

Effect of Crude Extract and Fractions from *Elephantopus scaber* on Hyperglycemia in Streptozotocin-diabetic Rats

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Abstract: The effect of the crude extract and acetone fractions from *Elephantopus scaber* (Asteraceae) on glycemia was investigated in streptozotocin (STZ) diabetic rats. Oral administration of crude extract significantly reduced serum glucose levels and increased the lowered insulin concentrations in diabetic animals at 150 mg kg⁻¹. Nevertheless, the hypoglycemic effect of the acetone fractions (mg kg⁻¹) of *Elephantopus scaber* in diabetic rats was evident at 2 h after treatment, where the sugar lowering effect of the most effective fraction was more than 69%. Phytochemical analysis revealed the compound to be a terpenoid. Administration of glibenclamide, a reference drug (0.6 mg kg⁻¹ bw) also produced a significant (p<0.005) reduction in blood glucose concentration in STZ-induced diabetic rats. Thus, the results of this experimental study shows that *Elephantopus scaber* has an anti-hyperglycemic action, is able to ameliorate the diabetic state and probably, is a source of potent hypoglycemic compounds.

Key words: *Elephantopus scaber*, diabetes, hypoglycemia, streptozotocin, insulin

INTRODUCTION

Diabetes mellitus is a chronic disorder of metabolism caused by an absolute or relative lack of insulin. It is characterized by hyperglycemia in the postprandial and/or fasting state and in its severe form is accompanied by ketosis and protein wasting (Bell, 1991; Dima *et al.*, 2007; Rotheri, 2007). Besides drugs classically used for the treatment of diabetes (insulin, sulphonylureas, biguanides and thiazolidinediones), several species of plants have been described in the scientific and popular literature as having a hypoglycaemic activity (Verspohl, 2002; De Sousa *et al.*, 2004; Colca, 2006). Because of their perceived effectiveness, minimal side effects in clinical experience and relatively low costs, herbal drugs are prescribed widely even when their biologically active compounds are unknown (Valiathan, 1998; Mahady, 2005). The present study investigated the acute effect of the oral administration of crude extract and fractions of acetone extract of *Elephantopus scaber* on serum glucose and insulin levels in diabetic rats. A number of investigators have shown that coumarins, flavonoids, terpenoids and a host of other secondary plant metabolites, including arginine and glutamic acid possess medicinal properties (Marles and Farnsworth, 1995; Ross, 2001).

Although, the medicinal properties of *Elephantopus scaber* have been well established (Nadkarni, 1954), yet there are no reports available for the hypoglycemic activity of *Elephantopus scaber* in STZ-induced diabetic rats and also on the compounds responsible for the activity. So the aim of the study was to investigate the hypoglycemic effects of the crude extract of *Elephantopus scaber* and its role on insulin concentrations and also evaluate the hypoglycemic effects of the fractions of the acetone extract of *Elephantopus scaber* (using a bioassay guided separation) in streptozotocin induced diabetic rats.

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MATERIALS AND METHODS

Elephantopus scaber Linn. popularly known as Elephant's foot, (Asteraceae) is a scabrescent aromatic herb distributed in the moist deciduous forests of the central Western Ghats. As per the traditional claims, the roots were used as an antipyretic, cardiogenic and diuretic (Nadkarni, 1954). Decoction of the roots and leaves is used as emollient and given in dysuria, diarrhea, dysentery and in stomach pain (Kirtikar and Basu, 1991). The aqueous extract of leaves is applied externally to treat eczema and ulcers (Chopra *et al.*, 1956). Phytochemically the plant has been reported to contain sesquiterpene lactones deoxyelephantopin, isodeoxy-elephantopin and scabertopin (Paul Pui-Hay But *et al.*, 1997). It also contains epifriedelinol, lupeol and stigmasterol (De-Silva *et al.*, 1982). The pharmacological properties of the leaf extracts have been evaluated for diuretic, (Poli *et al.*, 1992) anti-inflammatory, (Tsai and Lin, 1999) and hepatoprotective properties (Lin *et al.*, 1995). *Elephantopus scaber* plants were collected from Kerala, India. The species was identified and authenticated at the Department of Botany of the College and the voucher specimen had been deposited.

Isolation and Identification of the Active Compound

The shade-dried plants were powdered and 1 kg was extracted using acetone in a soxhlet apparatus and was evaporated to dryness under reduced pressure in rotary evaporator. The yield of the acetone extract was 12.1 g %. The dry residue of the crude extract obtained was stored at 4°C for further use. The acetone extract was further chromatographed on a silica gel column (Merck 70-230 mesh, 400 g, 3.5i.d.×60 cm) and successively eluted with stepwise gradient of ethyl acetate and hexane, then with acetone and ethyl acetate, with acetone and chloroform and finally with chloroform and hexane. Seventeen fractions were collected and each fraction was spotted on a precoated Silica gel 60 F254, 0.25 mm thick TLC plate (Merck) and fractions with similar R_f values in TLC pattern were pooled together.

Experimental Animals

Male albino rats (Wistar strain, weighing 150-220 g) bred in the Laboratory of Animal Medicine, Centre for Animal Health Studies, Tamilnadu Veterinary and Animal Sciences Studies, Madhavaram, Chennai, Tamil Nadu, India were used. All the animals were kept and maintained under laboratory conditions of temperature (22±2°C), humidity (45±5%) and 12 h day: 12 h night cycle and were allowed free access to food (standard pellet diet) and water *ad libitum*.

Induction of Diabetes in Rats

Diabetes was induced by a single intraperitoneal injection of streptozotocin (single dose of 60 mg kg⁻¹ b.wt.) dissolved in freshly prepared 0.01 M citrate buffer (pH 4.5) in a volume of 1 mL kg⁻¹ b.wt. After 7 days of STZ administration, rats with blood sugar levels of 280-350 mg dL⁻¹ and above, were considered as diabetic and were employed in the study. Blood was collected from the tail vein.

Experimental Design and Treatment Schedule

The rats were randomly divided into nine groups of five animals each. Group I served as control Group II was the untreated diabetic group. Groups I and II received 0.1% carboxy methyl cellulose (CMC) orally. Groups III received acetone extracts of *E. scaber*, orally at a dose of 150 mg kg⁻¹ by gastric intubation, while Groups IV and V served as positive controls and received humulin (Schmidt *et al.*, 1999) and glibenclamide (Dhanabal *et al.*, 2006). The treatment was continued for 60 days by administering the various crude extracts suspended in 0.1% CMC once daily. The rats were

sacrificed at the end of 60 days for estimation of glucose and insulin levels. Groups VI-IX received fractions (F1-F4; 2 mg/animal) and blood sugar was determined after 2 h of administration of the fractions. The experiment was carried out in the college during 2005-2006.

Estimation of Glucose and Biochemical Parameters

Blood samples were collected from tail vein in Eppendorff tubes (1.5 mL) at 0th, 15th, 30th and 60th days and serum was separated by centrifuging the samples at 5000 rpm for 10 min and immediately analysed for glucose content.

Statistical Analysis

Students t-test and a probability level of $p < 0.05$ were chosen as the criterion of statistical significance. Values reported are mean \pm SD.

RESULTS

Administration of STZ produced diabetes in rats after a week. The effects of the various extracts of *E. scaber* on blood glucose, serum insulin are depicted in Table 1 and 2.

Determination of the Serum Glucose Concentration and Serum Insulin

Blood samples from the tail vein of the anesthetized rat were collected, centrifuged and the serum was used to determine the glycemia by the glucose oxidase method (De Sousa *et al.*, 2004). Serum insulin was assayed using Radio immunoassay kit purchased from Disasorin, Italy.

Effect of the Acetone Crude Extract of *E. scaber* on Serum Glucose Levels in STZ-Induced Diabetic Rats

The blood glucose level of untreated rats was 84.4 ± 0.55 mg dL⁻¹. In the STZ diabetic rats, the level increased to 534.6 ± 2.07 mg dL⁻¹. In the diabetic rats treated with the acetone extract of the plant, the blood glucose decreased significantly and was to a level closer to the untreated control and within the normal range.

Effect of the Acetone Fractions of *E. scaber* on Serum Glucose Levels in STZ-Induced Diabetic Rats

Oral administration of the various fractions of *E. scaber* with doses of 20 mg kg⁻¹ significantly reduced the hyperglycemia in STZ-induced diabetic rats and maximum reduction of serum glucose levels was observed at 2 h after treatment. Fraction 3 brought about 69% decrease in the blood sugar levels as compared to the diabetic control (Table 3).

Table 1: Effect of the acetone extract of *Elephantopus scaber* on blood sugar levels in STZ-diabetic rats

Parameters	0 day	15th day	30th day	60th day
Normal	84.40 \pm 0.550	84.80 \pm 0.440	85.40 \pm 1.14	85.60 \pm 1.14
Diabetic	534.60 \pm 2.070	538.80 \pm 2.770	532.60 \pm 3.71	534.60 \pm 4.88
Humulin	543.38 \pm 0.455	85.60 \pm 0.477	85.56 \pm 0.52	85.48 \pm 0.64
Glibenclamide	521.70 \pm 1.320	387.34 \pm 0.931	165.80 \pm 2.17	94.20 \pm 2.28
ESA	532.96 \pm 2.126	330.28 \pm 2.390	121.68 \pm 0.93	89.14 \pm 1.74

Table 2: Effect of the acetone extract of *Elephantopus scaber* (after 60 days treatment) on serum insulin levels in STZ-diabetic rats

Treatments	Insulin (μ U mL ⁻¹) (mean \pm SD)
Normal	15.10 \pm 1.180
Diabetic	6.14 \pm 0.140
Humulin (6 U kg ⁻¹ b.wt.)	6.98 \pm 0.072
Glibenclamide (600 μ g kg ⁻¹ b.wt.)	12.68 \pm 0.880
<i>Elephantopus scaber</i> acetone extract (150 mg kg ⁻¹ b.wt.)	17.82 \pm 1.150

Table 3: Effect of the two compounds on blood sugar levels in STZ-diabetic rats

Parameters	Before treatment	2 h after treatment
Normal	84.4±3.550	84.8±0.440
Diabetic	534.6±12.07	538.8±2.770
Fraction 1	547.9±11.79	298.0±9.230
Fraction 2	522.0±4.620	395.8±6.910
Fraction 3	532.6±2.920	156.8±5.110
Fraction 4	5078.0±9.020	435.4±11.31

All values are means±SD of six rats

Serum Insulin by Radioimmuno Assay

The serum insulin level of untreated rats was 51.06±1.18 $\mu\text{U mL}^{-1}$). The insulin level decreased to 6.14±0.14 $\mu\text{U mL}^{-1}$ in the STZ-diabetic rats. In the ESA-treated diabetic rats the serum insulin levels increased to 38.26±1.15 $\mu\text{U mL}^{-1}$, which was close to the normal untreated rats (Table 2).

DISCUSSION

Single dose administration of STZ produces diabetogenic effects in the first 24 h in rats. β -cells underwent conspicuous regression after treatment with streptozotocin (Szkudelski, 2001). Insulin-dependent diabetes mellitus (IDDM) is a disease caused by progressive destruction of the insulin secreting β -cells. Despite meticulous insulin therapy, the appearance of micro- and macro-angiopathy complications after 15 to 20 years of the disease is difficult to prevent in some patients. Presently, the only option to achieve permanent normoglycemia in diabetic patients is renewal of the β -cells or stimulate insulin secretion by medicinal plant (Robertson, 1993). The lowering of blood sugar level in streptozotocin-induced hyperglycemic animals through the administration of *Elephantopus scaber* extract may be due to insulin release from regenerated β -cells of the pancreas or by stimulation of insulin secretion (Palanichamy *et al.*, 1988). Similar reports are available on the oral administration of aqueous extract of leaves of *G. sylvestre* normalized blood sugar levels of diabetic animals through β -cell regeneration (Shanmugasundaram *et al.*, 1981).

In this study we have found that *E. scaber* decreases blood glucose in STZ diabetic rats. The possible mechanism by which *E. scaber* brings about its hypoglycemic action may be potentiating the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the β -cells of islets of Langerhans or its release from bound insulin. The present study was conducted to evaluate the hypoglycemic and antidiabetic activity of the compounds from *E. scaber*, a very new herbal drug first time identified by us to get a berth in the group of antidiabetic herbal drugs. *E. scaber* did not exhibit any sign of toxicity. Since the main purpose of the preliminary acute toxicity study is to get some idea on conspicuous behavioral changes and death, if any and the alcoholic extract of *E. scaber* did not exhibit any toxic symptoms in the limited toxicity evaluation in male rats. A perusal of literature shows that studies were not carried out on this plant with regard to other pharmacological properties and phytochemistry. Type 1 diabetes occurs in a genetically susceptible human population as a result of the loss of the insulin-producing pancreatic beta cells. This accounts for the drastic drop in the insulin level in the diabetic rats. The serum insulin level decreased in diabetic animals, whereas *E. scaber* acetone extract treatment brought about a marked increase in serum insulin in streptozotocin-induced diabetic rats. This increase may be a consequence of the stimulation of insulin synthesis and secretion and or inhibition of insulin degradation, since many compounds present in plants have been demonstrated to produce these effects (Venkateswaran and Pari, 2003). For instance, benzoic acid-related molecules inhibit insulinase and enhance insulin effects (Aybar *et al.*, 2001). The increased levels of insulin in extract-treated diabetic rats indicated that *M. charantia* extract stimulates insulin secretion from regenerated β -cells (Kameswara Rao *et al.*, 2003). In the present study also, serum insulin level of diabetic animals treated with the extracts of *Elephantopus scaber* increased when

compared to the diabetic controls. The biochemical mechanism of action appears to be through regeneration of the damaged β -cells and thereby, stimulate the secretion of insulin in β -cells as revealed by insulin assay.

The significant and consistent hypoglycemic effect of crude extract of *E. scaber*, as well as the acetone fractions, in diabetic rats indicates that this effect can be mediated by stimulation of glucose utilization by peripheral tissues. However, phytochemical and pharmacological studies, performed indicated the most effective fraction to be a terpenoid. Terpenoids appear to involve stimulation of pancreatic β -cells and subsequent secretion of insulin (Marles and Farnsworth, 1995). Further studies to isolate and to characterize the terpenoid compound found in the acetone fraction are underway to further elucidate the mechanism involved in the hypoglycemic effect.

We conclude that the crude acetone extract of *E. scaber* was effective in decreasing the serum glucose level, also increasing serum insulin by stimulating the pancreatic β -cells in diabetic rats and also, the acetone fractions showed the hypoglycemic effect in severe STZ-diabetic rats and it was more potent than the crude extract and that the plant was more effective than the reference drug, Glibenclamide.

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