Antidiabetic Activity of tuber extract of *Eulophia epidendraea* (Retz.) Fisher (Orchidaceae) in alloxan diabetic rats

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Summary

This study was aimed to determine the antidiabetic potential of ethanolic tuber extract of *Eulophia epidendraea* (Retz.) Fisher (Orchidaceae). Diabetes was induced after a single intraperitoneal dose of alloxan monohydrate (70mg/kg body wt), while hyperglycemia was determined 24hr upon injection. The antidiabetic activity of the ethanolic tuber extract was compared with that of a reference drug Tolbutamide. 100mg and 200mg injection (i.p) of methanolic extract on diabetes induced rats could able to reduce the sugar level notably. Maximum hypoglycaemic activity (51.56% reduction) was observed in the wistar rats administered with 200mg of extract within 4.5 hours.

Keywords: *Eulophia epidendraea;* Orchidaceae; Tuber extract; Antidiabetic activity

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Introduction

Diabetes is a serious ailment caused by the failure of pancreas to produce the hormone, insulin that is essential to carry out carbohydrate metabolism (1). The cause of diabetes may be modified by a variety of compounds, including alkaloids, glycosides, polysaccharides, peptidoglycans, hypoglycans, and terpenoids. Despite considerable progress in the management of diabetes by conventional synthetic drugs, a search for natural anti-diabetic has been progressing well. There are many hypoglycemic plant ingreadients known through the folklore but their introduction into the modern therapy awaits animal test trials that closely parallel to the pathological course of diabetes in human. Hypoglycemic activity of plant ingredients has been reported during the last twenty years (2). The ethnobotanical information identifies about 800 Indian plants (among more than 45,000 species found in India) which may have anti-diabetic potential (3). Orchid *Eulophia epidendraea* (Retz.) Fisher (Orchidaceae) is a terrestrial plant, which is growing wild scrub forest of Western Ghats, South India. There is no phytochemical and pharmacological work on this plant. Hence, in the present study the influence of intraperitonial administration of *Eulophia epidendraea* tuber extract on the blood glucose levels in normal and alloxan diabetic inbred Wistar rats was evaluated.

Materials and Methods

Plant material

Fresh tubers of *Eulophia epidendraea* (Retz.) Fisher (Orchidaceae) were collected from Courtallam, Tirunelveli District, Tamil Nadu, India.

Extraction

The tubers of *Eulophia epidendraea* were air- dried, and powdered. About 250g of the powder was extracted with ethanol (66 ^{o}C) in a Soxhlet apparatus for 8 hours. The solvent was evaporated under reduced pressure. After determining the yield, sediment was stored at 4^{o}C for further use.

Induction of diabetes

A freshly prepared solution of alloxan hydrate (Purchased from Spectrochem, PVT , Mumbai, India) in normal saline was injected into the caudal vein of inbred Wistar rats (70 mg/kg body wt), three times on every third day ([4). Seven days after administration, blood samples were drawn from the caudal vein by the pinch clip method. Animals with a blood sugar level of 250 mg/dL were considered to be diabetic and they were used in this study. The animals were divided into several groups and kept under uniform husbandry conditions. Blood glucose was determined by the O-toluidine method. The blood glucose concentrations were measured at 1.5, 3.0, 4.5 and 24h in Shimadzu UV 160 spectrophotometer at 620 nm. The blood glucose was calculated using the formula given below

Calculation

$$\frac{\text{O.D. of unknown}}{\text{O.D. of standard}} \times \text{Concentration of standard} = \text{mg glucose per 100ml in unknown}$$

Normal inbred Wistar rats

The normal range of blood glucose levels in fasting wistar rats ranged from 80 to 120 mg per 100ml of blood. The tuber extract was then administered intraperitoneally (i.p.) and, at the same time, a control test was carried out using only saline solution. The dosage of ethanolic tuber extract administered was 100 mg, 200mg and 400mg/kg. The percentage decrease in glycaemia was determined as a function of time (5).

Evaluation of Hypoglycaemic Activity

To evaluate hypoglycaemic activity, a study was carried out on the variation of blood glucose levels after the administration of tuber extract (i.p) to Inbred Wistar rats. The animals were put under fasting for 12 hours prior to each experiment, but water was allowed *ad libitum.* The temperature of room where the animals were maintained was kept constant at 22^0C. Blood glucose concentration was determined and noted as the initial glycaemia (G_0) and blood samples were drawn from the caudal vein at 1, 3, 6 and 24 h (G_x). The percentage of ethanol extract induced glycaemia was calculated as a function of time by the following formula:

$$\% \text{ induced glycaemia} \quad \frac{G_x - G_0}{G_0} \times 100$$

Where G_0 is the initial blood glucose level and G_x the blood glucose level at 1.5, 3, 4.5 and 24 hour interavals[6]. The liver and kidney were carefully dissected out, washed in ice cold isotonic saline and blotted individually on ash-free filter paper and organ weights were measured.

Histological studies

Animals were sacrificed on 28th day during prolonged treatment. Pancreas was dissected out, washed with cold saline and preserved in 10% buffered formalin. Blocks from tissues were routinely processed and embedded in paraffin. Thin sections were cut (4-5µm) using rotary microtome and stained with hematoxylin and eosin for histomorphological evaluation.

Results

Anti - diabetic activity

The ethanolic tuber extracts of *Eulophia epidendraea* showed significant (P<0.05) increase in glucose tolerance of Wistar rats (Fig1). The blood glucose levels were reduced considerably within 1hr of the drug administration. The ethanolic extract was significantly brought down the glucose levels to control value.

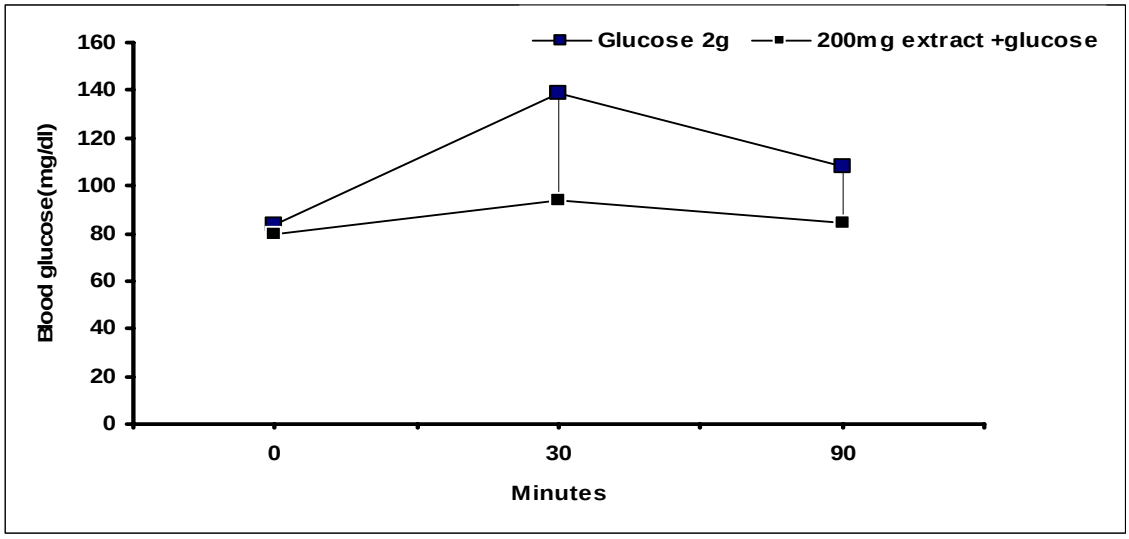

Fig. 1: Influence of ethanolic tuber extract of *Eulophia epidendraea* Fischer (Retz.) Orchidaceae on oral glucose tolerance in inbred Wistar rats

The hypoglycaemic effect of the ethanolic tuber extracts of *E. epidendraea* was determined at a dose of 100mg, and 200mg /kg by intraperitonial administration, comparing the levels of blood glucose reduction with the activity produced with tolbutamide at the 100mg dose. The effect of 100mg, 200mg, and tolbutamide on blood glucose levels of diabetic rats are shown in Table -1.

Table 1: Effect of ethanol tuber extract of *Eulophia epidendraea* Fischer (Retz.) (Orchidaceae) on alloxan - induced hypoglycemic inbred Wistar rats

Treatment	% of hypoglycemic activity			
	1.5h	3h	4.5h	24h
Normal	-5.77 ± 0.88	+2.70 ± 0.122	+8.47 ± 0.120	-6.44 ± 0.200
Diabetic	+4.70 ± 0.172	+5.72 ± 0.170	+1.59 ± 0.084	+17.70 ± 0.225
100 mg Extract	+1.44 ± 0.172	-10.59 ± 0.217	-37.87 ± 0.846	-19.67 ± 0.278
200 mg Extract	-3.50 ± 0.248	-22.15 ± 0.102	-51.597 ± 0.244	-16.643 ± 0.268
Tolbutamide	-25.66 ± 0.388	-24.35 ± 0.19	-32.32 ± 0.611	-2.66 ± 0.169

The administration of 100mg of ethanolic tuber extract brought about the maximum hypoglycaemic activity (37.87% reduction) within 4hours and thereafter glucose concentration increased which further indicates that the dose of 100 mg extract did not prolonged the effect for 24 hours. Maximum hypoglycaemic activity (51.597%

reduction) was observed in the wistar rats administered with 200mg of extract in 4.5 hr. The maximum reduction in blood glucose level obtained by using identical amounts of tolbutamide (50mg/kg of body weight) which lowered blood glucose level to 32.32%.

Table 2 shows the liver and kidney weight in control and alloxan induced diabetic rats. The dose of 200mg of tuber extract of *Eulophia epidendraea* restored the liver weight to near normal condition. The kidney weight in diabetic rats administered with *E. epidendraea* tuber extract regained the normal weight as in alloxan induced diabetic rats.

Table 2: Influence of ethanolic tuber extract of *Eulophia epidendraea* on liver and kidney weight of control and experimental animals.

Test Group	Treatment mg/kg (i.p)	Liver wt (g)	Liver wt/ 125-150 g body wt	Kidney wt (g)	Kidney wt/ 125-150 g body wt
I	Control	5.717±0.386[a]	2.787 ± 0.119[c]	1.123 ± 0.088[a]	0.583 ± 0.05[c]
II	Diabetic control	3.653 ± 0.152[b]	2.513 ± 0.221[c]	1.673 ± 0.276[b]	0.757 ± 0.09[c]
III	Diabetic + extract 100mg	3.993 ± 0.076[d]	2.77 ± 0.053[c]	1.553 ± 0 .054[d]	0.780 ± 0.017[c]
VI	Diabetic + extract 200mg	4.37 ± 0.05[d]	2.863 ± 0.161[c]	1.307 ± 0.035[d]	0.647 ± 0.061[c]
V	Diabetic + Tolbutamide	5.28 ± 0.174[d]	3.037 ± 0.094[c]	1.41 ± 0.026[d]	0.75 ± 0.058[c]

Values not sharing a common superscript (a,b,c and c) differ significantly at $p < 0.05$, Duncan's Multiple Range Test (DMRT) NO.3; Values are given as means ± SD of six animals in each group

Histologically, the islets of Langerhans usually appeared normal, and the pancreas, contained 60-70% insulin producing beta cells and 20-30% glucagon producing alpha cells (Plate-1 A). These cells may be functionally normal or abnormal, but morphologically they appeared normal. Diabetic conditions of rats showed the endocrine

cell types, irrespective of the islet size and intra-islet position was necrotic, along with the exocrine parenchymal cells (Plate-1 B). The alpha cells and delta cells could no longer be identified by their typical secretory granule morphology because of the disintegration of the granular membranes of the cells and of the intracellular organelles (Plate-1 C). In contrast to the other cell types, endothelial cells were recovered from damage when section of treated groups compared with diabetic control (Plate-1 C).

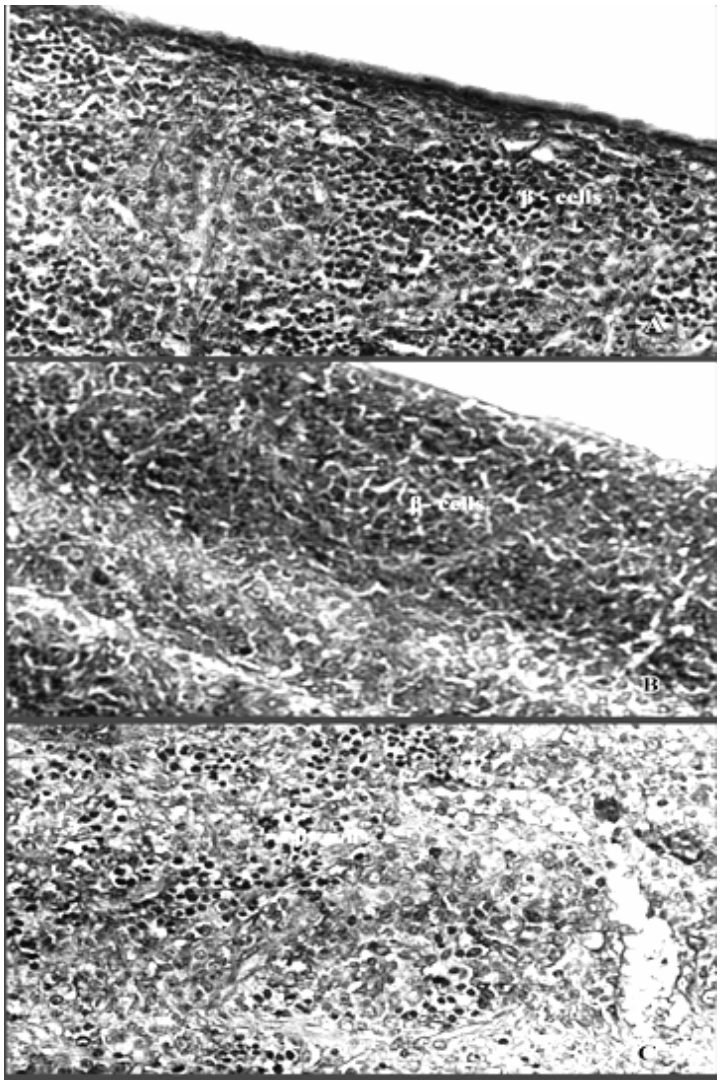

Plate 1: (a) Pancreatic histology of normal :(b) Diabetic (c) Extract treated rat models (H&E(x200)

Discussion

Diabetes mellitus is possibly the world's largest growing metabolic disease, and as the knowledge on the heterogeneity of this disorder is advanced, the need for more appropriate therapy increases (7). The present investigation indicates the hypoglycemic and protective effects of *E. epidendraea* in liver and kidney of alloxan - induced diabetic rats. It was observed that there was a significant weight gain in *E. epidendraea* - treated diabetic rats when compared with untreated diabetic rats. The optimum dosage (100 and 300 mg/kg) was standardized and confirmed by the previous study with a significant weight gain (8,9,10). This observation showed as anabolic effect of *E. epidendraea* on body weight of the diabetic animals.

The results of the present study showed that the 200mg of ethanolic - tuber extract of *Eulophia epidendraea* reduced the blood glucose levels as in alloxan induced diabetic rats. Earlier experimental studies demonstrated that plant extract treatment in allaxan - induced diabetic rat lead to a diabetic condition which resemble type 2 *diabetes mellitus*. Recent experiments demonstrated that the alcoholic extract of *B. forficata* from its leaves did not reduce glucose in diabetes induced by streptozotocin in rats (11). The methanolic extract of the aerial part of *Barleria lupulina* showed significant anti-diabetic activity in the streptozotocin- induced hyperglycemic rats (12). Mori *et al.* showed that, after oral administration, the leaf extract from *C. sicyoides* presented a potential hypoglycemic activity in hereditary diabetic mice, normal rats and rats with streptozotocin-induced diabetes(13). Similar results of hot water extract of *Camellia sinensis* (Black tea leaf) significantly reduced the blood glucose level (14). Pepato *et al.* demonstrated that 31- day administration of *B. forficata* decoction to the diabetic group caused a significant

reduction in plasma glucose. The beneficial effect of this decoction on plasma glucose level appeared around the 18th day of treatment (15). Recently, Lino, *et al*., reported that the oral administration of *Bauhinia forficata* extracts had a beneficial effect on the diabetic state reducing the hyperglycemia in allaxan - induced diabetic rat (16). The present study confirmed the ability of *E. epidendraea* to reverse the hyperglycemia of alloxan treated rats in sub-acute treatment. Evidence was presented to show that, in addition to its hypoglycemic activity, *E. epidendraea* also reduced the kidney and liver weights in diabetic rats.

Babu *et al*., reported that the oral administration of 400 to 500 mg/100 ml, of *Cassia kleinii* leaf extract reduced the blood glucose levels of allaxan - diabetic rats (17). Nammi *et al*., reported that the leaf juice of *Catharanthus roseus* L. reduced the blood glucose levels of alloxan diabetic rats (18). It was recently reported that oral administration of 20mg/kg aqueous extract of *Momordica charantia* L. fruit reduced the blood glucose levels (19). Eshrat, reported that 300mg of aqueous extract of *Abroma augusta* (L.) and leaf extract of *Coccinia indica* (L.) in equal proportions was given in Streptozotocin (STZ) induced diabetic rats which significantly both extract reduced blood glucose level after treatment of 8 weeks of treatment (20). The intake of fruits and seed powder of *Eugenia jambalana* decreased the urinary and blood glucose levels(21,22). De Paula *et al*. reported that 4*M* KOH fraction of *Rhynchelytrum repens* injected into streptozotocin - induced diabetes rats showed hypoglycemic activity, reducing blood sugar to normal levels within 24 h. This performance was better than that obtained with pure *ß*-glucan from barley, which decreased blood sugar levels within 4h(23). The present results showed that the intraperitoneal administration of ethanolic tuber extract of

E. epidendraea had a beneficial effect on the diabetic state reducing the BGL. A 200 mg dose of tuber extract of *E. epidendraea* (Retz.) Fischer (Orchidaceae) restored the liver weight to near normal condition.

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