REGENERATION OF THE β-CELLS IN THE ISLETS OF LANGERHANS BY *EUGENIA JAMBOLANA* IN STREPTOZOTOCIN-DIABETIC RATS

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Summary

Eugenia jambolana (Myrtaceae) is widely used in traditional system of medicine to treat diabetes in India. The present study was carried out to investigate the effect of methanol extract of *E.jambolana* on glucose concentrations, serum insulin and histopathology of pancreatic β-cells in STZ-induced diabetic rats. Oral administration of the methanol extract of *E.jambolana* (EJ) (150mg/kg bw) for 60days to streptozotocin (STZ) (60mg/kg bw)-induced male diabetic wistar rats was able to significantly ($p<0.05$) decrease the blood glucose concentration, comparable with the normal rats. In addition, oral administration of EJ significantly ($p<0.05$) increased serum insulin concentration by regenerating the β-cells in STZ-induced diabetic rats with the elapse of the experiment. Administration of glibenclamide, a reference drug (0.6mg/kg 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 *E.jambolana* demonstrates significant hypoglycemic effect, partly due to amelioration in the β-cells of pancreatic islets causing an increase in insulin secretion.

Keywords*: Eugenia jambolana ;D*iabetes; Hypoglycemia; Streptozotocin; β-cell.

Introduction

Diabetes mellitus, a complex syndrome is characterized primarily by the imbalance in blood glucose homeostasis leading to hyperglycemia (high glucose blood sugar) and a series of secondary complications caused by an absolute or relative lack of insulin. In conventional therapy, type 1 diabetes is treated with exogenous insulin and type 2 with oral hypoglycemic agents (1,2). Many of the oral antidiabetic agents have a number of serious adverse effects; thus, managing diabetes without any side effects is still a challenge. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of investigation. 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 hypoglycemic activity (3,4). 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 (5). The present study investigated the acute effect of the oral administration of different crude extracts of *Eugenia jambolana* on serum glucose, insulin levels and also on the histology of pancreas in STZ- diabetic rats.

The antidiabetic property of *Eugenia jambolana* has already been well established (6). Although *E.jambolana* has been used widely as a folk-lore medicine in India for diabetes for a long time, yet its effect on the histology of the β-cells of the islets of Langerhans has not been reported. Hence the study was undertaken to investigate the effect of *E.jambolana extracts* on histopathology of pancreatic β-cells, serum insulin and glucose concentrations in STZ-induced diabetic rats.

Materials and methods

Plant material

The plant used in this study, *Eugenia jambolana* seeds (EJS) were obtained commercially and were identified and authenticated by the Botany department of Holy Cross College, Tiruchirappalli and the voucher specimen is available at the Department. The air-dried seeds were powdered and 1kg powder was extracted using methanol in a soxhlet apparatus and were evaporated to dryness under reduced pressure in rotary evaporator. The yield of the methanol extract was 14.6gm%. The dry residue of the crude extract obtained was stored at 4°C for further use.

Experimental animals

Male albino rats (Wistar strain, weighing 150-220g) 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 12h day:12h 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 60mg/kg body weight) dissolved in freshly prepared 0.01M citrate buffer (pH4.5) in a volume of 1ml/kg bw. After 7 days of STZ administration, rats with blood sugar levels of 280-350 mg/dl 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 five groups of five animals each. Group I served as normal control Group II was the untreated diabetic group. Groups I and II received 0.1% carboxy methyl cellulose (CMC) orally. Group III received methanol extract of *E. jambolana*, orally at a dose of 150 mg/kg by gastric intubation, while Groups IV and V served as positive controls and received humulin (0.3IU/kg) (7) and glibenclamide (0.6mg/kg bw) (8). The treatment was continued for 60 days by administering the crude extract suspended in 0.1% CMC once daily. The rats were sacrificed at the end of 60 days for biochemical estimation.

Estimation of glucose
Blood samples were collected from tail vein in Eppendorff tubes (1.5mL) at 0^{th},15^{th}, 30^{th} and 60^{th} days and serum was separated by centrifuging the samples at 5000 rpm for 10 min and immediately analysed for glucose content by the glucose oxidase method (3).

Estimation of serum insulin
Serum insulin concentrations were determined by radioimmunoassay kit (Pharmacia, Uppsala, Sweden) with a beta metric counter (Cronex, Dupont, France). The kit included human insulin as standard and ^{125}I-labeled human insulin antibody, which cross-reacts similarly with rat insulin.

Histopathological procedure
Pancreatic tissues were harvested from the sacrificed animals on the 60^{th} day of the treatment with EJ methanol extract and the fragments from pancreatic tissues were fixed in 10% neutral buffered formaline, embedded in paraffin and then stained with haematoxylin and eosin. The histological specimens were examined under light microscopy.

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) (9). All the results were expressed as mean ± SD for six rats in each group. P-Values <0.05 were considered as significant.

Results

Serum glucose levels measured in normal and experimental rats at the end of 15, 30,45 and 60 days of treatment are given in table I. STZ-treated diabetic rats showed significant increase in the levels of blood glucose as compared to normal rats. However, oral administration of the methanol extract of EJ showed significant ($p<0.05$) hypoglycemic effect in 60 days treatment. However, the sugar lowering effect was much similar to the normal and was also comparable with the reference drug, glibenclamide. Table II presents the effect of the methanol extract on serum insulin levels in STZ-induced diabetic rats. Oral administration of the methanol extract increased the serum insulin levels significantly.

TABLE I –Effect of the different crude extracts of *Eugenia jambolana* seeds on blood glucose levels (mg/dl) in fasting normoglycemic and STZ induced hyperglycemic rats at varying days

PARAMETERS	0-DAY	15^{TH} DAY	30^{TH} DAY	60^{TH} DAY
Normal	84.4±0.55	84.8±0.44	85.4±1.14	85.6±1.14
Diabetic (STZ-60mg/kg bw)	534.6±2.07	538.8±2.77	532.6±3.71	534.6±4.88
Diabetic +Humulin (0.3 IU/ kg bw)	536.38±0.45	85.6±0.477	85.56±0.52	84.48±0.64

Diabetic + Glibenclamide (0.6mg/kg bw)	524.7±1.32	345.34±0.93	224.8±2.17	99.2±2.28
Diabetic + *Eugenia jambolana* methanol extract treated (150mg/kg bw)	523.74±3.56	330.7±4.6	206.8±5.8	84.22±1.94**

Values are means ± SD of six rats. *P<0.05

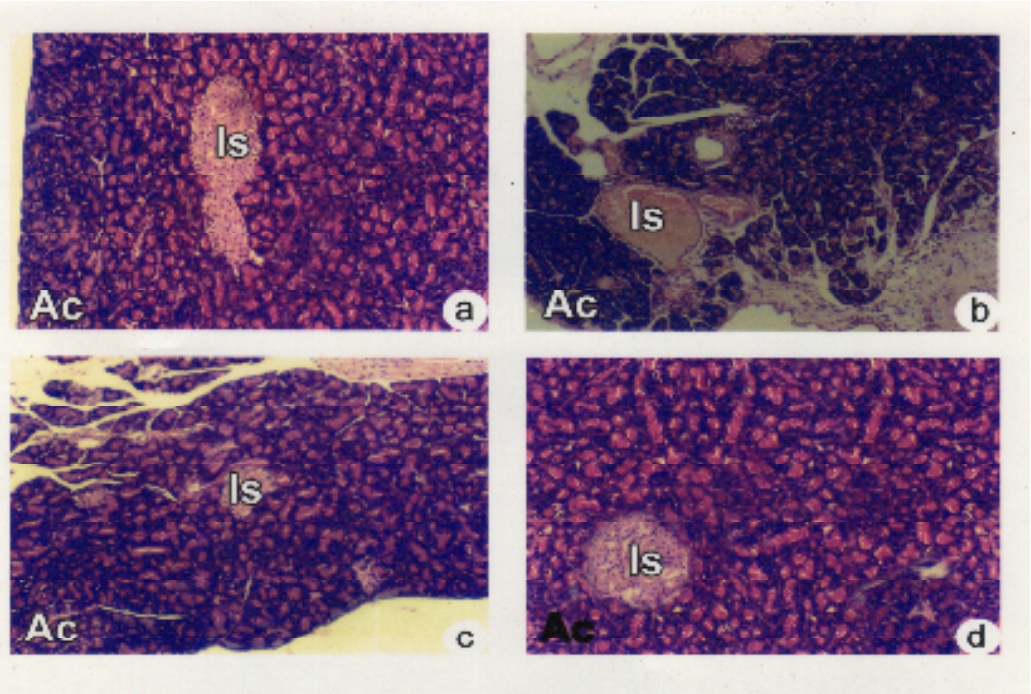
TABLE II–Effect of the different crude extracts of *Eugenia jambolana* seeds on serum insulin levels in fasting normoglycemic and STZ induced hyperglycemic rats

PARAMETERS	**Insulin** (μU/ml) (mean±SD)
Normal	15.1±1.18
Diabetic (STZ-60mg/kg bw)	6.14±0.14
Diabetic +Humulin (0.3 IU/ kg bw)	6.98±0.072
Diabetic + Glibenclamide (0.6mg/kg bw)	12.68±0.88
Diabetic + *Eugenia jambolana* methanol extract treated (150mg/kg bw)	14.84±1.96*

Values are means ± SD of six rats. *P<0.05

Hematoxylin and eosin stained sections of pancreas of untreated rats revealed that each islet of Langerhans appeared lightly stained when compared with the surrounding acinar tissue (fig a). The Islet cells were round to ovoid with round vesicular nuclei and pale pink cytoplasm. Capillaries were found in between the islet cells. Islets from the pancreas of diabetic control rats showed an entirely different picture in hematoxylin and eosin stained sections (Fig. b). They were devoid of granulated cells, showing the loss of beta cells, while the stained paraffin sections of islets from methanol extract of EJ-treated diabetic rats showed the restoration of normal architecture of the islet cells and were granulated (Fig.d). Unlike the extract treated rats, the drug (glibenclamide) treated diabetic rats failed to show total restoration of normal architecture of the islet cells (Fig.c).

Fig: Pancreatic section from a) Untreated b) diabetic control c) diabetic control treated with drug d) diabetic rat treated with plant extract.
Is- Islet of Langerhans, Ac- Acini (Hematoxylin and Eosin stain; X 100).



Discussion

Streptozotocin leads to the damage of β-cells, DNA fragmentation, decrease of glucose oxidation, impaired glucose-insulin secretion and decreased insulin action and proinsulin biosynthesis and breaks nuclear deoxyribose nucleic acid strands of the islet cells (10). The breakdown of DNA strands activates polyadenosine diphosphate ribose synthetase. This enzyme uses cellular nicotinamide adenine dinucleotide as a source of ADP ribose for DNA repair. The decline in cellular NAD concentrations ultimately results in the death of the β-cells (11,12). The loss of blood glucose homeostasis increases the sugar levels, leading to a hyperglycemic condition. The islets of man and animals exposed to toxic chemicals introduced into the environment are known to undergo destruction particularly in respect to their β–cells. Similarly, under experimental conditions too, β–cells cytotoxicity has been reported. Loss of islet mass is associated with experimental diabetes brought about by chemicals. β–cells underwent conspicuous regression after treatment with streptozotocin (13, 14, 15). As compared to a homogenously normal configuration in non-diabetic rats, the islet tissues of diabetic animals depict profound distortion in its structural organization. Streptozotocin diabetes results in degenerative and lytic changes in the islets of Langerhans of the pancreas. 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 (16, 17, 18, 19).

The loss of the insulin-producing pancreatic beta cells in diabetes accounts for the drastic drop in the insulin level in the diabetic rats. The serum insulin level decreased in diabetic animals, whereas EJ methanol 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 (20). For instance, benzoic acid-related molecules inhibit insulinase and enhance insulin effects (21). The increased levels of insulin in extract-treated diabetic rats indicated that *M. charantia* extract stimulates insulin secretion from regenerated β-cells (22). In the present study also, serum insulin level of diabetic animals treated with the extracts of EJ increased when compared to the diabetic controls. The biochemical mechanism of action appears to be through stimulation of the secretion of insulin in the regenerated β-cells as revealed by insulin assay.

However, EJ extract 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 EJ did not exhibit any toxic symptoms in the limited toxicity evaluation in male rats.

Our findings show that oral administration of EJ produces significant antihyperglycemic effect, increases serum insulin and restores the architecture of the islets of Langerhans by regenerating the β-cells in STZ-induced diabetic rats. Restoration of the pancreatic architecture by *E.jambolana* ethanol extract was reported by Sharma *et al.* (23). This investigation reveals the potential of *E.jambolana* for inducing regeneration of β-cells in STZ-induced diabetic rats and gains significance since presently, the only option to achieve permanent normoglycemia in diabetic patients is renewal of the β-cells (24).

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