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Hypoglycemic and Hypolipidemic Activity of *Eugenia jambolana* in Streptozotocin-diabetic Rats

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Abstract: The present study was carried out to evaluate the hypoglycemic and its related hypolipidemic property of *Eugenia jambolana* in streptozotocin-induced diabetic rats. *Eugenia jambolana* (EJ) methanol extracts were administered for (150 mg kg^{-1} b.w.) for 60 days to streptozotocin (STZ) (60 mg kg^{-1} b.w.) induced male diabetic wistar rats. It was found that serum glucose concentration was significantly ($p<0.05$) decreased compared to the control. In addition, oral administration of EJ significantly ($p<0.05$) decreased serum total cholesterol, LDL-cholesterol, VLDL-cholesterol, triglycerides and at the same time markedly increased serum insulin and HDL-cholesterol levels. Administration of glibenclamide, a reference drug (0.6 mg kg^{-1} b.w.) 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 *Eugenia jambolana* possesses hypoglycemic and hypolipidemic effects and is able to ameliorate the diabetic state and is a source of potent hypoglycemic agent.

Key words: *Eugenia jambolana*, diabetes, hypoglycemia, hypolipidemia, streptozotocin

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. Abnormalities in lipid profile are one of the most common complications in diabetes mellitus, which is found in about 40% of diabetics (Ravi *et al*., 2005). Diabetes induction causes increase in the cholesterol, triglycerides, LDL and VLDL (Soltani *et al*., 2007). The level of serum lipids is usually elevated in diabetes mellitus and such an elevation represents the risk factor for coronary heart disease (Rajasekaran *et al*., 2006). 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 present study investigated the acute effect of the oral administration of acetone extract of *Eugenia jambolana* on serum glucose, insulin levels and lipid profile in diabetic rats.

The medicinal properties of *Eugenia jambolana* have been well established (Sridhar *et al*., 2005). Although *Eugenia jambolana* has been used widely as a folk-lore medicine in India, yet more scientific validation of the hypoglycemic and hypolipidemic activity of the seeds needs to be established. Hence this study was undertaken to evaluate the hypoglycemic and hypolipidemic activity of *Eugenia jambolana* in STZ-induced diabetic rats.

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MATERIALS AND METHODS

The plant used in this study, *Eugenia jambolana* seeds (EJS) were obtained commercially and were identified and authenticated by the Department of Botany 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, 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} 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 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^{-1} by gastric intubation, while Groups IV and V served as positive controls and received humulin (Robert Schmidt *et al*., 1999) and glibenclamide (Dhanabal *et al*., 2006). The treatment was continued for 60 days by administering the acetone 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 (1.5 mL) at 0,15, 30 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 Cholesterol Levels

Serum total cholesterol, triglycerides, LDL,HDL and VLDL-cholesterol 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 Duncan's Multiple Range Test (DMRT). All the results were expressed as mean±SD for six rats in each group. p-values <0.05 were considered as significant.

Table 1: Effect of the different crude extracts of *Eugenia jambolana* seeds on blood glucose levels (mg dL^{-1}) in fasting normoglycemic and STZ induced hyperglycemic rats at varying days

Parameters	0th day	15th day	30th day	60th day
Normal	84.40±0.55	84.80±0.44	85.40±1.14	85.60±1.14
Diabetic (STZ-60 mg kg^{-1} b.w.)	534.60±2.07	538.80±2.77	532.60±3.71	534.60±4.88
Diabetic +Humulin (0.3 IU kg^{-1} b.w.)	536.38±0.45	85.60±0.477	85.56±0.52	84.48±0.64
Diabetic + Glibenclamide (0.6 mg kg^{-1} b.w.)	524.70±1.32	345.34±0.93	224.80±2.17	99.20±2.28
Diabetic + *Eugenia jambolana* methanol extract treated (150 mg kg^{-1} b.w.)	523.74±3.56	330.70±4.60	206.80±5.80	84.22±1.94**

Values are means±SD of six rats; *p<0.05

Table 2: 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^{-1}) (mean±SD)
Normal	15.10±1.18
Diabetic (STZ-60 mg kg^{-1} b.w.)	6.14±0.14
Diabetic +Humulin (0.3 IU kg^{-1} b.w.)	6.98±0.072
Diabetic + Glibenclamide (0.6 mg kg^{-1} b.w.)	12.68±0.88
Diabetic + *Eugenia jambolana* methanol extract treated (150 mg kg^{-1} b.w.)	14.84±1.96*

Values are means±SD of six rats; **p<0.05

Table 3: Effect of the different crude extracts of *Eugenia jambolana* seeds on serum cholesterol and triglyceride levels in fasting normoglycemic and STZ induced hyperglycemic rats

Parameters	Cholestrol	Triglyride	HDL	LDL	VLDL
Normal	89.36±0.432	66.68±0.912	59.6±0.45	78.2±0.19	21.8±0.36
Diabetic (STZ-60 mg kg^{-1} b.w.)	201.50±0.58	149.20±0.56	14.9±0.29	144.0±0.67	47.8±0.30
Diabetic +Humulin (0.3 IU kg^{-1} b.w.)	90.22±0.21	72.60±0.53	52.2±0.32	86.6±0.20	20.9±0.26
Diabetic + Glibenclamide (0.6 mg kg^{-1} b.w.)	98.00±0.34	76.20±0.72	49.2±0.19	92.6±1.28	22.9±0.33
Diabetic + *Eugenia jambolana* methanol extract treated (150 mg kg^{-1} b.w.)	96.10±0.89**	76.70±0.35**	56.46±0.22**	85.84±0.22**	22.1±0.32**

Values are means±SD of six rats; **p<0.05

RESULTS

Administration of STZ produced diabetes in rats after a week. STZ-treated diabetic rats showed significant increase in the levels of blood glucose as compared to normal rats (Table 1). 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 acetone extract lowered the glucose content similar to the normal and was also comparable with the reference drug, glibenclamide.

Oral administration of the acetone extract increased the serum insulin levels better than the other two extracts (Table 2).

There was a significant decrease in HDL-cholesterol and a significant increase in the levels of LDL, VLDL, total cholesterol and triglycerides in diabetic rats when compared to normal rats (Table 3). Administration of the acetone extract restored the levels of serum lipids to normal and was even better than the reference drug.

DISCUSSION

The aim of the present study was to evaluate the antihyperglycemic and hypolipidemic 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 the methanol extract of *E. jambolana*. 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 (Tiedge and Lenzen, 1995). 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.

Lipids play a vital role in the pathogenesis of diabetes mellitus. The level of serum lipids is usually elevated in diabetes mellitus and such an elevation represents the risk factor for coronary heart disease (Rajasekaran *et al*., 2006). High levels of total cholesterol and, more importantly LDL-cholesterol, in blood are major coronary risk factors (Bhavapriya *et al*., 2001; Hannan *et al*., 2003). The abnormal high concentration of serum lipids in the diabetic subjects is due, mainly to the increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia (Pepato *et al*., 2002; Kameswara Rao *et al*., 2003). Significant lowering of total cholesterol and rise in HDL-cholesterol is a very desirable biochemical state for prevention of atherosclerosis and ischaemic conditions (Sachdewa and Khemani, 2003). Several studies show that an increase in HDL-cholesterol is associated with a decrease in coronary risk and most of the drugs that decrease total cholesterol also decrease LDL-cholesterol (Kameswara *et al*., 2003; Nagappa *et al*., 2003).

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 flavonoids, saponins and traces of steroids and phenols. Saponins appear to involve stimulation of pancreatic β-cells and subsequent secretion of insulin (Marles and Farnsworth, 1995). *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, lowers both cholesterol and triglyceride levels and, at the same time, increases HDL-cholesterol in STZ-induced diabetic rats. This investigation reaffirms the potential of *E. jambolana* for use as a natural oral agent, with both hypoglycemic and hypolipdemic effects. Further studies to isolate and to characterize the active compound and to further elucidate the mechanism involved in the hypoglycemic effect are underway.

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