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Role of *Elephantopus scaber* on the Glucose Oxidation in Liver and Skeletal Muscles of Streptozotocin (STZ) Induced Diabetic Adult Male Rats

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Abstract: In type-II diabetic individuals, there is an increase in hepatic glucose production impaired insulin signaling, decreased glucose transport and phosphorylation and diminished glycogen synthesis contribute to insulin resistance in target tissues. Liver and skeletal muscles are major target sites for insulin-mediated glucose uptake, metabolism and utilization in humans. Indeed, impaired insulin action in liver and skeletal muscles is responsible for the majority of the decreased levels of non-oxidative glucose disposal observed in type II diabetes. The influences of *Elephantopus scaber* (ES) (roots and leaves) on glucose oxidation in liver and skeletal muscles were studied. The present study shows that *E. scaber* extracts have a positive role in glucose oxidation and corrects the metabolic alterations caused by diabetes effectively. A significant reduction in the blood glucose levels and a corresponding increase in the serum insulin levels further proves the hypoglycemic activity of the plant by oxidation of glucose. These observed effects of *E. scaber* on glucose oxidation in liver and skeletal muscles are comparable to the effects of insulin and suggest a possible therapeutic effect of *E. scaber* on glucose oxidation in diabetes. Further studies on the isolation of active compounds and determining their mode of action would be of great interest.

Key words: Streptozotocin, glucose oxidation, *Elephantopus scaber*, insulin

INTRODUCTION

Glucose is the primary energy source used in animal kingdom and multiple mechanisms have evolved for its synthesis, storage and metabolism (Watson *et al.*, 2004). Too low or very high glucose concentrations can lead to medical emergency as seizures, unconsciousness, death, long-term organ damage such as retinopathy and neuropathy (Srivastava *et al.*, 2005). Hence it is important for the body to keep the blood glucose level within narrow limits (Shuldiner, 1998). Increase in glucose level stimulates the secretion of insulin and suppresses the secretion of glucagon (Atkinson and Maclaren, 1995). Thus the glucose level acts as a signal that initiates the hormonal response. Liver and skeletal muscles have an important role in the maintenance of normal glucose homeostasis and in regulating the whole-body carbohydrate metabolism (Sinacore and Gulve, 1993). They are major target sites for insulin-mediated glucose uptake, metabolism and utilization in humans. Splanchnic tissues take up one third of the ingested carbohydrate (Ferrannini *et al.*, 1980). Of the remaining two thirds of the ingested carbohydrate that enters the systematic circulation, some is extracted by liver, but peripheral tissues take up the major portion (Klip *et al.*, 1990). Liver and skeletal muscles are the predominant sites for peripheral glucose disposal and have been responsible for about one fourth of the ingested carbohydrate (DeFronzo, 1988; Baron *et al.*, 1998). Since only recently its antidiabetic property had been reported (Daisy *et al.*, 2007), we undertook this work to study the efficiency of the plant in glucose oxidation, both in the liver and tissues of STZ-induced diabetic rats.

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MATERIALS AND METHODS

Plant Material

Elephantopus scaber (Asteraceae) is a small herb, which grows in the wild throughout the hotter parts of India. The major phytochemical constituents of the plant are elephantopin, triterpenes, stigmasterol, epifriedelinol and lupeol (Rastogi and Mehrotra, 1990; Kritikar and Basu, 1991). The plant has been used in the Indian system of Medicine as an analgesic, diuretic, astringent and antiemetic. The leaves of the plant are used for bronchitis, small pox and diarrhoea and as a brain tonic (Sankar *et al.*, 2001). Recently it has been shown to possess anti-inflammatory and antitumour activity in animal models (Reico, 1989) and also found to have antibacterial activity against selected standard (ATCC) strains (Avani and Neeta, 2005). Only recently its antidiabetic property had been reported (Daisy *et al.*, 2007). Hence, we undertook this study to study the efficiency of the plant in glucose oxidation, both in the liver and tissues.

Preparation of Plant Extract

E. scaber plants were collected from Kerala, India and authenticated at the Department of Botany of Holy Cross College, Tiruchirappalli, India. The plants were thoroughly washed and dried under shade. The dried roots and leaves were powdered and extracted in a Soxhlet apparatus using methanol as solvent (1 kg powder in 3 L solvent). The extract was filter-sterilized through a 0.45 μ membrane filter and evaporated to dryness in a rotary vacuum evaporator at 40°C. The yield of the acetone extract was 14.3 g %. The extracts were dried and 150 mg kg⁻¹ b.w. was suspended in 5 mL of 1.0% of carboxy methyl cellulose, commonly used as a suspending solvent.

Animals

Feeding, Housing Conditions and Ethical Approval

All rats were fed with a standard pellet diet (Durga feeds, Bangalore, India) and tap water ad libitum and housed in a 12:12 h light: dark cycle at 22-25°C. The experimental protocols were conducted in accordance with internationally accepted principles for laboratory animal use and care as found in the US guidelines.

Induction of Diabetes and Experimental Design

Diabetes was induced in rats by intraperitoneal injection of streptozotocin (single dose of 60 mg kg⁻¹ body weight) dissolved in freshly prepared 0.01 M citrate buffer (pH 4.5). Rats with fasting blood glucose more than 300 mg dL⁻¹ were included in the study (Grover *et al.*, 2001).

The rats were randomly divided into five groups of five animals each as:

- **Group 1:** Normoglycemic control. Received only vehicle (1.0% CMC) (5 mL kg⁻¹)
- **Group 2:** Diabetic control. Received only vehicle (1.0% CMC) (5 mL kg⁻¹)
- **Group 3:** Diabetic. Administered methanol extract of *E. scaber* roots once a day throughout 60 days with a dose of 150 mg kg⁻¹ b.w.
- **Group 4:** Diabetic. Administered methanol extract of *E. scaber* leaf once a day throughout 60 days with a dose of 150 mg kg⁻¹ b.w.
- **Group 5:** Diabetic. Glibenclamide was given once a day throughout 60 days with a dose of 0.6 mg kg⁻¹ b.w.

After the 60th day treatment, the rats were sacrificed by decapitation and liver and skeletal muscles were dissected and removed to measure glucose oxidation and blood was collected for determination of serum insulin and blood glucose.

Determination of the Blood Glucose Levels

Blood glucose was determined on the 0, 15, 30 and 60th days, using Glucose Oxidase/Peroxidase method (Trinder, 1969).

Determination of Serum Insulin Levels

Serum insulin was assayed on the 60th day of treatment. The rats were sacrificed and blood samples were collected, serum separated and insulin levels were determined using Radio immunoassay kit purchased from Disasorin, Italy.

¹⁴C-Glucose Oxidation

¹⁴C-glucose oxidation in liver and skeletal muscles was estimated by the method of Johnson and Turner (1971) and Kraft and Johnson (1972).

Statistical Analysis

Statistical analysis was performed using SPSS software package, version 6.0. The values were analyzed by one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) (Duncan, 1957). All the results were expressed as mean±SD for five rats in each group. p-values <0.05 were considered as significant.

RESULTS

Effect on Blood Glucose

Serum glucose levels in the normal untreated rats did not show any significant variation in the blood glucose throughout the experimental period. Administration of STZ (60 mg kg⁻¹ b.w.) led to an elevated blood glucose level, almost 5 fold increase than the untreated group, which was significant (p<0.05). Blood glucose levels measured in extracts-treated experimental rats at the end of 15, 30 and 60 days of treatment are given in Table 1. The decrease in blood glucose in the extract-treated groups could be observed from the 15th day onwards, registering a significant decrease on the 30th day, while a near total restoration comparable to the drug treated and untreated were observed on the 60th day of treatment, showing a time response pattern. Oral administration of the methanol extracts of *E. scaber* root and leaf for a period of 60 days, significantly decreased the blood glucose levels (p<0.05).

Serum Insulin Levels

Table 2 summarizes the effect of STZ and treatment with methanol extracts of ES on serum insulin levels. As evidenced, the serum level decreased in diabetic control as compared to untreated rats. As against the decrease in insulin levels of diabetic control rats, the extract treated rats registered a significant increase after the experimental period. Both the extracts registered an increase in the serum

Table 1: Effect of the methanol extracts of the root and leaf of *Elephantopus scaber* on blood glucose levels (mg dL⁻¹) in fasting normoglycemic and STZ induced hyperglycemic rats at varying days

Parameters	Days			
	0	15	30	60
Normal	84.40±0.550	84.80±0.44	85.40±1.14	85.60±1.14
Diabetic (STZ-60 mg kg ⁻¹ b.w.)	534.60±2.070	538.80±2.77	532.60±3.71	534.60±4.88
Diabetic+Glibenclamide (0.6 mg kg ⁻¹ b.w.)	524.70±1.320	345.34±0.93	224.80±2.17	99.20±2.28
Diabetic+ <i>Elephantopus scaber</i> methanol root extract treated (150 mg kg ⁻¹ b.w.)	532.96±2.126	330.28±2.39	121.68±0.93	86.14±1.74
Diabetic+ <i>Elephantopus scaber</i> methanol leaf extract treated (150 mg kg ⁻¹ b.w.)	521.96±2.930	337.84±6.63	139.88±1.42	97.84±1.17

Values are means±SD of five rats

Table 2: Effect of the methanol root and leaf extracts of *Elephantopus scaber* for 60 days on serum insulin levels in fasting normoglycemic and STZ induced hyperglycemic rats

Parameters	Insulin ($\mu\text{U mL}^{-1}$) (mean \pm SD)
Normal	15.10 \pm 1.18
Diabetic (STZ-60 mg kg ⁻¹ b.w.)	6.14 \pm 0.14
Diabetic+Glibenclamide (0.6 mg kg ⁻¹ b.w.)	12.68 \pm 0.88
Diabetic+ <i>Elephantopus scaber</i> root methanol extract treated (150 mg kg ⁻¹ b.w.)	14.82 \pm 1.15*
Diabetic+ <i>Elephantopus scaber</i> leaf methanol extract treated (150 mg kg ⁻¹ b.w.)	12.80 \pm 0.26

Values are means \pm SD of five rats, *p<0.05

Table 3: Role of *E. scaber* root and leaf extracts on glucose oxidation in liver and muscles on the 60th day of treatment

Treatments	Liver	Muscle	
		Gracilis	Triceps
Normal	2508.0 \pm 3.44	2213.0 \pm 5.44	2648.0 \pm 5.80
Diabetic (STZ-60 mg kg ⁻¹ b.w.)	903.0 \pm 1.80	917.0 \pm 5.25	985.6 \pm 5.80
Diabetic+ <i>Elephantopus scaber</i> root methanol extract treated (150 mg kg ⁻¹ b.w.)	2450.4 \pm 3.16	2386.2 \pm 1.72	2207.2 \pm 2.28
Diabetic+ <i>Elephantopus scaber</i> leaf methanol extract treated (150 mg kg ⁻¹ b.w.)	2286.0 \pm 1.50	2190.0 \pm 1.60	2067.2 \pm 4.96

Values are means \pm SD of five rats

insulin levels of STZ-diabetic rats. However, a significant increase was observed in *E. scaber* root treated group, whose values were comparable and even better than the drug treated group, but on comparison, both the extracts had activity in restoring the serum insulin levels to normal in STZ-treated diabetic rats.

Effect on ¹⁴C-Glucose Oxidation

Table 3 depicts the effects of the methanol extract of the root and leaf of *E. scaber* on ¹⁴-C glucose oxidation in liver and skeletal muscles of adult male diabetized rats. In control rats, liver possessed a maximum ability to oxidize glucose when compared to the skeletal muscles. STZ caused a remarkable decrease in glucose oxidation both in liver and skeletal muscles. ESR treatment restored the rate of glucose oxidation to that of control, while the increase in ESL treated samples did not match the control level.

DISCUSSION

Glucose is an important fuel for liver and muscle metabolism. Liver and skeletal muscles are major sites of carbohydrate metabolism. Hormones are the major regulator of metabolic activities in skeletal muscles. Among the hormones, insulin is the major regulator of liver and skeletal muscle glucose homeostasis. Experimental data have demonstrated that insulin act at the level of target organs (Rajkhowa *et al.*, 1994; Sheffield-Moore and Urban, 2004). Induction of diabetes resulted in a significant reduction of serum insulin and treatment of *E. scaber* extracts restored the same suggesting the positive relationship between the extracts and the insulin. Liver and Skeletal muscles are the major sites of insulin mediated glucose metabolism. Studies on rats suggest that insulin maintains glucose homeostasis by enhancing glycolysis and glycogen synthesis in liver and skeletal muscles (Fell *et al.*, 1982). The observed effects of the plant extracts on glucose oxidation in liver and skeletal muscles are comparable to the effects of insulin and suggest a possible mediatory effect of the plant on insulin-induced changes in liver and skeletal muscles. However, the direct effect of the plant on insulin receptor expression could not be ruled out. Further studies on these lines would be of great interest.

In view of the decrease in glucose levels by the plant extracts, it became pertinent to investigate the effect of the plant on glucose oxidation by both liver and different muscles. The plant extracts significantly decreased the rate of glucose oxidation by the liver and muscles. Impairment of fuel

oxidation was also decreased in the liver and muscle of STZ injected rats, but was reversed in extract treated rats. These results suggest that the decreased glucose utilization by the liver and muscle assume a greater role in the metabolism of this substrate. The reduction of glucose oxidation by liver and muscle as well as decreases in glycogen content of both liver and muscle in STZ treated rats (Table 3) may be related in part to a decrease in insulin secretion during diabetes, which has been previously reported (Eaton, 1973) and was also confirmed in this study (Table 2). It is rather interesting to note that the reversals occurring in extract treated rats prove the treatments effective.

Thus the present study suggests that insulin deficiency has adverse effect on glucose oxidation in liver and skeletal muscles of diabetic rats and this metabolic disorder can be corrected effectively by *E. scaber*. When compared, the *E. scaber* root was more effective than *E. scaber* leaf in correcting the metabolic disorder and the underlying mechanism and the active principle involved have to be studied extensively.

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