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Hypoglycemic and Hepatoprotective Activity of *Eugenia jambolana* in Streptozotocin-Diabetic Rats

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Abstract: *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 liver enzymes in STZ-induced diabetic rats. Oral administration of the methanol extract of *E. jambolana* (EJ) (150 mg kg^{-1} b.w.) for 60 days to streptozotocin (STZ) (60 mg kg^{-1} b.w.)-induced male diabetic wistar rats was able to significantly ($p<0.05$) decrease the blood glucose concentration and restore normal functioning of liver comparable with the normal rats. Thus, the results of this experimental study shows that *Eugenia jambolana* possesses hypoglycemic and hepatoprotective effects and is able to ameliorate the diabetic state and is a source of potent hypoglycemic agent.

Key words: Diabetes, hypoglycemia, hepatoprotection, streptozotocin, *Eugenia jambolana*

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 (Rosak, 2002). Many of the oral antidiabetic agents have a number of serious adverse effects; thus, managing diabetes without any side effects is still a challenge (Radermecker and Scheen, 2007). 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 (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). The increase in the activities of serum alkaline phosphatase (ALP), Serum Glutamate Oxaloamino Transferase (SGOT) and Serum Glutamate Pyruvate Transferase (SGPT) indicated liver dysfunction in diabetes. Insulin deficiency leads to various metabolic aberrations in the animals namely increased blood glucose, decreased protein content, increased levels of cholesterol and triglycerides (TG), increased alkaline phosphatase (ALP) and increased activities of SGOT and SGPT (Huseini, 2006). Ohaeri (2001) also found that liver was necrotized in STZ-induced diabetic rats. The present study investigated the acute effect of the oral administration of the methanol extract of *Eugenia jambolana* on serum glucose and liver functioning in diabetic rats.

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Although *Eugenia jambolana* has been used widely as a folklore medicine in India, yet scientific validation of its hypoglycemic and hepatoprotective properties need to be established. Hence this study was undertaken to evaluate the hypoglycemic and restoration of normal liver functioning activity of *Eugenia jambolana* in STZ-induced diabetic rats.

MATERIALS AND METHODS

Preparation of Extracts

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 1 kg 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 14.6 g%. The dry residue of the crude extract obtained was stored at 4°C for further use.

Experimental Animals

Male albino rats (Wistar strain, weighing 150-220 g) bred in the Laboratory of Animal Medicine, Center 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 intra peritoneal injection of streptozotocin (single dose of 60 mg kg^{-1} body weight) dissolved in freshly prepared 0.01 M citrate buffer (pH 4.5) in a volume of 1 mL kg^{-1} b.w. 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 five groups of five animals each. Group I served as normal control Group II was the untreated diabetic control group. Groups I and II received 0.1% carboxy methyl cellulose (0.1 g CMC in 10 mL distilled water) orally. Group III received methanol extract of *E. jambolana*, orally at a dose of 150 mg kg^{-1} (dosage determined earlier) by gastric intubation, while Groups IV and V served as positive controls and received (0.3 IU kg^{-1} b.w.) humulin (Schmidt *et al*., 1999) and (0.6 mg kg^{-1} b.w.) glibenclamide (Dhanabal *et al*., 2006). The treatment was continued for 60 days by administering the plant extract 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 (1.5 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 by the glucose oxidase method (De Sousa *et al*., 2004).

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.

Measurement of Serum Glutamate Transferases (SGOT, SGPT) and Alkaline Phosphatase

Serum glutamate transferases and alkaline phosphatase enzymes were determined using commercial kits (Dialab, Austria).

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 Duncans Multiple Range Test (DMRT) (Duncan, 1957). All the results were expressed as mean±SD for six rats in each group. P-values <0.05 were considered as significant.

RESULTS AND DISCUSSION

Administration of STZ produced diabetes in rats after a week. Serum glucose levels measured in normal and experimental rats at the end of 15, 30 and 60th days of treatment are shown in Table 1. STZ-treated diabetic rats showed significant increase in the levels of blood glucose as compared to normal rats. Oral administration of 150 mg kg^{-1} b.w. of the various extracts showed significant ($p<0.05$) effect in 60 days treatment. However, the methanol extract lowered the glucose content similar to the normal and was also comparable with the reference drug, glibenclamide.

Table 2 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 better than the other two extracts.

Table 3 shows the levels of serum liver enzymes in normal and experimental rats. Administration of the methanol extract restored the levels of the serum enzymes to normal and the results were comparable with the reference drug.

Table 1: Effect of the methanol extract of *E. jambolana* on blood sugar levels in STZ-diabetic male wistar rats

	Serum glucose levels (mg dL^{-1})			
Groups	Diabetic	15th day	30th day	60th day
Normal	84.40±0.550	84.80±0.440	85.40±1.14	85.60±1.14
Diabetic control	534.60±2.070	538.80±2.770	532.60±3.71	534.60±4.88
Diabetic+humulin (0.3 IU kg^{-1})	536.38±0.455	85.60±0.477	85.56±0.52	84.48±0.64
Diabetic+glibenclamide (0.6 mg kg^{-1} b.w.)	524.70±1.320	380.28±2.390	191.68±0.93	116.14±1.74
Diabetic+EJM* (150 mg kg^{-1} b.w.)	523.74±3.560	330.70±4.600	206.80±5.80	84.22±1.94

Table 2: Effect of the 60 days treatment of the methanol extract of *E. jambolana* on serum insulin in STZ-diabetic male wistar rats

Treatments	Insulin (μU mL^{-1}) (Mean±SD)
Normal	14.06±1.180
Diabetic control	6.14±0.140
Diabetic+humulin (0.3 IU kg^{-1})	6.98±0.072
Diabetic+glibenclamide (0.6 mg kg^{-1} b.w.)	12.68±0.880
Diabetic+EJM* (150 mg kg^{-1} b.w.)	12.14±0.140

Table 3: Effect of the 60 days treatment of the methanol extract of *E. jambolana* on liver enzymes in serum in STZ-diabetic male wistar rats

Parameters	SGOT (U dL^{-1})	SGPT (U dL^{-1})	Alkaline phosphatase (U I^{-1})
Normal	5.58±0.033	6.5±0.03	114.98±0.180
Diabetic control	25.06±0.360	31.2±0.13	314.46±0.640
Diabetic+humulin (0.3 IU kg^{-1})	6.02±0.016	7.1±0.33	125.86±0.901
Diabetic+glibenclamide (0.6 mg kg^{-1} b.w.)	6.34±0.020	7.7±0.03	130.74±0.890
Diabetic+EJM* (150 mg kg^{-1} b.w.)	5.9±0.0700	7.7±0.16	131.38±1.400

*: EJM: *E. jambolana* methanol extract

The aim of the present study was to evaluate the hypoglycemic and hepatoprotective effects of the methanol extract of *E. jambolana* in STZ-induced diabetic rats. Diabetes mellitus causes a disturbance in the uptake of glucose as well as glucose metabolism. The increased levels of serum glucose in STZ-induced diabetic rats were lowered by *E. jambolana* extract. The serum glucose lowering activity of the methanol extract was compared with glibenclamide, a standard hypoglycemic drug. Glibenclamide has been used for many years to treat diabetes, to stimulate insulin secretion from pancreatic β-cells (Tian *et al*., 1998). The possible mechanism by which *E. jambolana* 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. It may be suggested that the mechanism of action of *E. jambolana* is similar to glibenclamide.

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. jambolana* 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 (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 *E. jambolana* increased when compared to the diabetic controls. The biochemical mechanism of action appears to be through stimulation of the secretion of insulin in β-cells as revealed by insulin assay.

The increase in the activities of serum alkaline phosphatase (ALP), Serum Glutamate Oxaloamino Transferase (SGOT) and Serum Glutamate Pyruvate Transferase (SGPT) indicated that diabetes may be induced due to liver dysfunction. Therefore increase in the activities of ALP, SGOT SGPT may be mainly due to leakage of these enzymes from the liver cytosol into the blood stream (Navarro *et al*., 1993), which gives an indication on the hepatotoxic effect of STZ. Treatment of the STZ-diabetic rats with the plant extract caused reduction in the activity of these enzymes compared to the diabetic and consequently may alleviate liver damage caused by STZ-induced diabetes. These results are in agreement with those obtained by El-Demerdash *et al.* (2005) diabetic in rats. Hepatic marker enzymes like ALP, SGOT and SGPT were found to be in a highly increased state in STZ-induced diabetic rats. A three fold and a five fold increase was observed in the levels of ALP and glutamate transferases, respectively. The increase in serum ALP, SGOT and SGPT levels concentrations, considered as a marker of liver dysfunction, has been rectified in STZ-induced diabetic rats, by administration of the methanol extract for a period of 60 days. This result indicates the hepatoprotective property of the plant extract.

The significant and consistent hypoglycemic effect of crude extract of *E. jambolana* 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 methanol extract to contain saponins, flavonoids and traces of steroids and phenols. Treatment of the STZ-diabetic rats with the plant extracts caused reduction in the activity of the liver enzymes compared to the diabetic and consequently may alleviate liver damage caused by STZ-induced diabetes.

E. jambolana 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. jambolana* did not exhibit any toxic symptoms in the limited toxicity evaluation in male rats.

Present findings show that oral administration of *E. jambolana* produces significant antihyperglycemic effect, restores liver functioning and, at the same time, increases serum insulin in STZ-induced diabetic rats. This investigation validates the potential of *E. jambolana* for use as a natural oral agent, with both hypoglycemic and hepatoprotective effects. Further studies to isolate and to characterize the active compound found in the methanol extract are underway to further elucidate the mechanism involved in the hypoglycemic effect.

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