Research Article

Evaluation of Antidiabetic, Antihyperlipidemic and Histopathologic Effect of Dry Fruit Powders of *Phyllanthus emblica* Linn (Euphorbiaceae) in Alloxan Induced Diabetic Albino Rats

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ABSTRACT

Numerous traditional treatments have been recommended in the substitute system of medicine for the treatment of diabetes mellitus. Amla (*Phyllanthus emblica,* Euphorbiaceae) fruits are an edible and natural therapeutic agent shown to possess antioxidant, hypotensive, and diuretic properties. The purpose of this present investigation was to examine the antidiabetic, antihyperlipidemic and histopathologic effects of *P.emblica* fruit powder on the pancreas of diabetic rats. The animals were treated with *P. emblica* fruit powder at dosages of 200 and 400 mg/kg body weight for 28 days. The various parameters studied included blood glucose, Haemoglobin, Glycosylated haemoglobin, Plasma Insulin, Total Cholesterol, Triglyceride, HDL-C, Phospholipids, LDL and the morphological nature of pancreas in all groups. The dry powders of amala fruits shown more significant ($p<0.01$) reduction in blood glucose level, serum lipid level, and increase the plasma insulin in alloxan induced diabetic Wistar rats compared to control and glipizide at a dose of (10mg/Kg). In this study, Alloxan which selectively destroy B cells of the islet was used to induce type 1 diabetes mellitus. Histology showed regenerative changes of pancreatic islet cell at a dose of 400 mg/kg/day. This also suggests that the plant extract at this dose has the ability of inducing the quiescent cells to proliferate to replace the lost cells. The present study demonstrates the herbal powder exhibits promising antidiabetic activity and helps to maintain the good glycemic and metabolic control.

Keywords: Alloxan, diabetes mellitus, Wistar rats, antidiabetic, serum lipids, plasma insulin.

INTRODUCTION

The Diabetes mellitus is a metabolic disorder in which the body does not produce or properly utilize insulin. It causes disturbance in carbohydrate, protein and lipid metabolism and complications such as retinopathy, microangiopathy and nephropathy.

In practical terms, diabetes mellitus is a condition in diabetes, a profound alteration in the concentration and composition of lipid occurs[1].

Almost 1.3% of the population suffers from this disease throughout the world and number of diabetics is increasing by 6% per year.

Approximately 300,000 deaths each year are attributed to diabetes. The global figure of people with diabetes set rise from the current estimate of 150-220 million in 2010 and 300 million in 2025[2-3].

Despite the immense strides that have been made in the understanding and management of diabetes the disease and disease related complications are increasing unabated[4]. Insulin and oral hypoglycemic agents like sulphonyl ureas and biguanides are still the major players in the management but there is quest for the development of more effective anti-diabetic agents.

In spite of the presence of known anti-diabetic medicine in the pharmaceutical market, remedies from medicinal plant are used with success to treat this disease. Many traditional plants treatments for diabetes are used throughout the world and there is an increasing demand by patients to use the natural products with anti-diabetic activity[5].

Ayurveda and other traditional system of medicine supports *S. sesban* bark, Leaves of *G. asiatica* and *L. acutangula* fruit as anti-diabetic[6], which are efficacious and economical, as compared to synthetic drugs, but not evaluated systematically till date.

P. emblica is highly nutritious and is an important dietary source of vitamin C, minerals, and amino acids, such as calcium, phosphorus, iron, carotene, thiamine, riboflavin, and niacin[7].

The important constituent of plant leaves have the anti-neutrophilic activity and anti-platelet properties *in vitro*[8].

The extracts also posses several pharmacological properties like anti-viral (HIV, AIDS, Herpes Virus, CMV) antimutagenic, anti-allergic, anti-bacterial activities[9-10].

The study also showed that the plant leaves have antineutrophilc and antiplatelet properties *in vitro*[11].

The fresh or dry fruit is widely used for the treatment of diarrhea, jaundice and inflammatory disorder.

It is also shown that the fruits of this particular plant can be used as a glucose lowering agent with minimal differentiation of pre-adipocytes and so can be used in treating Type II diabetes very effectively without the fear of obesity[12].

Hence the present investigation is undertaken to the study the effect of powder *Amla* on changes in Body

weight, Plasma glucose, Hemoglobin and glycosylated hemoglobin and lipid profile of alloxan induced diabetic albino rats.

MATERIALS AND METHODS

Plant Materials

The fruit of amala were collected from local market of Sivaganga and authenticated at Dept of Botany, Thiyagarajar College, Madurai.

Collected fruits were washed in two times with distilled water and allowed to air dried. The dried fruits sample were crushed to coarse powder by mortor and pestle and stored at $4^{\circ}C$ at refrigerator for future use.

Selection & Acclimatization of Animals

Wistar strains of male albino rats weighing between 180-220gm are used for this study. The animals were housed in large spacious cages and they were fed with commercial pellets and access to water *ad libitum*.

The animals were well acclimatized to the standard environmental condition of temperature ($22^{\circ}c \pm 5^{\circ}C$) and humidity (55 ±5%) and 12 hr light dark cycles throughout the experimental period.

Induction of Diabetes Mellitus

Diabetes mellitus is induced in Wistar rats by single intraperitoneal injection of freshly prepared solution of Alloxan monohydrate (150mg/kg BW) in physiological saline after overnight fasting for 12hrs[1].

Alloxan is commonly used to produce diabetes mellitus in experimental animals due to its ability to destroy the β-cells of pancreas possibly by generating the excess reactive oxygen species such as H_2O_2, O_2 and HO^-.

The development of hyperglycemias in rats is confirmed by plasma glucose estimation 72 hrs post alloxan injection.

The rats with fasting plasma glucose level of 160-220mg/dl were used for this experiment.

Experimental Procedure

In the experiment a total of 30 rats (24 diabetic surviving rats & 6 normal rats) were used. Diabetes was induced in rats 3 days before starting the experiment.

The rats were divided into 6 groups after the induction of alloxan diabetes. In the experiment 6 rats were used in each group.

Treatment Protocol

Group-I

(Normal control) consist of normal rats given with 10ml/Kg of normal saline, orally.

Group-II

(Toxic control) Diabetic control received 150mg/Kg of Alloxan monohydrate through I.P.

Group-III

Diabetic control received glipizide at a dose of (10mg/Kg orally) for 28 days.

Group-IV

Diabetic control received powder of amla at a dose of (200mg/Kg orally) for 28 days.

Group-V

Diabetic control received powder of amla at a dose of (400mg/Kg orally) for 28 days.

Sample Collection

After 28 days of treatment, body weight, blood glucose, haemoglobin, glycosylated haemoglobin, plasma insulin, total cholesterol, triglycerides, HDL-cholesterol and phospholipids were determined.

Blood was collected from the eyes (venous pool) by sino-ocular puncture[4] in EDTA coating plasma tubes for the estimation of blood parameters.

Biochemical Analysis

Estimation of Blood Glucose

Blood glucose was estimated by commercially available glucose kit (One Touch Ultra) Johnson Johnson based on glucose oxidase method[13].

Plasma Insulin

Plasma insulin was determined by ELISA method using a Boehringer–Mannheim kit[14] with an ES300 Boehringer analyzer (Mannheim, Germany).

Estimation of Total Haemoglobin and Glycosylated Haemoglobin

Total haemoglobin and glycosylated haemoglobin was determined by the method of Sudhakar Nayak and Pattabiraman (1981)[15].

Estimation of Lipid & Lipoprotein

Plasma lipids were determined by auto analyzer according to the method of Parkeh and Jung (1970)[16] (total cholesterol), Gidez and Webb (1950)[17] (HDL-cholesterol), Zilversmith and Davis (1950)[18] (phospholipids) and Rice (1970)[19] (triglycerides).

Dissection and Homogenization

On the 29th day all animal were killed by euthanasia. The pancreatic tissues were quickly excised and washed immediately with ice-cold physiological saline (0.9% NaCl) and one part immediately stored at -5°C until analysis of biochemical parameters. The other part of the tissue was taken and washed in normal saline then they were fixed in 10% formalin.

The fixed specimens were further processed and they were embedded in paraffin and sectioned at 4μm thickness then they were stained with Hematoxylin and eosin for histopathological examination.
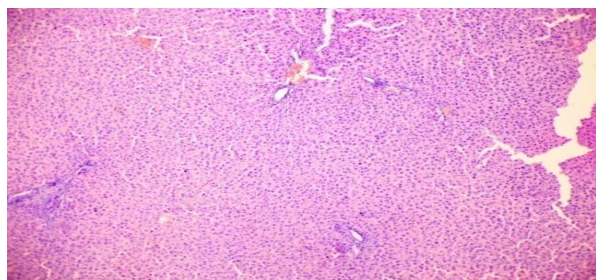
Statistical Analysis

The data for various biochemical parameters were analyzed using analysis of variance (ANOVA), and the group means were compared by Newman-Keuls multiple range test (NKMRT). Values were considered statistically significant at p<0.01.

RESULTS AND DISCUSSION


Figure 1A: Photomicrograph of a pancreatic section from the control group showing the islets of Langerhans, with scattered β cells and red blood cells visible in the vicinity.
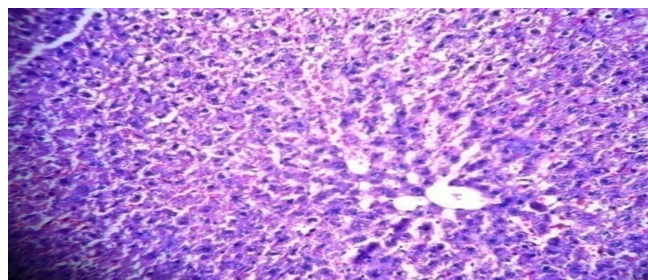
**Figure 1B:** Photomicrograph of alloxan induced type 1 diabetic pancreatic section showing the islets of Langerhans with damaged β cells due to necrosis and a decreased number of β cells.
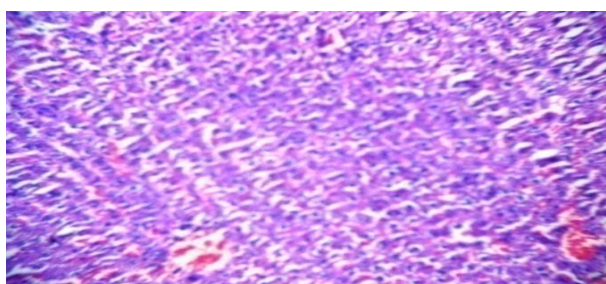
Figure 1C: Photomicrograph of type 1 diabetic islet treated with 10mg/Kg of glipizide orally showing evenly distributed β cells and an increased number of β cells.
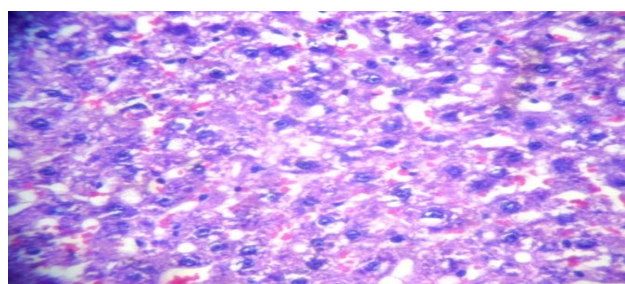
Figure 1D: Photomicrograph of type 1 diabetic islet treated with 200 mg/kg of dry fruits powder of *P. emblica* showing evenly distributed β cells and an increased number of β cells
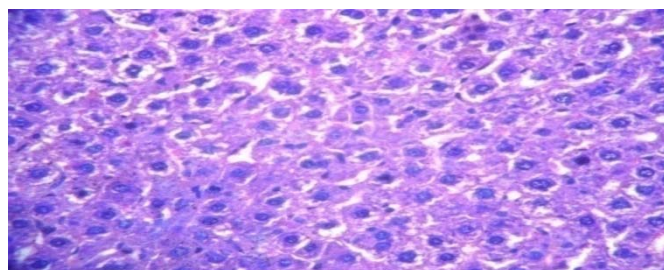
Figure 1E: Photomicrograph of type 1 diabetic islet treated 400 mg/kg of dry fruits powder of *P. emblica* showing evenly distributed β cells and an increased number of β cells

Figure 1: Effect of dry fruits powders of *P. emblica* on histopathology studies on pancreas of both normal and alloxan induced diabetic rat

Table 1: Effect of dry fruits powders of *P. emblica* on initial and final body weight and blood glucose in normal and alloxan treated albino rats

Group	Body Weight (g)		Blood Glucose (mg/100ml)	
	Initial	Final	Initial	Final
G1	222 ± 7.30	235 ± 7.60	83.65 ± 3.30	87.70 ± 3.75
G2	220 ± 6.70	172 ± 4.50**[(a)]	84.75 ± 3.85	210.45 ± 6.70**[(a)]
G3	230 ± 7.50	225 ± 7.25	87.55 ± 4.22	118.30 ± 4.42**[(b)]
G4	222 ± 7.25	240 ± 8.10	84.80 ± 3.60	135.45 ± 5.20**[(b)]
G5	230 ± 7.30	232 ± 7.40	86.50 ± 3.80	124.40 ± 4.65**[(b)]

Values are expressed as mean ± SEM.

Values were compare by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests.

** (a) Values are significantly different from normal control G1 at P<0.001.

** (b) Values are significantly different from Diabetic control G2 at P<0.01.

Table 2: Effect of of dry fruits powders of *P. emblica* on plasma insulin, Hemoglobin & Glycosylated hemoglobin in normal and alloxan treated albino rats.

Groups	Haemoglobin (gm/100ml)	Glycosylated haemoglobin HbA$_1$ (%)	Plasma Insulin (µU/ml)
G1	11.80 ± 1.60	0.33 ± 0.06	32.50 ± 2.90
G2	6.10 ± 0.70**(a)	0.99 ± 0.14**(a)	12.60 ± 1.70**(a)
G3	11.10 ± 1.22**(b)	0.44 ± 0.06**(b)	28.45 ± 2.40**(b)
G4	10.30 ± 0.92**(b)	0.42 ± 0.09**(b)	24.60 ±2.30**(b)
G5	10.96 ± 1.12**(b)	0.43 ± 0.05**(b)	27.95 ± 2.35**(b)

Values are expressed as mean ± SEM.

Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests.

** (a) Values are significantly different from normal control G1 at P<0.001.

** (b) Values are significantly different from Diabetic control G2 at P<0.01.

Table 3: Effect of dry fruits powders of *P. emblica* on *s*erum lipids in normal and alloxan treated albino rats.

Groups	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-C (mg/dl)	Phospholipids (mg/dl)	LDL (mg/dl)
G1	82.95 ± 2.55	86.55 ± 2.50	49.30 ± 1.80	122.70 ± 2.45	15.40 ± 1.26
G2	220.25 ± 6.80**(a)	154.65 ± 4.60**(a)	30.66 ± 1.30**(a)	200.35 ± 6.22**(a)	38.60 ± 2.35**(a)
G3	110.80 ± 3.30**(b)	93.85 ± 2.60**(b)	43.95 ± 1.45	148.50 ± 3.88	22.30 ± 1.90**(b)
G4	125.65 ± 3.60**(b)	106.75 ± 2.95**(b)	38.45 ± 1.42**(b)	157.65 ± 4.02**(b)	28.30 ± 1.92**(b)
G5	116.55 ± 3.30**(b)	99.30 ± 2.60**(b)	42.45 ± 1.62**(b)	148.30 ± 3.85**(b)	24.35 ± 1.70**(b)

Values are expressed as mean ± SEM.

Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests.

** (a) Values are significantly different from normal control G1 at P<0.001.

** (b) Values are significantly different from Diabetic control G2 at P<0.01.

Table 1 illustrates effect of dry powders of Amla fruits on levels of initial and final blood glucose, and change in body weight, in both normal and treatment rat animals in each group. The mean body weight of diabetic rats (G2) was significantly decreased as compared to normal control rats. The body weight of diabetic control rats treated with Amla powder at a dose of 200mg/kg and 400mg/kg was increased the body weight non-significantly as compared to normal control animals.

Fasting blood glucose level was significantly increased 210.45 ± 6.70 in diabetic animals as compared to normal animals. However the level of fasting blood glucose, returned to near normal range in diabetic rats treated with Amla powder at a dose of 200mg/kg and 400mg/kg.

Table 2 illustrates the levels of total hemoglobin, glycosylated hemoglobin and plasma insulin in normal rat and treatment control animals in each group. The levels of total hemoglobin, and plasma insulin levels were decreased significantly where as glycosylated heamoglobin levels were increased significantly as compared to normal control rats. However the level of total hemoglobin, glycosylated hemoglobin and plasma insulin, returned to near normal range in diabetic rats treated with Amla powder at a dose of 200mg/kg and 400mg/kg.

Table 3 shows the level of serum total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), Low density lipoprotein (LDL) and phospholipids of normal and experimental animals in each group. Total cholesterol, triglycerides, high density lipoprotein, Low density lipoprotein (LDL) and phospholipids levels were significantly increased, where as HDL-C level was decreased in alloxan induced diabetic rats as compared to normal rats. Treatment of normal and alloxan induced diabetic rats with Amla powder at a dose of 200mg/kg and 400mg/kg for 28 days resulted in marked decrease in total cholesterol, triglycerides, Low density lipoprotein(LDL) and phospholipids levels and increase in HDL-C levels as compared to alloxan induced diabetic rats.

Alloxan causes massive reduction in insulin release, through the destruction of β-cells of the islets of Langerhans. The mechanism of alloxan action was fully described elsewhere[20-21]. In the present investigation observed a significant increase in the plasma insulin level when alloxan induced diabetic rats were treated with Amla powder at a dose of 200mg/kg and 400mg/kg this could be due to potentiating of the insulin effect of plasma by increasing the pancreatic secretion of insulin from existing β-cells of islets of Langerhans or its release from bound insulin.

In uncontrolled or poorly controlled diabetes there is an increased glycosylation of a number of proteins including haemoglobin and α-crystalline of lens[22]. Glycosylated haemoglobin (HbA$_1$C) was found to increase in patients

with diabetes mellitus to approximately 16%[23] and the amount of increase is directly proportional to the fasting blood glucose level[24]. During diabetes the excess glucose present in blood reacts with haemoglobin. Therefore, the total haemoglobin level is decreased in alloxan induced diabetic rats[25]. Administration of Amla powder at a dose of 200mg/kg and 400mg/kg for 28 days prevents a significant elevation in glycosylated haemoglobin there by increasing the level of total haemoglobin in diabetic rats. This could be due to the result of improved glycaemic control produced by Amla powder at a dose of 200mg/kg and 400mg/kg.

The body weight was decreased in alloxan diabetic rats. Administration of Amla powder at a dose of 200mg/kg and 400mg/kg increases the body weight in alloxan induced diabetic rats. The ability of Amla powder at a dose of 200mg/kg and 400mg/kg to protect massive body weight loss seems to be due to its ability to reduce hyperglycemia.

The level of serum lipids are usually elevated in diabetes mellitus and such an elevation represents the risk of coronary heart disease (CHD).

Lowering of serum lipids concentration through diet or drug therapy seems to be associated with a decrease in the risk of vascular disease. The abnormal high concentration of serum lipids in diabetic subject is mainly due to increased mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase.

However, glucagon, catecholamines and other hormones enhance lipolysis. The marked hyperlipidaemia that characterized the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots.

In the alloxan-induced diabetes mellitus, the rise in blood glucose is accompanied by an increase in serum cholesterol and triglycerides. The levels of cholesterol and triglycerides and Low density lipoprotein (LDL) levels were brought to near normal by the treatment with Amla powder at a dose of 200mg/kg and 400mg/kg in alloxan induced diabetic rats.

The effect of dry fruits powder at a dose of 200mg/kg and 400mg/kg on diabetic hypertriglyceridemia could be through its control of hyperglycaemia. This is in agreement with the facts that:

1. The level of glycaemic control is the major determinant of total and very low density lipoprotein (VLDL), triglyceride, concentrations[20].
2. Improved glycemic control following sulfonylurea therapy decreases the levels of serum VLDL and total triglycerides[26].

The main 'anti-atherogenic' lipoprotein (HDL) is involved in the transport of cholesterol from peripheral tissues into liver[27] (Segal) and thereby it acts as a protective factor against coronary heart disease (CHD)[28,5].

The level of HDL-cholesterol was decreased in diabetic rats when compared with normal rats. Our results clearly show that the level of HDL-cholesterol was increased in alloxan induced diabetic rats when treated with Amla powder at a dose of 200mg/kg and 400mg/kg.

Figure 1 A showed the photomicrograph of a pancreatic section from the control group of rat showing the islets of Langerhans, with scattered β cells and red blood cells visible in the vicinity. The islet shows a large number of β cells (hematoxylin-stained blue cells) distributed throughout the islet. A few α cells (phloxine stained pink cells) can also be observed. Figure 1 B showed photomicrograph of alloxan induced type 1 diabetic pancreatic section showing the islets of Langerhans with damaged β cells due to necrosis and a decreased number of β cells.

The islet of Langerhans shows that the β cells did not differ in group II, neither in number nor in diameter, from those of the control group (I). In the diabetic group, a decrease in the number of β cells of the islets of Langerhans was observed in comparison to the control group (Figure 1A and 1B). The damage or necrosis of β cells was caused by the alloxan used to induce diabetes. A small number of functional β cells were observed and β cells were more prominent.

Figure 1C showed photomicrograph of type 1 diabetic islet treated with 10mg/Kg of glipizide orally showing evenly distributed β cells and an increased number of β cells. The damaged β cells seen after the initial induction of diabetes were no longer observed after treatment with glipizide. Figures 5D and 5E show the islets of Langerhans from the diabetic groups treated with dry fruits powder of *P. emblica* at concentrations of 200 and 400 mg/kg body weight respectively. It showed that β cells were evenly distributed and an increased number of β cells due to treatment.

The damaged β cells seen after the initial induction of diabetes were no longer observed after treatment with dry fruit powders of *P. emblica*. The recovery of necrotic β cells was especially more pronounced after treatment with 400 mg/kg of dry fruit powders of *P. emblica* than in the group treated with 200 mg/kg of the fruit.

The dry fruit powders of *P. emblica* were found to be effective in controlling hyperglycemia in diabetic rats. Although many suggestions have been made about plants' roles, the precise mechanism is unclear[29]. Plants may act on blood glucose through different mechanisms, some of them may have insulin-like substances, some may inhibit insulin activity, and others may increase β cells in the pancreas by activating the regeneration of these cells[30].

The fiber of plants may also interfere with carbohydrate absorption and thereby affect blood glucose[31]. Different studies have demonstrated that certain plant extracts of *Scoparia dulcis*[32], *Teucrium polium*[33], and *Salvadora oleoides*[34] effectively increased the number of β cells in

streptozotocin- and alloxan-induced diabetes mellitus. The diminished islet size and β cell number resulted in the histopathology of the diabetic pancreas[35]. A report showing that adult cell numbers are formed by self-duplication/self-proliferation supports our finding of an increase in the β cell number in diabetic islets after plant extract treatment[36-39].

CONCLUSION

In conclusion, the histopathological studies undertaken on the islets demonstrated the recovery of damaged islets and an improvement in the number of β cells after treatment with the plant extract. These results suggest that Amla powder at a dose of 200mg/kg and 400mg/kg has protective effect against alloxan-induced diabetes and its complications. It can thus be assumed that dry powder of fruits of *P. emblica* has a therapeutic effect that alleviates diabetes mellitus. The actual chemical compound that is responsible for this effect requires additional exploration.

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