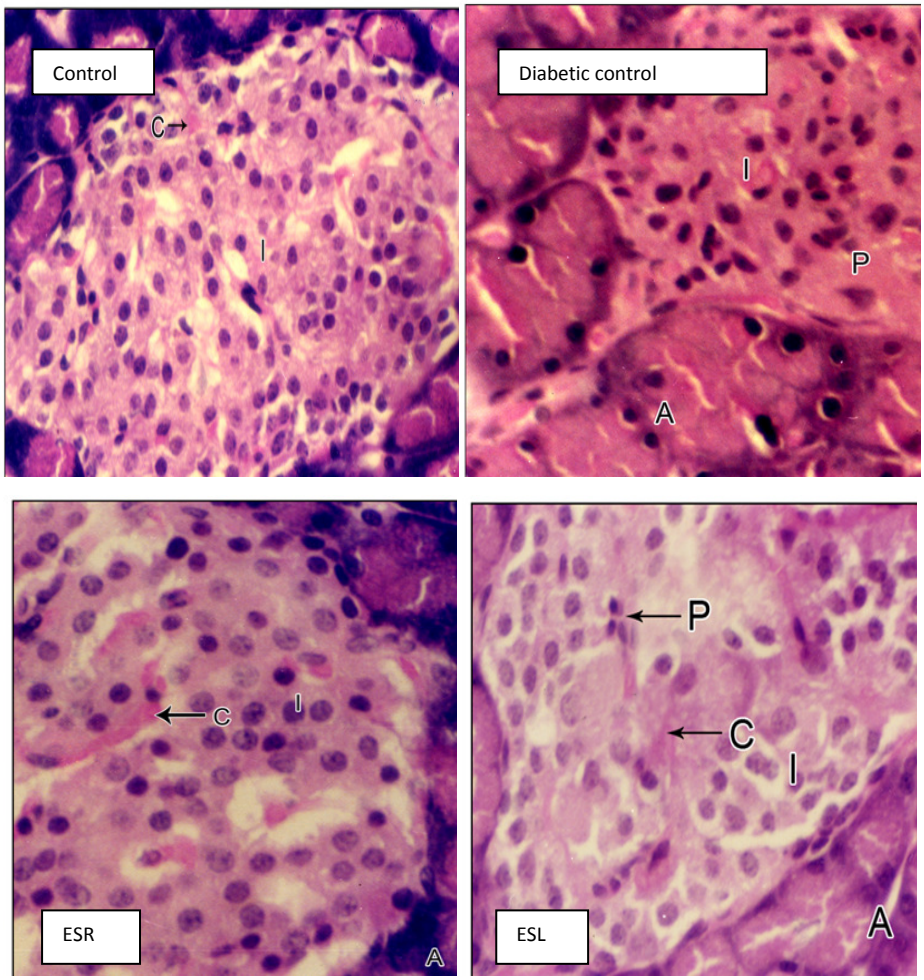

Figure 1: Paraffin section of an Islet of Langerhans of a control rat and alloxan-induced diabetic rat I, islet of Langerhans; A, acinar tissue, C, capillary. ; P, pyknotic nuclei; C, capillary [Paraffin section, Hematoxylin & Eosin (H & E); x 400]

RESULTS

A significant increase in blood glucose, glycosylated hemoglobin and a decrease in serum insulin were observed in diabetic control rats when compared to control rats (Table I). Administration of ESL and ESR to diabetic rats significantly decreased the level of blood glucose and glycosylated hemoglobin, increased serum insulin to near control level. Table II shows the content of liver glycogen and skeletal muscle glycogen in control and diabetic rats. The content of liver glycogen and skeletal muscle glycogen was reduced in diabetic animals when compared to control animals. The lowered content of liver glycogen and skeletal muscle glycogen, increased to near control after ESL and ESR treatment. Light microscopic sections of pancreas of control rats showed no strikingly individual characteristics, they appeared as islands of lightly stained cells surrounded by a thin layer of reticular fibres (Fig. I). The islets appeared lightly stained when compared with the surrounding acinar tissue, there were numerous cells found in the islet. The limiting membrane between the islet and acinar tissue is well differentiated. The islet cells were round to ovoid with round vesicular nuclei and pale pink cytoplasm. Capillaries were found in between the islet cells. Section of pancreas from diabetic control rat showed a different picture, most of the islet cells possessed pyknotic nuclei, limiting membrane between the islet and acinar cells is found to be disrupted, and number of cells in the islet is also decreased. Administration of ESL and ESR to diabetic rats for 84 days had resulted in the improvement of the architecture of pancreatic section of diabetic rats; the limiting membrane between the islet and acinar cells seems to be more prominent, number of cells in the islet has also increased. Most of the islet cells were round to ovoid with round vesicular nuclei and pale pink cytoplasm, whereas only few cells showed pyknotic nuclei, capillaries were observed in between the islet cells.

DISCUSSION

The currently available drug regimens for management of diabeties mellitus have certain drawbacks and therefore, there is a need to find safer and more effective anti-diabetic drugs [17]. Diabetes mellitus of long duration is associated with several complications such as atherosclerosis, myocardial infarction, nephropathy etc. These complications have long been assumed to be related to chronically elevated glucose level in blood [18]. Alloxan causes a massive reduction in insulin release by the destruction of β-cells of the islets of langerhans and thereby induces hyperglycemia [19, 20]. Daily administration of aqueous extracts of ESL and ESR for 84 days had resulted in a decrease in blood glucose level in alloxan-induced diabetic rats. The possible hypoglycemic mechanism of ESL and ESR may be through potentiation of pancreatic secretion of insulin from β-cell of islets or due to enhanced transport of blood glucose to the peripheral tissues.

Glycosylated hemoglobin is produced through glycosylation of hemoglobin. Glycosylated hemogolobin is formed progressively and irreversibly over a period of time and is stable till the life of the RBC and is unaffected by diet, insulin or exercise on the day of testing. Therefore glycosylated hemoglobin can be used as an excellent marker of overall glycemic control. Since it is formed slowly and does not dissociate easily, it reflects the real blood glucose level [21, 22]. In this study, the diabetic rats had higher levels of glycosylated hemoglobin, the significant decrease of glycosylated hemoglobin in alloxan- induced diabetic rats due to ESL and ESR therapy indicates that the overall blood glucose level is controlled which must be due to improvement in insulin secretion.

Serum insulin level of diabetic animals treated with the extracts of ESL and ESR increased when compared to the diabetic control animals. Administration of aqueous extract of ESL and ESR increased the serum insulin level in alloxan-induced diabetic rats suggesting its possible action by increasing insulin release. This result is in agreement with other previous studies on *Gymnema sylvestre* [23], *Momordica charantia* [24], *Enicostemma littorale* [25].

The use of light microscopy to study the morphometry of control and experimentally or pathologically altered pancreatic islets of Langerhans provides data pertaining to the number, size and distribution of the cell types [26, 27]. In non-diabetic rats, the islet tissues show a homogenously normal configuration, whereas, the islet tissues of diabetic animals depict profound distortion in its structural organization. The islet is considerably reduced and shrunken, there is destruction of some β cells with central hyalinization, a few cells show pyknotic nuclei and the number of cells is lower [28, 29]. Histopathological abnormalities in the islet of Langerhans of alloxan-induced diabetic rats

were improved on administration of aqueous extracts of ESL and ESR for 84 days, this is in agreement with other previous studies on D-400 (a herbomineral formulation) [30], *Cassia alata* [31], *Gymnema sylvestre* [32], *Terminalia catappa* [33], *Eugenia jambolana* [34].

CONCLUSION

The results of the present investigation clearly indicate that the leaves and roots extract of *Elephantopus scaber* have hypoglycemic effect on alloxan-induced diabetic rats. The extracts were highly effective in managing the complications associated with diabetes mellitus as revealed by biochemical and histological studies. Therefore, *Elephantopus scaber* leaves and roots extract show therapeutic action against the development and progression of diabetic complications mentioned above. Further studies are in progress to isolate the active principle (S) and elucidate the exact mechanism of action of *Elephantopus scaber* leaves and roots.

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