Advance Research in Pharmaceuticals and Biologicals

(A Peer Reviewed International Journal for Pharmaceutical and Allied Research)

ISSN 2250-0774 USA CODEN: ARPBGZ

PREVENTION OF DIABETIC CARDIOMYOPATHY BY A NOVEL PROTEIN OF *EUGENIA JAMBOLANA* SEEDS ON STREPTOZOTOCIN INDUCED DIABETES IN RATS.

***G. Renuga[1], A. B. Thandapani[1] and A. K. J. Arul[2]**

[1]Department of Pharmaceutical Biotechnology, Ultra College of Pharmacy, Madurai, Tamil Nadu, India.

[2]Department of Oral Pathology, Ultra Best Dental science college, Madurai, Tamil Nadu, India.

Received on 10/12/2013 Revised on 19/12/2013 Accepted on 26/12/2013

ABSTRACT:

In the present study the antioxidant effect of oral administration of purified protein from ethanolic extract of *Eugenia Jambolana* seed kernel on protective effect of streptozotocin-induced diabetic rats was evaluated. Administration of a novel protein to diabetic rats significantly decreased the levels of blood glucose, glycosylated hemoglobin and increased body weight gain and biochemical parameters. The diabetic rats showed the low activities of glutathione peroxides and reduced glutathione content heart tissues which were restored to near normal levels by experimental treatment with protein of *Eugenia Jambolana*. The increased levels of lipid peroxidation and hydroperoxides in diabetic rats were reverted back to near normal levels after the treatment and restored almost normal microarchitecture of heart which was confirmed by histopathological examination. The present study reveals the efficacy of *Eugenia Jambolana* seed kernel in the amelioration of diabetes cardiomyopathy which may be attributed to its hypoglycemic property along with its antioxidant potential.

Keywords: Diabetes, *Eugenia Jambolana*, Glibenclamide, Streptozotocin.

***Corresponding Author:**
Dr. G. Renuga M.Sc. Ph.D
Professor& Research coordinator,
Department of Pharmaceutical Biotechnology
Ultra College of Pharmacy,
Madurai, Tamil Nadu, India
Mob : 9443795813
Email: renugabiog@gmail.com

INTRODUCTION

Diabetes mellitus is the most common metabolic disorder characterized by hyperglycemia that results from an absolute or relative insulin deficiency. Diabetes mellitus is classified into two types, insulin dependent diabetes mellitus (IDDM, Type 1) and non-insulin-dependent diabetes mellitus (NIDDM, Type 2). Type I diabetes is an autoimmune disease characterized by a local inflammatory reaction in and around islets that is followed by selective destruction of insulin-secreting β cells. Type II diabetes is characterized by peripheral insulin resistance and impaired insulin secretion. Diabetes is characterized by increased metabolism of free fatty acids due to reduced glucose utilization and associated with long-term complications affecting the eyes, kidneys, heart and nerves[1].

Diabetes causes various cardiovascular complications, which have become the major cause of morbidity and mortality in the diabetic population. Moreover, mortality from cardiac diseases is approximately two- to fourfold higher in patients with diabetes than in those who have the same magnitude of vascular diseases without diabetes, and diabetic cardiomyopathy can occur without any vascular pathogenesis. Several studies have shown that hyperglycemia as an independent risk factor directly causes cardiac damage, leading to diabetic cardiomyopathy but the mechanisms for the pathogenesis remain unclear. Hyperglycemia is the underlying abnormality characterizing the diabetic condition. Chronic hyperglycemia introduces a plethora of complications such as cardiovascular disease, which is the most frequent cause of death in the diabetic

population[2]. Diabetic patients have a poorer prognosis post-myocardial infarction as well as an increased risk of subsequent heart failure.

Diabetes mellitus, a well recognized risk factor for the development of heart failure, was initially linked to heart disease 30 years ago with the Framingham Heart Study[3].Cardiac failure in diabetes is primarily due to ischemic heart disease caused by coronary artery disease[4]. However, it is proposed that the incidence of heart failure in diabetes except for atherosclerotic coronary lesions was 4-5 times higher compared to nondiabetics[5]. Rubler et al. propounded the concept that diabetic cardiac failure without coronary lesions is classified as diabetic cardiomyopathy and it is caused by systolic and diastolic dysfunction due to diabetes itself [6]. Conversely, patients with heart failure are also at a higher risk of developing diabetes[7].

Eugenia Jambolana LAM (Fam: Myrtaceae) is commonly called as Jamun, Black plum or Indian Black berry. The present study reveals the efficacy of *Eugenia jambolana* seed kernel on streptozotocin- induced diabetic rats in the amelioration of diabetes, which may be attributed to its hypoglycemic property along with its antioxidant potential. The efficacy was compared with a standard hypoglycemic drug glibenclamide.

MATERIALS AND METHOD

Experimental Animals

Wistar rats of both sexes weighing 100-150gm were used for study (Mahaveer Enterprises, Hyderabad). All animals were maintained under standard laboratory conditions (temperature 22±2°C 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 (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 intraperitoenal injection of a freshly prepared solution of streptozotocin (STZ, 55 mg/kg body weight) in 0.1M citrate buffer pH 4.5[8]. 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 250mg/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[9]. 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 Soxhletion. 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.

Experimental Design

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 5 d Group IV: STZ-diabetic animals given glibenclamide (10mg/kg b.w/d) in aqueous solution orally for 5 d. All animals were fasted for 18 hr, before experimentation, but allowed free access to water. Blood samples were collected for the measurement of blood glucose level by puncture of retero-orbital plexus at 0hr, 2hr, 4hr and 6hr from control and test group animals after feeding the plant extract. At the end of the experimental period, the rats were anaesthetized and sacrificed by cervical dislocation. The heart tissue samples 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[10]. The levels of glycosylated hemoglobin were estimated using the diagnostic kit from Biosystems, Spain. The heart tissues were 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[11] Protein was estimated by the method of[12].

ISSN 2250-0774
(RESEARCH ARTICLE)

The activity of Lipid peroxidase (GPx) was assayed according to the method of [13].

Histopathological Studies:

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

RESULTS AND DISCUSSION

Eugenia jambolana seed extracts were passed on various level of chromatography for separation of different fraction and precipitated the protein which were analyzed by SDS gel electrophoresis using silver staining of DEAE column fraction separated bands confirmed its size as 182KDa novel protein (Fig 1).The particular protein fraction alone could be eluted from gel and then used for experimental animals as treatment drug for the prevention of diabetic cardiomyopathy in rats induced by streptozocin.

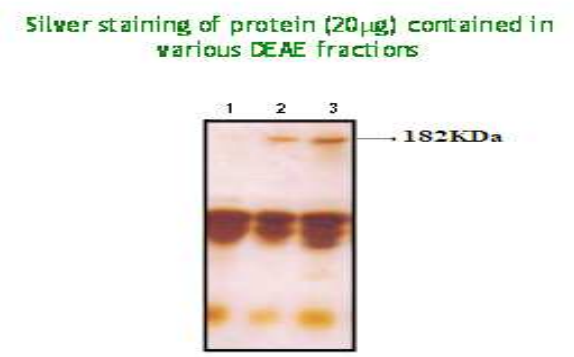

Fig. 1: TLC of Novel Protein of *Eugenia jambolana*

Figure 2 showed the various fractions collected from chromatography separations of *Eugenia jambolana* extract and samples were pooled then purified protein compounds analyzed by HPLC. The data confirmed the presence of novel molecules which has size as182 KDa protein and its potential properties exhibited when administrated into experimental animals.

Table1: Effect of purified protein on experimental animals

	Heart wt. in mg	Body wt. in g	Heart wt. wt.x100 / Body
Normal	124	33.3	372
Diabetic			
1st week	105	25	454
5th week	95	21.7	454
7th week	97	20	454
10th week	83	16.7	484
12th week	81	11.6	490
Protein injected 7th week 25µg	117	26.1	420

The data are expressed as mean ± SEM (standard error of mean) of six experiments.*P<0.05, when compared to control.

The STZ control group did not show reduced body weight gain, but the diabetic group showed a significant decrease in body weight gain, which was prevented by supplementation of protein purified from *Eugenia jambolana* seed*s* extract that also significantly reduced glucose levels in the diabetic rat (Table 1).

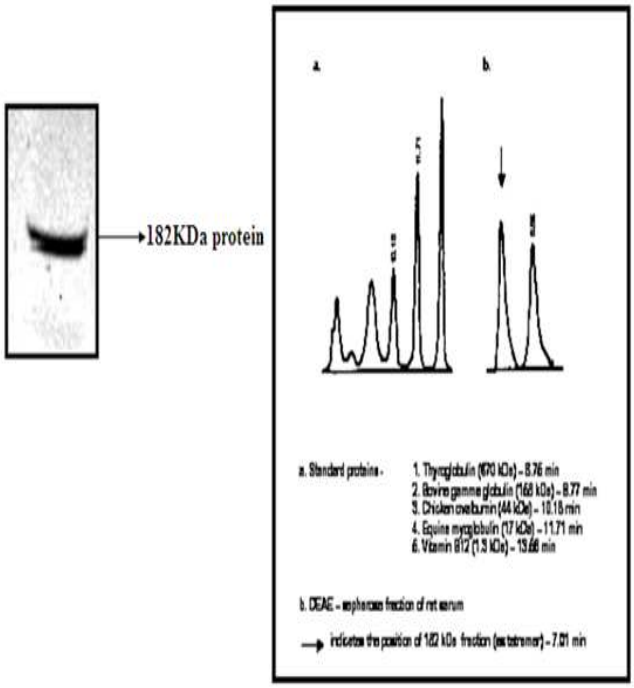

Fig.2: HPLC of Purified *Eugenia jambolana*

Figure 3 revealed the level of glucose in control and experimental groups of diabetic rats induced by streptozocin. There was a significant increase in the level of blood glucose in diabetic rats and administration of 182KDa protein prepared from *Eugenia jambolana* seed*s* and glibenclamide to diabetic rats significantly decreased the level of blood glucose. After a carbohydrate containing meal 70% of the glucose load is delivered via the portal circulation. Some of the glucose is oxidized and some is converted to glycogen for use as a fuel under fasting conditions. Glucose in excess of requirement was partly converted by the liver to fatty acids and triglycerides, which are then incorporated into VLDLs and transported to adipose tissue stores.

Figure 4 showed the concentration of lipid peroxide of control and experimental groups of rats. There was a slight elevation in diabetic rats which restore near to normal level by treatment of natural protein purified

ISSN 2250-0774

from plant and results were compared with standard drug.

Figure 5 demonstrate the concentration of GSH in heart tissues of both control and experimental groups of rats. A significant decrease in the content of glutathione was observed in diabetic rats when compared to control group of rat Administration of 182KDa protein prepared from *Eugenia jambolana* seed*s* and glibenclamide to diabetic rats tends to bring the concentration of GSH to near normal level. Intra-peritoneal administration of STZ selectively destroys the pancreatic insulin secreting ß- cells living less number of active cells and resulting in type-1 diabetic state. Similar results were obtained in the present study by both histopathological examinations and by serum glucose estimation.

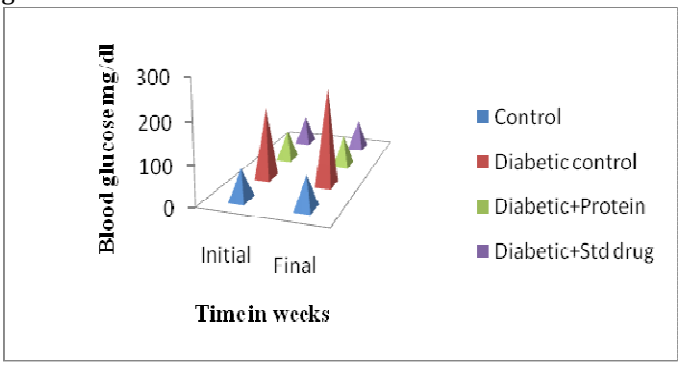

Fig 3: Blood Glucose Level in control and Experimental rats.

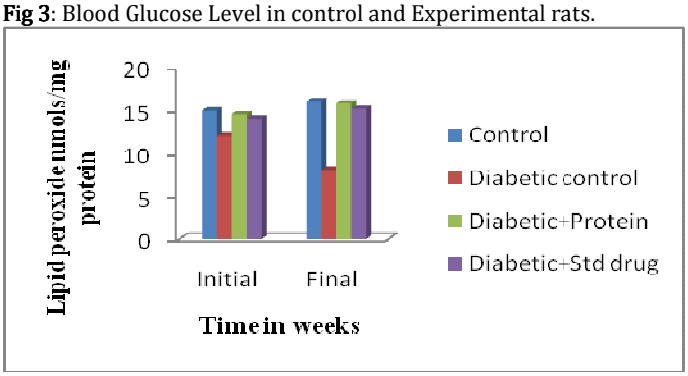

Fig. 4: Lipid Peroxide level in heart tissues of Rats

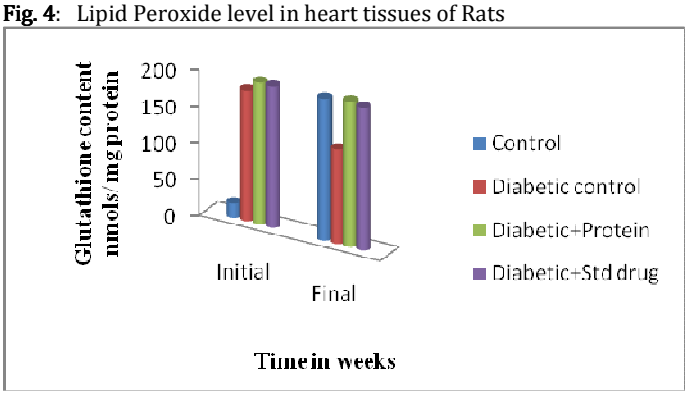

Fig 5: Reduced Glutathione content in experimental Rats

Legend: Common for fig 3, 4 &5 .Values are given as average mean of groups of six animals each. X-indicated period of experiments in which Initial means 1st week of in experimental period; Final means 4th weeks of experimental period. Y-indicates measurement biochemical parameters such as Blood glucose (Fig 3), Lipid Peroxide (Fig 4) and Glutathione content in heart in tissues of control and experimental rats.

The results of the present study have shown that of *Eugenia jambolana* extract have potent antidiabetic effects in the treatment of STZ induced diabetic in rat and compared with standard glibenclamide was effective in moderate diabetic rats not in severe diabetic animals. However, the purified protein effect of the extracts was more potential than glibenclamide.

Hematoxylin and eosin (H & E) staining of the heart tissues (Figure 6) showed that compared with vehicle control diabetic hearts displayed structural abnormalities but the hearts from the STZ control group did not show the same alterations.

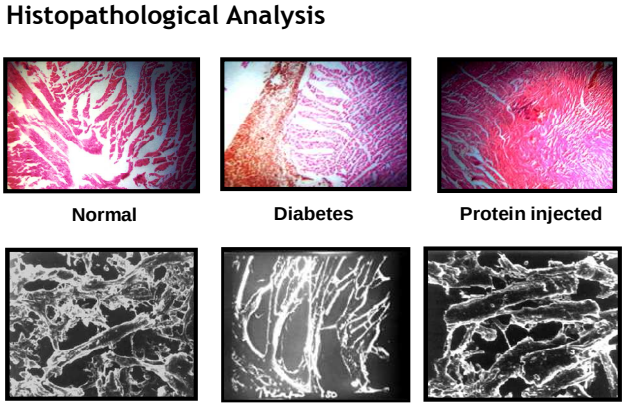

Fig.6: Histopathology of *Eugenia jambolana* extracts

More important, structural abnormalities in the heart of diabetic rats were completely prevented by *Eugenia jambolana* extracts administration. Diabetic cardiomyopathy is characterized by a reduction in cardiac mass over time, myocardial hypertrophy and interstitial and perivascular fibrosis at late phase. Because myocytes rarely proliferate in adult cardiac muscles, the loss of cardiac muscle cells would eventually lead to compromised cardiac function.

Moreover, elucidation of the mechanisms concerning obesity and diabetes mellitus contributes to the prevention of metabolic syndrome and the appearance of effective new medications available to treat diabetic cardiomyopathy formulated as drugs that could be used by clinicians in the treatment of their diabetic patients.

REFERENCE

1. E. L. Barr, P. Z. Zimmet, T. A. Welborn, D. Jolley, and D. J. Magliano. Risk of cardiovascular and all-cause mortality in individuals with diabetes mellitus, impaired fasting glucose, and impaired glucose tolerance: the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab), Circulation 116: 151-157 (2007).
2. A. G. Bertoni, A. Tsai, E. K. Kasper, and F. L. Brancati. Diabetes and idiopathic cardiomyopathy: a nationwide case-control study, Diabetes Care 26: 2791- 2795 (2003).
3. B. K. William, H. Marthana, and P. C. William. Role of diabetes in congestive heart failure: The Framingham Study, Am J Cardiol., 34: 29-34 (1974).
4. Y. Kohda, T. Tanaka, H. Matsumura. Effects of Thiamine Repletion on Cardiac Fibrosis and Protein *O*-Glycosylation in Diabetic Cardiomyopathy, J Diabetes Metabolism S7:001. doi:10.4172/2155-6156.S7-001 Page ISSN: 2155-6156 (2012).
5. S. K. Mukherjee, L. K. Klaidman, R. Yasharel, J. D. Adams. Increased brain NAD prevents neuronal apoptosis in vivo, Eur J Pharmacol., 330: (1) 27-34 (1997).
6. J. P. Piccini, L. Klein, M. Gheorghiade, and R. O. Bonow. New insights into diastolic heart failure: role of diabetes mellitus, Am J Med., 116: 64S-75S (2004).
7. M. A. Weber, G. L. Bakris, K. Jamerson, M. Weir, and S. E. Kjeldsen. Cardiovascular events during differing hypertension therapies in patients with diabetes, J Am Coll Cardiol., 56: 77-85 (2010).
8. N. Sekar, S. Kanthasamy, S. William, S. Subramanian, and S. Govindasamy. Insulinic actions of vanadate in diabetic rats, Pharmacol. Res,. 22: 207-217 (1990).
9. R. Burcelin, M. Eddouksm, J. Maury, J. Kande, R. Assan, J. Girard. Excessive glucose production, rather than Insulin resistance, accountfor hyperglycemia in recent onset streptozocin-diabetic rats, Diabetologia 35: 283-290 (1995).
10. A. Relander, C. E. Raiha. Differences between the enzymatic and toluidine methods of blood glucose determination, Scandinavian Journal of Clinical and Laboratory Investigation 15: 221–224 (1963).
11. J. Sed gb']-+lak, R. H. Linday. Estimation of total protein bound and non protein sulhydryl groups in tissue with Ellman's reagents, Analytical Biochemistry 25: 192-205 (1968).
12. O. H. Lowry, N. H. Rosebrough, A. L. Farr and R. J. Randal. Protein measurement with the Folin phenol reagent, *J. Biol. Chem.*, 193: 256-275 (1951).
13. D. Braham and P.Tinder. An improved color reagent for the determination of blood glucose by the oxidase system, Analyst 97: 142-45 (1972).