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Research Article

COMPENSATORY HYPOGLYCEMIC EFFECT OF *EUGENIA JAMBOLANA* SEED DERIVED BIO MOLECULES IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT
The research was designed to investigate Streptozotocin (STZ 55 mg/kg body weight) induced diabetic rats can be used as a model to design a drug for the treatment of diabetes. A novel compound purified from *Eugenia jambolana* seeds extract showed protein band on SDS PAGE which has the potential action of hypoglycemic so that it can be considered as drug for experimental groups. A single dose of purified protein was administered after induction of diabetes with Streptozotocin. The induction of diabetic in rats were checked by biochemical analysis revealed increase blood glucose levels in STZ induced rats than normal control, protein administrated showed decreased level of glucose and tend to bring down the level to control animals. Decreased in the content of reduced GSH in diabetic rats than control groups of rats were confirmed the compensation by the administration protein, this results has equal response with standard drug glibenclamide. Administrated of protein from *Eugenia jambolana* recovered the activities of antioxidants such as superoxide dismutase (SOD) Catalase (CAT) these enzymes activities restore to the level of normal activity in diabetic rats and protein could tends to compensate the damage in the liver and protect the liver from free radicals generated during diabetes. Western blot analysis showed the glucokinase expression increased at end of the experimental period in diabetic rats. Diabetic rats treated with administration of medicinal plant extract of *Eugenia jambolana* seed resulted in activation of β -cells and also restored almost normal architecture of liver was confirmed by histopathological examination.
Keywords: Antioxidant, Diabetes, *Eugenia jambolana* extract, SDS gel electrophoresis, Streptozotocin, western blot analysis.

INTRODUCTION

Diabetes mellitus (DM) is the most challenging metabolic disorder as it cannot be cured but needs only to be managed. Diabetes mellitus (DM) is a common metabolic disease characterized by the increased circulating glucose concentrations. It is correlated with abnormalities in variety of micro vascular, macro vascular, neurologic and infectious complications including carbohydrate, fat, and protein metabolism. It has been revealed that there is a significant overlap in the development of both diabetes and cardiac failure. Diabetes is characterized by increased metabolism of free fatty acids due to reduced glucose utilization. Oxidative stress plays an important role in chronic complications of diabetes and it is postulated to be associated with increased lipid peroxidation[1]. Enhanced oxidative stress and changes in antioxidant capacity, observed in both clinical and experimental diabetes mellitus, are thought to be the etiology of diabetic complications[2]. Mechanisms by which increased oxidative stress is involved in the diabetic complications are partly known, including activation of transcription factors, advanced glycated end products (AGEs) and protein kinase C. Elevated glucose level causes slow but significant non-enzymatic glycosylation of proteins in diabetes. There are several potential sources of increased free radical production in diabetes including auto-oxidation of plasma glucose, activation of leucocytes and increased transition metal bioavailability. Many indigenous Indian medicinal plants have been found to be useful to successfully manage diabetes[3]. Despite the introduction of hypoglycemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major problem in the world population. Streptozotocin (STZ) is an antibiotic that can cause pancreatic β-cell destruction, so it is widely used experimentally as an agent capable of inducing insulin-dependent diabetes mellitus (IDDM), also known as type 1 diabetes mellitus (T1DM). Diabetes mellitus is the world's fastest-growing disease with high morbidity and mortality rates predominantly as results of cardiovascular diseases[4]. Prospective studies have documented increased likelihood of sudden cardiac death and unrecognized myocardial infarctions in patients with diabetes[5]. Recently, various studies indicated multiple sites of impaired insulin signaling in various animal models and all the findings clearly support the existence of myocardial insulin resistance[6] insulin-stimulated glucose uptake, protein synthesis and glycogen synthesis have been shown to be reduced in the heart and cardiomyocytes of diabetic rats[7]. The management of diabetes without any side effects is still a challenge to the medical system. It is commonly known that the treatment for diabetes is relatively limited with significant side effects[8]. There is growing interest in the use of natural health products as an alternative approach to current medications. Plant source has become a target to explore new drugs and in searching biologically active compounds[9]. The streptozotocin-induced diabetic rat is still considered as an important means for the pathophysiology and pharmacology studies of diabetes mellitus. In traditional medicine plants are generally used for treatment of various acute and chronic disease and abnormalities in the body. Due to the present fast life of the human a drastic increase in chronic disease conditions mainly diabetes has been determined. The present study therefore justifies its use in the folklore remedies as an anti diabetic drug of natural origin. *Eugenia jambolana* is called as Black plum or Indian Black berry. *Eugenia jambolana* seeds have hypoglycemic, anti-inflammatory, neuropsycho-pharmacological, anti-bacterial, anti-HIV and anti-diarrheal effects and also contain several active constituents such as flavonoids, gallic acid, ellagic acid and tannins. The present study was designed to investigate the anti diabetic activity of extract of *Eugenia jambolana* seed kernel on streptozotocin- induced diabetic rats in the protective role of diabetes, which may be efficiently used to

design a drug for the treatment of streptozotocin-induced diabetic rat model.

MATERIALS AND METHODS
Experimental Animals
Wistar rats of both sexes weighing 100-150 g were used for study (Mahaveer Enterprises, Hyderabad, India). All animals were maintained under standard laboratory conditions (temperature 22 ± 2^oC and humidity 50 ± 15 %) with 12 hours day: 12 hours night cycle. The animals were fed with normal laboratory diet and allowed to drink water *ad libitum.* All protocols were performed in accordance with the Institutional Animal Ethical Committee (IAEC) as per the directions of the CPCSEA approved number UCP/IAEC/2008/027 (Committee for the purpose of Control and Supervision of Experiments on Animals).

Experimental induction of Diabetes
The animals were fasted overnight and diabetes was induced by a single intra peritoneal injection of a freshly prepared solution of streptozotocin (STZ 55 mg/kg body weight) in 0.1M citrate buffer pH 4.5[10]. The animals were allowed to drink 5 % glucose solution overnight to overcome the drug - induced hyperglycemia. Control rats were injected with citrate buffer alone. The animals were considered as diabetic, if their blood glucose values were above 250 mg/dl on the 3rd day after STZ injection. The treatment was started on the 4th day after STZ injection and this was considered as 1st day of treatment. On the third day of STZ-injection, the rats were fasted for 6 h and blood was taken from tail artery of the rats[11]. Rats with moderate diabetes having hyperglycemia were taken for the experiment. The blood was collected from sinocular puncture. Streptozotocin was purchased from Sigma-Aldrich, St. Louis, USA. All other commercial reagents used were of analytical grade.

Preparation of Plant Extract
Eugenia jambolana fruits were collected from a tree in Alagarkoill Hills, Tamil Nadu, India. The fruits of *jambolana* pulp was removed and washed with distilled water to remove the traces of pulp from the seeds. The seeds were dried and the kernel was powdered in an electrical grinder and stored at 5°C until further use. Kernel powder (100 g) was extracted with petroleum ether (60-80°C) to remove lipids. It was then filtered and the residue was extracted with 95 % ethanol by Soxhlation. Ethanol was evaporated in a rotary evaporator at 40—50°C under reduced pressure. The yield of kernel was 5 g/100 g of dried seeds. *Eugenia jambolana* extracts were used to precipitated protein and purified using DEAE-sepharose CL6B column chromatography and HPLC by the method of[12].

Experimental Design
In the experiment, a total of 24 rats (18 diabetic surviving rats and 6 normal rats) were used. The rats were divided into 4 groups comprising of 6 animals in each group as follows: The rats were divided into four groups comprising of five animals in each group and designated as follows: Group I: Control animals receiving 0.1 M citrate buffer (pH 4.5) ,Group II: Diabetic Control animals Group III: STZ-diabetic rats given *Eugenia jambolana* extract (100 mg / kg b.w/d) in aqueous solution orally for 3 times at 10 days interval for 30 days, Group IV: STZ-diabetic animals given glibenclamide (10 mg/kg b.w/d) in aqueous solution orally 3 times at 10 days

interval for 30 d. At the end of the experimental period, the rats were anaesthetized and sacrificed by cervical dislocation. The liver tissues were taken for morphological investigations and for biochemical analyses, respectively.

Biochemical Analysis
The body weight of all rats was measured at days 0 and 15. Blood samples were collected through the tail vein of the experimental animals at 0 and 15 days. In all samples, 18 h period of fasting blood glucose levels were determined by the O-toluidine method of[13]. The liver tissues was excised, rinsed in ice cold saline and then homogenized in Tris–HCl buffer (pH 7.4). The tissue homogenates were used for the following estimations: Reduced glutathione (GSH) was estimated by the method of[14] Protein was estimated by the method of[15]. The activity of superoxide dismutase (SOD) was assayed by the method of[16]. Catalase (CAT) activity was assayed by the method of[17].

Western Blot Analysis
The expression of proteins in liver tissues was detected by Western blot analysis. The proteins examined were glucokinase and glucose-6-phosphatase. The primary antibodies and HRP conjugated secondary antibodies were all purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Tissues were lysed in 600 μl lysis buffer containing 1 % protease inhibitor solution, and then centrifuged at 12,000 × g for 30 minutes to collect the soluble protein extracts from the supernatant. The protein concentration was determined by using bovine serum albumin as the standard. Protein samples were resolved on 10 % SDS-PAGE gels and transferred to PVDF membranes (Bio-Rad, Hercules, CA, USA). The PVDF membranes were then incubated with the indicated primary antibodies diluted at 1:3,000 in PBS buffer for 1 h at room temperature with HRP-conjugated secondary antibodies (1:5,000) in PBS-T blocking buffer containing 5 % dry milk. Protein bands were then detected with the Odyssey infrared imaging system (LI-COR, Lincolin, NB, USA). An antibody to β-actin at 1:10,000 was used as the loading control. The normalized expression levels of target proteins are presented as fold changes over the expression of the control samples.

Histopathological Studies
A portion of the liver tissues was taken under ether anesthesia from animals has fixed with Bouin's fixative and embedded in paraffin, solid sections were cut at 5 mm and stained with haematoxylin Eosin Masson's trichrome. The sections were examined under light microscope and photomicrographs were taken.

RESULTS AND DISCUSSION
A novel compound prepared from *Eugenia jambolana* seeds extract might posses potential hypoglycemic action through stimulation of surviving β-cells to release more insulin. Figure 1 showed protein bands had separation by SDS PAGE in which mixture on molecules were loaded under various fraction of preparation. It was evidence as visible band in all wells loaded with *Eugenia jambolana* seeds extract and indicated 182 KDa protein in the 6th well separated in the form of purified samples having intense band. The particular protein fraction alone could be administrated to experimental animals resulted its hypoglycemic potential.
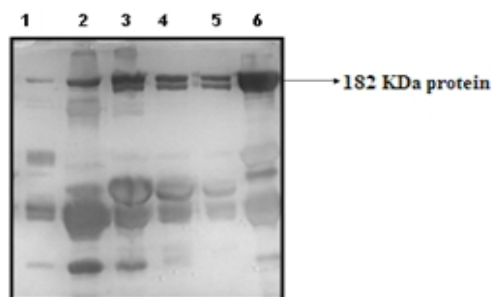
Figure 1: SDS PAGE showed *Eugenia jambolana* extracted various fraction of protein
Comparative analysis of *Eugenia jambolana* extracts were passed through column and collected fraction samples separated on 12 % SDS PAGE. Sharp intense band was found on Gel indicates 182 KDa protein. The purified protein has given to diabetic rat to confirm its anti diabetic activity in rat model.
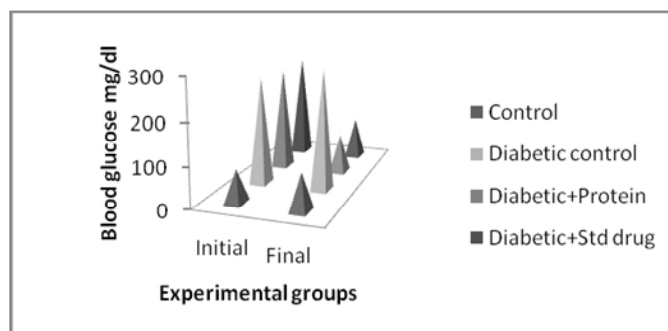

Figure 2: Level of Blood Glucose
In experimental groups 1st day of treatment indicated as Initial and 30th day mention as final in the x axis and the y axis marked the glucose content of the experimental animals. Values are given as average for experimental groups of six animals each.
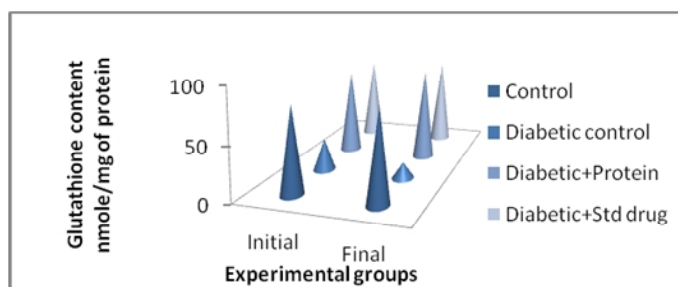

Figure 3: Glutathione content in Liver of experimental animals
In experimental groups 1st day of treatment indicated as Initial and 30th day mention as final in the x axis and the y axis marked the glutathione content of the experimental animals. Values are given as average for experimental groups of six animals each.
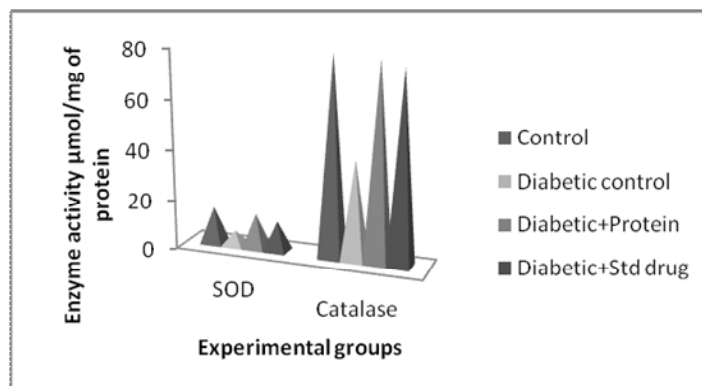

Figure 4: Activities of SOD and Catalase
Values are given as average for experimental groups of six animals each.
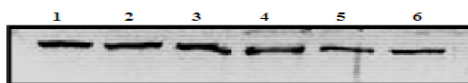
Figure 5: Western Blot analysis of 182KDa injected in Diabetic rats
Lane: 1-6 indicates the expression of liver protein in the presence 182KDa administrated in Diabetic rats during experimental period. A single dose of injections was administered after induction of Diabetes with Streptozotocin, the glucokinase level increased at end of the experimental period as compared with control.
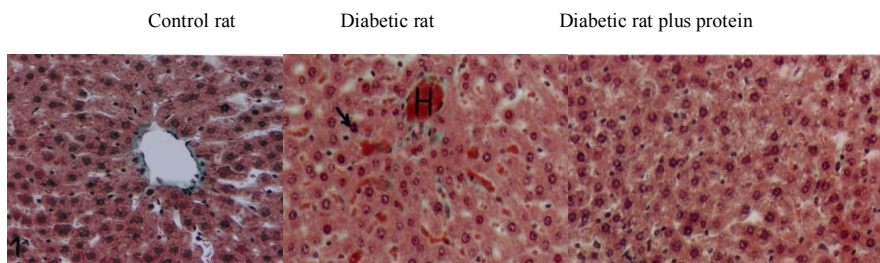
Control rat   Diabetic rat   Diabetic rat plus protein

Figure 6: Liver section for histopathological analysis
Microscopic section of histological appearance of hepatocytes. Masson X270, Pyknotic nuclei (→) in hepatocytes, hyperemia (H) in sinusoids and central vein, sinusoidal dilations (

The concentration blood glucose in control and experimental animals were measured (Figure 2) in STZ- induced diabetes rats. Marked increase in the level of blood glucose mainly associated with STZ-induced diabetes in rats showed decreased level of insulin. Extracted proteins were administrated into the diabetic animal revealed significantly decreased the level of glucose and tend to bring down the level to control animal. Figure 3 revealed the concentration of GSH in liver of control and experimental groups of rats. There was rapid decrease in the content of GSH in diabetic rats than control groups of rats. When compared the results of protein administration or glibenclamide to diabetic rats tends to compensate the concentration of glutathione almost near to normal level. Reduced GSH is known to protect the cellular system against the toxic effects of free radicals. GSH plays a significant role as a direct free radicals scavenger and also as a cofactor for many enzymes which forms conjugates in endo and xenobiotic reactions. The significant recovery of GSH content by treatment in the presence of novel protein purified from *Eugenia jambolana* seed extracts indicates the hypoglycemic activities than standard drug glibenclamide. Figure 4 showed the decreased activities of SOD and CAT in liver tissue of diabetic rats. The reduction in the activities of enzymes might be due to increased production of reactive oxygen radicals that can themselves reduce the enzyme activities. Administrated of protein from *Eugenia jambolana* recovered the activities of these enzymes to the level of normal activity in diabetic rats and protein could help to avoid the free radicals generated during diabetes.

Western Blot analysis
(Figure 5) of diabetic Liver protein with rat injected plant based protein antibody. In order to identify the presence of novel protein in *Eugenia jambolana* extract, 100 μg of liver proteins from control, diabetic rat (positive control) were resolved on 15 % SDS-PAGE and transferred on to nitrocellulose membrane (NC). The transferred proteins were allowed to react with antidiabetic protein antibody and anti rabbit IgG HRP conjugated antibody. The activity of glucokinase in liver decreased markedly, whereas the activity of glucose-6-phosphatase increased significantly in diabetic control rats. Treatment with *Eugenia jambolana* extract in

diabetic rats increased the Glucokinase activity and decreased the glucose-6-phosphatase activity nearly equal to normal whereas comparatively equal to standard hypoglycemic drug glibenclamide. It can be concluded that plant extract of *Eugenia jambolana* regenerate the damaged endocrine pancreas and thereby stimulation of insulin secretion in β cells as revealed by insulin and C-peptide.

Histopathological Examination
Liver were fixed in 10 % formalin and the sections were stained with hematoxylin eosin. The control animals showed the same microstructure (Figure 6). Excessive cytoplasmic vacuolization and pycnotic nuclei in hepatocytes were observed in diabetic rats compared to the control groups. In addition, liver sections of this group revealed sinusoidal dilations and hyperemia in sinusoids and central veins (Figure 6). In diabetic group receiving protein an ordinary histological appearance was accompanied by minor vacuolization in some cells. In diabetic group receiving *Eugenia jambolana* extract ordinary histological appearance was accompanied by minor vacuolization in some cells. Liver might also be affected by the changes in the levels of insulin. It is known that structural changes occur in the liver as a result of the absence of insulin in diabetes. Excessive cytoplasmic vacuolization, pyknotic nuclei and a lot of lipid droplets are accepted as an indicator of hepatotoxic effect. The increases in the vacuolization and pyknotic nuclei are important findings indicating that hepatocytes were either dying or heading for necrosis.

CONCLUSION
Administration of purified 182 KDa compound from extract of *Eugenia jambolana* seeds to diabetic rats significantly recovered the levels of blood glucose, antioxidant enzyme properties and liver structural organization resulted in an increased in body weight compared to untreated diabetic rats. Experimental results (data not shown) revealed a decrease in body weight in STZ-diabetic rats was compensated in treatment with extract from *Eugenia jambolana* thereby improved the body weight in diabetic rats, which could be attributed to its anti diabetic effect of compound present in *Eugenia jambolana* seeds. Present studies showed 10 mg/kg

doses protein was injected in streptozotocin induced diabetic it showed compensatory effects by minimizing the cellular damage and also to know whether it has any protective effect by inducing diabetic in rat performed experiments by injecting purified protein in Diabetic Rats. The prepared compound formulated as drugs that could be used by clinicians in the treatment of their diabetic patients. .

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