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Research Article

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Antidiabetic and Nephroprotective of Emblica Officinalis on Streptozotocin Induced Toxicity in Swiss Albino Mice

Pramod Shankar[1], Arun Kumar[2]*, Ranjit Kumar[2], Mohammad Ali[2] and Sudhir Kumar Srivastava[1]

[1]*Department of Zoology, Patna University, Patna Bihar, India*

[2]*Mahavir Cancer Sansthan and Research Centre, Patna, Bihar, India*

***Corresponding author:**
Arun Kumar, Mahavir Cancer Sansthan and Research Centre, Phulwarisharif, Patna, Bihar-801 505, India, Phone: 091- 612- 2258510/ Extn-307; Mobile Phone: +91- 9334740800/ 8294439121; Tele Fax: 091-612- 2253957; E-mail: arunk31@rediffmail.com, arunk3@rediffmail.com

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Abstract

Diabetes mellitus in the present date has become a major health concern problem in the population worldwide. The drugs available for the medication are very limited and have side effects hence new drug discovery is required. The present study is aimed to investigate the anti- diabetic and antitoxic effects of Emblica officinalis (Amla) fruit extract on Streptozotocin induced diabetic mice.

The study was approved through the Institutional Animal Ethics Committee of the institute. Mice were grouped into 3 groups – Control (n=6), Streptozotocin treated (n=12) and Emblica officinalis administered group (n=6). Treated group mice were administered with Streptozotocin 100 mg/kg body weight intraperitoneally. After the development of diabetes in mice the aqueous fruit, extract of Emblica officinalis at the rate of 100 mg/kg body weight was administered for 4 weeks to evaluate its anti- hyperglycemic activity. There serum glucose levels as well as the Kidney Function Tests (KFT) – urea, uric acid and creatinine levels were analyzed statistically using ANOVA and Dunnett's tests. In the present study, there was significant increase in the serum glucose levels in the Streptozotocin induced diabetic group in comparison to control group while in the Emblica officinalis group there was significant decrease in the glucose denotes the antidiabetic effect. The KFT showed significant elevation in the levels in Streptozotocin induced groups while the Emblica officinalis showed significant decrease in in the KFT levels denotes the antitoxic effects. Thus, from the entire study it can be concluded that Emblica officinalis can be used as a potent natural anti-diabetic drug, which can control the diabetes at much extent. Furthermore, it also prevents the kidney from the diabetic damage and restores of cellular status of the kidney.

Keywords: Antidiabetic, Emblica Officinalis, Streptozotocin, Kidney Function Test

Introduction

Diabetes mellitus (DM) is currently the major metabolic endocrine disorder causing serious health related issues in the population worldwide. Presently, 7% population worldwide are affected with the disease [1]. It is estimated that 422 million adults were living with diabetes in 2014, compared to 108 million in 1980 [2]. This disease is either inherited or a lifestyle related auto immune disorder which causes insufficient amount of insulin secretion in the individual leading to cause diabetes type-I or type II. It furthermore causes, nephropathy, neuropathy and retinopathy in the individuals who are have very high blood glucose levels. In addition to the classical condition of elevated sugar levels, diabetes also affects the metabolisms of carbohydrate, fat and protein, which in the long term causes severe complications to the individual, which is more fatal than the primary disease [3]. In diabetes, oxidative stress has also been found as one of the major cause of the disease, which occurs due to increased production of oxygen free radicals and failure of antioxidant defense mechanism [4, 5]. Antioxidants thus play a very vital role to protect the human body against the damage caused by free radicals [6]. Hence, compounds having both hypoglycemic and antioxidative properties can be a novel antidiabetic agent [7].

There are limited therapeutic agents being prescribed for the control of diabetes, which includes use of oral hypoglycemic and insulin injections. Hence, there has been search for the novel drug discovery, which has reduced toxicity and preferably are of dietary origin. A plethora of medicinal plants has been reported in the *Ayurveda* (Indian medicine system) which has potent antidiabetic effect with very least side effects. In them, *Emblica officinalis* (Amla) has been found to be a potent medicinal plant, which has been used commonly as Indian traditional medicine. It belongs to the family Euphorbiaceae and various parts of this plant such as fruit, seed, root, bark and flowers are widely used in preparation of *ayurvedic* medicines. One of the most popular is decoction of fruit, which is traditionally used against low immunity, common cold, fever, asthma, cholesterol and glucose levels. It contains phenolic compounds like

tannins, phyllembelic acid, phyllemblin, curcuminoides, rutin and emblicol [8]. Extracts of E. *officinal* is have been also reported to possesses hypolipidemic, anti-obesity, anti-diabetic, anti-cancer, hepatoprotective, antioxidant, and anti-inflammatory [9-14]. Hence, present study aims to know the anti-diabetic effect of *Emblica officinalis* on Streptozotocin treated mouse model.

Materials and Methods

Animals: Twenty four Swiss albino mice (28g to 32 g) were obtained from animal house of Mahavir Cancer Institute & Research Centre, Patna, India (CPCSEA Regd. No. 1129/bc/07/CPCSEA, dated 13/02/2008). The research work was approved by the IAEC (Institutional Animal Ethics Committee) with no. IAEC/2011/12/04. Food and water to mice were provided *ad libitum* (prepared mixed formulated feed by the laboratory itself). Animals were maintained in colony rooms with 12 hrs light/dark cycle at 22 ± 2°C.

Chemicals: Streptozotocin was purchased from the Himedia, India. Commercially available kit for chemical analyses like Serum Glucose levels and kidney function tests (KFT) – Urea, Uric acid and Creatinine were used of crest coral clinical system, Goa, India.

Plant Material: The fresh fruit of *Emblica officinalis* (Local name-Amla) were procured from local market (Patna). Dr. Ashok Kumar Ghosh (Botanist), Department of EWM, A.N. College, Patna, Bihar, India, confirmed the identity of the fruit. The fruit were washed with distilled water, dried completely through hot air oven, and crushed with electrical grinder coarse powder. Extract was made by dissolving it in distilled water using by mortal and pistal. The dose was finally made to 200 mg/kg body weight for oral administration.

Induction of Diabetes: Swiss albino mice were induced diabetes by administration of dose of Streptozotocin at the rate of 100 mg / kg body weight through *intra peritoneal* (i.p) method.

Experimental Design: In the present study 24 mice (18 diabetic surviving and 6 as control mice) were taken and divided into groups - control, streptozotocin treated and *Emblica officinalis* treated. The Streptozotocin at the rate of 100 mg /kg body weight were administered i.p for making the Streptozotocin induced diabetic mice model. To this Streptozotocin treated group aqueous rhizome, extract of *Emblica officinalis* at the rate of 100 mg / kg body weight was administered for 4 weeks. During the experimental period, the glucose levels were monitored in diabetic group as well as in *Emblica officinalis* treated group regularly. After the completion of the experiment blood samples were collected by orbital sinus puncture method and then serum was extracted.

Statistical Analysis: Results are presented as mean ± S.D and total variation present in a set of data was analysed through one-way analysis of variance (ANOVA). Difference among means has been analysed by applying Dunnet's't' test at 99.9% ($p< 0.001$) confidence level. Calculations were performed with the Graph Pad Prism Program (Graph Pad Software, Inc., San Diego, USA).

Results: The serum glucose levels shows inclination in the levels in the Streptozotocin induced diabetic group in comparison to control group while the *Emblica officinalis* shows the glucose lowering down activity denotes the antidiabetic effect. The kidney function test shows elevation in the levels of Urea, Uric acid and Creatinine levels in Streptozotocin induced groups while the *Emblica officinalis* shows declination in the KFT levels denotes the antitoxic effects (Figure1-4).

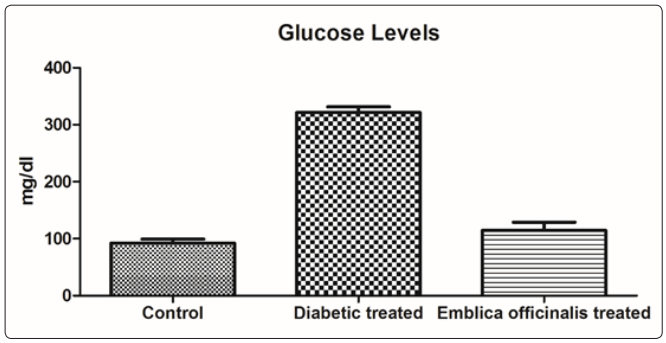

Figure 1: Effect of Emblica officinalis on Diabetic induced group showing Glucose levels (n=6 values are mean ± S.D)

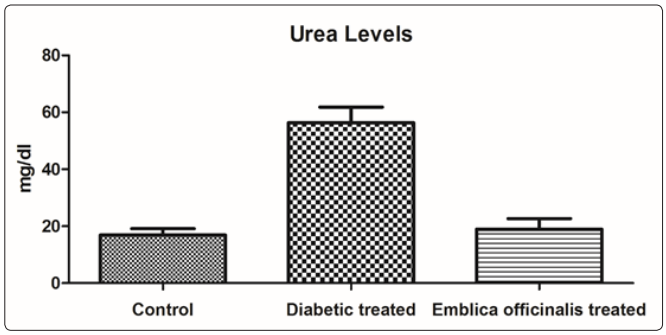

Figure 2: Effect of Emblica officinalis on Diabetic induced group showing Urea levels (n=6 values are mean ± S.D)

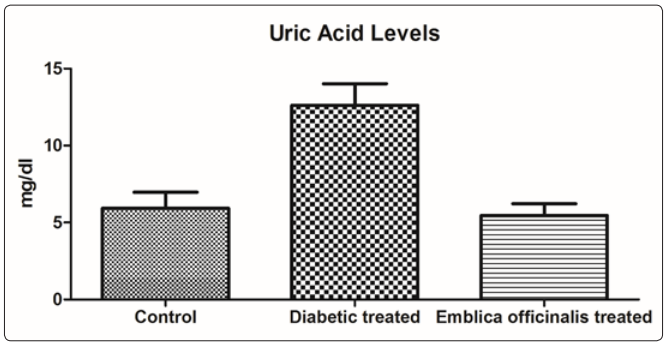

Figure 3: Effect of Emblica officinalis on Diabetic induced group showing Uric Acid levels (n=6 values are mean ± S.D)

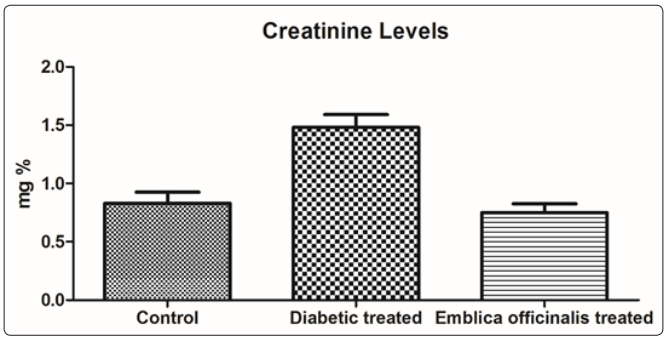

Figure 4: Effect of Emblica officinalis on Diabetic induced group showing Creatinine (n=6 values are mean ± S.D)

Discussion

Diabetes mellitus is a disease inherited or inadequate secretion of the hormone insulin type I or insulin dependent diabetes mellitus (IDDM) or due to inadequate response of target cells to insulin type II or non-insulin dependent diabetes mellitus (NIDDM) or combination of both the factors. In addition to the hyperglycemia, diabetes also affects the carbohydrate, fat and protein metabolism, which in long term leads to severe complications, which is more fatal than the primary disease. The chronic elevation of the blood glucose levels damages the blood vessels leading to microvascular and macrovascular disease. The microvascular disease in long term causes retinopathy, nephropathy and neuropathy while macrovascular disease in long term causes coronary artery disease leading to myocardial infarction or angina, stroke and peripheral vascular disease, which causes diabetic foot [3, 15].

The major regulatory hormone for intermediary metabolism of glucose is insulin, produced and secreted by the β-cells of the islets of Langerhans of the pancreas. Impaired control of blood glucose concentrations by insulin leads to diabetes mellitus. Many kidney diseases are also reported, however it is also well recognized that diabetes progressively affects systems such as kidneys, retina, heart, peripheral and central nervous system and possibly liver and is thus systemic in nature.

The diabetic nephropathy is the most common cause of end stage kidney disease (ESKD) and most of the diabetic patient with ESKD have diabetes type II. This kidney disease causes decline in the glomerular filtration rate (GFR), ranging from 2 – 20 ml min-1 yr-1 [16, 17]. Blood pressure control is known to be important in preventing adverse cardiovascular and renal outcomes in diabetic patients with hypertension [18]. However, it is not clear whether blood pressure is an important predictor of GFR decline in diabetic patients with CKD in whom blood pressure is controlled.

In the present study, there was significantly very high levels of glucose, and kidney function test levels – urea, uric acid and creatinine levels. As it is well known that uric acid is the final product of purine metabolism. And its concentration is determined largely by the efficiency of renal clearance and rate of purine metabolism [19-22]. While it has important antioxidant property *in vivo* and *in vitro* [23, 24]. Moreover, uric acid is formed from guanine and hypoxanthine via xanthine in reactions catalyzed by guanase and xanthine oxidase of liver, small intestine and kidney. Urea is the product of protein metabolism, is increased and serum level of urea increases [25, 19]. Revealed that production of oxygen free radicals by arsenic induces tubular necrosis, which in turn increases tubular permeability, resulting in diffusion and back leak of the filtrate across the tubular basement membrane back into the interstitium and circulation, leading to an apparent decrease in GFR. Under these circumstances, back leak of filtrate results in decreased excretion and increased retention of nitrogenous waste i.e. urea in serum [26, 27].

In the present study, there was significant normalization in the levels of glucose, urea uric acid and creatinine denotes the ameliorative effect of *Emblica officinalis*. Various studies have shown the similar effect as in a studies, it has been confirmed that, amla ameliorates alloxan, streptozotocin (STZ) and high fat diet fed to rats [11, 28-30]. In one of the earliest studies, observed that oral administration of the methanolic extract of the amla fruits (100 mg kg per Kg body weight) caused hypoglycemia in both normal and diabetic rats (alloxan-induced) [31]. The anti-hyperglycemic effects of amla were observed to be better when the extract was administered continuously for 11 days. In addition to the methanolic extract, studies have also shown that the aqueous extract of the fruits was effective in reducing the serum glucose and glycosylated hemoglobin (HbA1C), which are comparable to that of the anti-diabetic drug chlorpropamide. The fresh juice and the hydroalcoholic extracts were also shown to decrease the elevated levels of fasting blood glucose and increase the levels of serum insulin in STZ-induced diabetic rats [32, 33].

Diabetic nephropathy is the most common cause of chronic renal failure and end stage kidney disease and is linked with increased mortality and morbidity. Gallotanin is an important constituent of amla, which possesses nephroprotective effects on rats. It decreases the levels of plasma creatinine and to reduce apoptosis by inhibiting poly- ADP- ribose polymerase (PARP) cleavage [34, 35]. The hyperglycemia in cells is caused due to glycation of reactive dicarbonyls, which in turn causes pathogenesis of sensory neuron damage [36]. The Emblica officinalis have shown to be effective in ameliorating diabetic neuropathy and to decrease the behavioral, biochemical and molecular alterations in diabetic models [37-39].

Thus, from the entire study it can be concluded that *Emblica officinalis* can be used as potent natural anti-diabetic drug which can control the diabetes at much level furthermore it also prevents the kidney from the diabetic damage and restores of cellular status of the diabetes.

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