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# Hypoglycemic activity studies on aerial leaves of *Pongamia pinnata* (L.) in Alloxan-induced diabetic rats

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## ABSTRACT

*Diabetes mellitus (DM) is a metabolic disorder, characterized by absolute or relative deficiency in insulin secretion or insulin action. The synthetic oral hypoglycemic drugs used in clinical practice have significant unwanted effects. Plant based drugs are considered to be less toxic and free from adverse effects in comparison to modern allopathic drugs. Pongamia pinnata (L.), locally known as Karanja, is a mangrove plant belonging to the family, Fabaceae. The present work aimed to evaluate the antioxidant and antidiabetic potency of standardized ethanolic extract of Pongamia pinnata (EP) to flavonoids on alloxan-induced animals and its effect were compared with reference glibenclamide (GL). Inbreed adult male Charles-Foster (CF) albino rats were used in the experiment for hypoglycemic activity in oral glucose tolerance test (OGTT) and normoglycemic rats, and antidiabetic activity in alloxan induced rats. Preliminary phytochemical screening revealed that EP showed positive response to alkaloids, saponins and triterpenes, tannins, flavonoids, carbohydrates and sterols. Further, the flavoniod content of EP was found to be 28.1±0.7 mg in quercetin equivalent/ 1g extract. Results revealed in the present experiment that the routine post-treatment for 21 days with the EP showed potential hypoglycemic activity in OGTT and normoglycemic rats and antidiabetic activity in alloxanized rats. In conclusion, isolation and establishment of exact mechanism of action of specific compound from EP is to be carried out in the future.*

**Keywords:** *Pongamia pinnata*, diabetes, hyperglycemia, dyslipidemia and oxidative stress.

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## NTRODUCTION

Diabetes mellitus is a metabolic disorder of multiple etiological characteristics including chronic hyperglycemia with abnormal metabolism of carbohydrate, fat and protein resulting from defects either in insulin secretion or in insulin action or both. It has been reported that the chronic hyperglycemia of diabetes is associated with complications like nephropathy, cardiovascular disorder, neuropathy, retinopathy, limb amputation, long term dysfunctions and failure of various organs and eventually premature death [1]. It has been predicted by world health organization that Indian people are more genetically susceptible to diabetes accounting about 30 to 33 million and would go up to 40 million by the end of 2010 which further will reach to maximum of 74 million by 2025 [2]. Management of this disease may include lifestyle modifications, diet, exercise, and long – term use of oral hypoglycemic agents or insulin therapy [3]. It has been investigated that for a long time plants based herbal medicines or their extracts have been the major source of drugs for the treatment of diabetes mellitus (DM) in Indian medicine and other ancient systems in the world [4], because plant products are frequently considered to be less toxic and more free from side effects than modern synthetic drugs [5]. Ethnobotanical information suggests that about 800 medicinal plants possess hypoglycemic or antidiabetic potential [6]. Furthermore, after the recommendations made by the WHO on DM, investigations on hypoglycemic agents from medicinal plants have become more important and the search for more effective and safer hypoglycemic agents has continued to be an important area of active research. Many herbs and plants have been described as possessing hypoglycemic activity when taken orally [4, 7]. However, large floras are still waiting for investigation for their medicinal properties [8]. Medicinal plants possess antidiabetic potential or bioactive compounds such as glycosides, alkaloids, terpenoids, carotenoids and floavonoids are confirmed to be effective in both preclinical and clinical studies [9, 10].

*Pongamia pinnata* (L.), locally known as Karanja, is a mangrove plant belonging to the family, Fabaceae. It is a medium size glabrous tree with a short bole and attaining a height of round 18 m and its habitat is in the littoral regions of South East Asia, Australia and Fiji [11, 12]. In the Indian Ayurvedic literature, various parts of this plant have been recommended as a remedy for various ailments. Different parts of the plant have been used in traditional medicines for bronchitis, whooping cough, and rheumatic joints and to quench dipsia in diabetes. The leaves are digestive, laxative, anthelmintic and cure piles, wounds and other inflammations [8]. A hot infusion of leaves is used as a medicated bath for relieving rheumatic pains and for cleaning ulcers in gonorrhea and scrofulous enlargement [13]. Different extracts of leaves, roots and seeds (ethanol, petroleum ether, benzene extracts and others) of *Pongamia pinnata* have been reported to have anti-inflammatory activity and also used to treat infectious diseases such as leucoderma, leprosy, lumbago, muscular and articular rheumatism [14-16]. In addition, phytochemical examinations of this plant indicated the presence of furanoflavones, furanoflavonols, chromenoflavones, and flavones. furanodiketones and flavonoid glucosides [17,18]. It is a well-documented fact that most medicinal plants are enriched with phenolic compounds and bioflavonoids that have excellent antioxidant property.

On this context, we have standardized the ethanolic extract of *Pongamia pinnata* (EP) to flavonoids and then performed the hypoglycemic activity on alloxan-induced animals.

## MATERIALS AND METHODS

### 2.1. Chemicals and reagents

Alloxan monohydrate was obtained from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. Glibenclamide (GL) was provided as gift sample by Hoechst Pharmaceuticals, Mumbai, India. All other reagents and chemicals used were of analytical grade procured from Hi-media Labs, Mumbai, India.

### 2.2. Plant material and extraction

The aerial leaves of *Pongamia pinnata* were collected from local horticulture garden of Bilaspur (Chattissgarh) India in the month of July-August, 2008. The plant was identified and authenticated by Dr. Siddhamallaiah, Regional Research Laboratory, Bangalore (India) where Specimen was deposited in their herbarium (Voucher No. RRCBI/Mus/6). Plant material was carefully washed with tap water and left to dryness in dark at room temperature and finally stored in well-closed cellophane bags. The shade-dried and coarse powdered leaves (1 kg) were subjected to defat with petroleum-ether (bp 40-60°C) using Soxhlet extraction apparatus (Quickfit, England). The defatted sample was air dried in order to remove solvent residue. Extract was prepared by extracting the defatted powder with ethanol solvent (70% v/v) for period of 48 h, which was then concentrated to a semisolid mass under reduced pressure (Buchi Rotavor R-200, Switzerland) for 20 min at 70°C (yield: 10.2% w/w). Further, there was prepared a fresh suspension of 0.3% v/v carboxymethylcellulose (CMC) in distilled water. Thereafter, the ethanol extract was mixed with 0.3% CMC suspension separately to form ethanol suspension (EP) before administration to rats.

### 2.3. Preliminary phytochemical screening and standardization to total flavonoid content

Each plant extracts were subjected to identification tests for alkaloids, saponins and triterpenes, tannins, flavonoids, carbohydrates and sterols [19]. Total flavonoid content was determined by the Aluminium trichloride colorimetric assay procedure of Woisky and Salatino, 1998 [20] and Jung et al., 2006 [21]. Briefly, 1.5 ml of 2% aluminium trichloride ($AlCl_3$) was added to 1.5 ml ethanolic extract solution (500 ppm in 80% MeOH). After incubation of 1 hour at room temperature, the absorbance was estimated against blank using Double-Beam UV-Vis Spectrophotometer at 420 nm against a blank. Quercetin was used as a standard to plot the calibration curve. The amount of total flavonoid was expressed as quercetin equivalents (mg quercetin/g extract). All the samples and the standards were analyzed in triplicate.

### 2.4. Animals

Inbreed adult male Charles-Foster (CF) albino rats (150-200 g), 2-3 months old, were obtained from School of Pharmacy, Chouksey Engineering College, Bilaspur and were used in the study. The animals were grouped and housed in Poly-acrylic cages lined with husk under standard condition (24 ± 2°C temperature, 45-55% relative humidity and 12 h light: 12 h dark cycle) respectively. Animals were allowed to freely feed their standard pellet diet (Lipton India, Ltd., Mumbai) and water *ad libitum*. Fasted animals were deprived with their food for 16-18 h before experimentations but allowed to water *ad libitum*. All the experimental procedures utilized were performed in accordance with the approval of the Institutional Animal Ethics Committee

(1169/ac/08/CPCSEA) under strict compliance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for the experimental studies.

### 2.5. Oral toxicity studies

An acute oral toxicity study was performed according to "Organization for Environmental Control Development" guidelines (OECD: Guidelines 420; Fixed Dose Method) for oral administration of ethanol extract. Albino male rats (N=6, 150-200 g), overnight fasted for 18 h were used for the study. Each extract in increasing dose of 5, 50, 300 and maximum dose up to 2000 mg/kg was found tolerable in the experimental animals. EP (50, 100 and 200 mg/kg) were selected for assessment of hypoglycemic activity according to the previous report [22]. Thereafter, the rats were continuously observed for first 3 h for any gross behavioral, neurological or autonomic toxic effects and for any lethality after 24 to 72 h till 14 days.

### 2.6. Induction of diabetes in rats

Diabetes was induced by a single dose subcutaneous injection of freshly prepared alloxan monohydrate (120 mg/kg, Sigma chemicals, USA) dissolved in citrate buffer (pH 4.00) to overnight fasted male CF albino rats [23]. Blood glucose level was measured by using one-touch glucometer and diabetes was confirmed after 72 hr of alloxanisation. Rats with fasting blood glucose level more than 250 mg/dl were considered to be diabetic and were selected for studies.

### 2.7. Experimental Design

#### 2.7.1. Study on Oral Glucose Tolerance Test (OGTT)

Initially, hypoglycemic activity of plant extracts was carried out in overnight fasted normal rats, which were equally divided into five groups of six rats each. Normal control group received only vehicle (1 ml of 0.3% CMC; p.o.) and standard group received 1 ml of reference drug GL suspended in the vehicle (0.25 mg/kg, p.o.), while group from third to fifth were administered with 1 ml of EP (50, 100 and 200 mg/kg, p.o.) respectively. Following 30 min post extract administration all the animals were fed with glucose (2 g/kg). Blood samples were collected from tail vein prior to dosing and then at 30, 60, 90 and 120 min after glucose administration. The fasting blood glucose level was analyzed using glucose-oxidase-peroxide reactive strips (Accu-chek, Roche Diabnostics, GmbH, Germany).

#### 2.7.2. Study on normoglycemic rats

Normoglycemic studies were carried out in overnight fasted normal rats, which were equally divided into five groups of six rats each. Normal control group received only vehicle (1 ml of 0.3% CMC; p.o.) and 1 ml of standard group received reference drug GL suspended in the vehicle (0.25 mg/kg, p.o.), while group from third to fifth were administered with 1 ml of EP (50, 100 and 200 mg/kg, p.o.) respectively. Blood samples were collected from tail vein prior to dosing (day 0) and then at regular intervals of day 7, 14 and 21 respectively and subjected to fasting blood glucose level.

#### 2.7.3. Study on Alloxan-induced diabetic rats

In the experiment, diabetic rats were selected after alloxan induction and were equally divided into six groups of six rats each. Diabetic and control groups received only vehicle (1 ml of 0.3% CMC; p.o.) and standard diabetic group received 1 ml of reference drug GL suspended in the

vehicle (0.25 mg/kg, p.o.), while group from third to sixth were administered with 1 ml of EP (50, 100 and 200 mg/kg, p.o.) respectively once a day regularly up to 21$^{th}$ day. Blood samples were collected from tail vein prior to dosing (day 0) and then at regular intervals of day 7, 14 and 21 respectively and subjected to fasting blood glucose level. The body weight, food and fluid intake of all groups of animals were monitored on a daily basis for 21 days at regular time. Fixed amount of rat chow and fluid was given to each rat and replenished the next day. For experimental purposes, only the weights before treatment and at the end of the study were used for analysis. At the end of 21$^{th}$ day, all the rats were euthanized by pentobarbitone sodium (60 mg/kg) and sacrificed by cervical dislocation. Blood sample was withdrawn from abdominal aorta into fresh centrifuge tubes and centrifuged at 2,500 rpm for 15 min to obtain serum and plasma. Serum samples were stored at -20°C until utilized for further biochemical estimations.

### 2.8. Biochemical estimations

Plasma insulin was assayed by using commercial enzyme-linked immuno sorbent assay kit (ELISA, Boerhringer Mannheim, Germany). Serum total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT), alkaline phosphatase (ALP), urea, creatinine, protein and albumin were estimated in a Hitachi Auto analyzer using commercial kit (Ecoline, E-Merck, India; Roche Diagnostics, GmbH, Germany) following standard procedures. The level of hemoglobin (Hb) and glycosylated hemoglobin ($HbA1_C$) was estimated using Drabkin reagent.

### 2.9. Estimation of lipid peroxidase

Plasma malondialdehyde (MDA) content was estimated using the method described by Uchiyama and Mihara, 1978 [24] with minor modification by Sunderman et al., 1985 [25] based on the thiobarbituric acid reaction (TBAR) test. Plasma samples were added (1/10, v/v) to ice-cold 1.15% KCl solution and mixed with 0.1 ml of 8.1% sodium dodecyl sulfate (SDS), 0.75 ml of 20% acetic acid, and 0.75 ml of 0.8% TBA solution. The mixture was made up to 2.0 ml with distilled water and heated at 95$^0$C for 60 min. After cooling with tap water, 0.5 ml of distilled water and 2.5 ml of *n*-butanol/pyridine mixture (15:1, v/v) were added and the mixture shaken vigorously. The mixture was centrifuged at 4000×*g* for 10 min and the absorbance of the organic layer (upper layer) was measured at 532 nm. The TBAR reaction was standardized by the analysis of tetraethoxypropane standard solutions, which yield MDA, mole for mole, under the described reaction conditions.

### 2.10. Estimation of superoxide dismutase (SOD)

The SOD activity was estimated by the method described by Ukeda et al., 1997 [26]. Into 2.6 ml of 50 mmol/L sodium citrate buffer (pH 9.4) were added 0.1 ml each of 30 mmol/l xanthine (dissolved in 1 mol/L NaOH), 3 mmol/l EDTA, 0.8 mmol/L XTT (3-{1-[(phenylamino)-carbonyl]-3,4-tetrazolium}- bis(4-methoxy-6-nitro)-benzenesulfonic acid hydrate) dissolved in buffer at 50$^0$C and 0.05 ml of sample solution containing SOD or water. The reaction consists of the addition of 0.02 ml of xanthine oxidase solution (500 mU/ml) with the formation of a red formazan dye. The SOD activity was monitored for 30 s at 470 nm (25$^0$C) by the degree of inhibition of this reaction.

### 2.11. Estimation of catalase (CAT)

Catalase activity was measured by the method of Aebi, 1984 [27]. The homogenate was prepared with 50 mmol/l phosphate buffer, pH 7.0, with a drop of TritonX100 and centrifuged at 15,000×*g* for 15 min at $4^0$C. To 3.0 ml of phosphate buffer, 0.05 ml of 90 mmol/l hydrogen peroxide solutions and 0.02 ml of extract or water were added. The absorbance was read at 240 nm for 30 s.

### 2.12. Statistical Analysis

The results are expressed as mean±S.E.M. The statistical significance was determined by One-Way Analysis of Variance (ANOVA) followed by *Post-hoc* Student Newman Keuls test. $P < 0.05$ was considered to be statistically significant.

## RESULTS

### 2.13. Effect of EP (50, 100 and 200 mg/kg) on oral glucose tolerance test (OGTT) and plasma glucose level in normoglycemic and diabetic rats:

Table-1 illustrates the effect of EP (50, 100 and 200 mg/kg) on OGTT at different time points. Statistical analysis by One-way ANOVA showed that there was no significant difference among the groups at 0 min [F (4, 25) = 0.11, P>0.05]. Similarly, statistical analysis at 30 min showed that there was significant difference among the groups [F (4, 25) = 5.28, P<0.05]. Post-hoc test revealed that GL (0.25 mg/kg) and EP (100 and 200 mg/kg) showed significant attenuation in the plasma sugar level compared to control. Further, statistical analysis at 60 min showed that there was significant difference among the groups [F (4, 25) = 10.21, P<0.05]. Post-hoc by Student Newmann Keuls test revealed that GL and EP (100 and 200 mg/kg) showed significant decrease in the plasma sugar level compared to control. Furthermore, EP (200 mg/kg) showed significant increase in the sugar level (P<0.05) compared to GL and this trend was similar at 90 min [F (4, 25) = 7.36, P<0.05] and 120 min [F (4, 25) = 25.32, P<0.05].

**Table-1: Hypoglycemic effect in oral glucose tolerance test (OGTT)**

| Groups | 0 min | 30 min | 60 min | 90 min | 120 min |
|---|---|---|---|---|---|
| CON | 88.5±3.72 | 144.9±3.26 | 135.2±1.75 | 107.1±2.29 | 102.3±4.27 |
| GL | 84.7±1.52 | 112.8±3.64[a] | 98.7±4.75[a] | 80.7±1.79[a] | 67.3±3.17[a] |
| EP-50 | 83.7±1.76 | 122.7±3.69 | 111.7±2.65 | 90.4±4.86 | 82.6±1.75 |
| EP-100 | 84.6±2.71 | 120.6±2.75[a] | 110.7±3.45[a] | 87.3±4.72[a] | 74.6±2.10[a] |
| EP-200 | 85.3±1.54 | 125.4±3.45[a] | 115.1±2.66[a,c] | 92.4±3.45[a,c] | 77.3±1.76[a,c] |

*All values are Mean±SEM. [a]P<0.05 compared to control, [c]P<0.05 compared to GL (One-way ANOVA followed by Student Newmann keuls test).*

Time dependant effect of EP (50, 100 and 200 mg/kg) on the level of plasma glucose level in fasted normoglycemic rats is depicted in Table-2. Statistical analysis by One-way ANOVA revealed that there was no significant difference among the groups at 0 day [F (4, 25) = 0.12, P>0.05]. Similarly, statistical analysis at 7 day showed that there was significant difference among the groups [F (4, 25) = 4.3, P<0.05]. Post-hoc test revealed that GL and EP (200 mg/kg) showed significant decrease in the plasma sugar level compared to control. Further, EP (200

mg/kg) showed significant reduction in blood sugar levels compared to GL, EP (50 and 100 mg/kg). Furthermore, the similar trend like at 7 day was observed at 14 day [F (4, 25) = 9.25, P<0.05] and 21 day [F (4, 25) = 7.82, P<0.05].

**Table-2: Hypoglycemic effect of EP (50, 100 and 200 mg/kg) in fasted normoglycemic rats**

| Groups | Day 0 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|
| CON | 75.8±3.71 | 77.6±2.75 | 74.2±4.72 | 72.5±2.12 |
| GL | 72.7±1.65 | 55.8±3.48[a] | 49.6±3.47[a] | 51.4±1.42[a] |
| EP-50 | 75.6±3.45 | 66.4±3.21 | 62.2±5.31 | 66.1±1.44 |
| EP-100 | 73.5±1.78 | 64.1±2.46 | 63.4±2.36 | 58.9±1.35 |
| EP-200 | 73.2±2.71 | 59.7±5.39[a,c,d,e] | 51.7±3.15[a,c,d,e] | 51.6±3.05[a,c,d,e] |

*All values are Mean±SEM. [a]P<0.05 compared to control, [b]P<0.05 compared to diabetes, [c]P<0.05 compared to GL, [d]P<0.05 compared to EP-50 and [e]P<0.05 compared to EP-100 (One-way ANOVA followed by Student Newmann keuls test).*

Table-3 showed time dependant effect of EP (50, 100 and 200 mg/kg) on the level of plasma glucose level in alloxan treated rats. Statistical analysis by One-way ANOVA revealed that there was no significant difference among the groups at 0 day [F (5, 30) = 1.89, P>0.05].

**Table 3: Hypoglycemic effect of EP (50, 100 and 200 mg/kg) in Alloxan induced animals**

| Groups | Day 0 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|
| CON | 75.1±2.43 | 76.7±1.87 | 75.1±3.33 | 72.1±3.11 |
| DM | 343.2±2.75 | 422.6±4.06[a] | 396.3±9.45[a] | 363.3±9.23[a] |
| GL | 306.3±1.25 | 253.2±8.77[a,b] | 195.6±11.52[a,b] | 96.6±12.56[a,b] |
| EP-50 | 339.3±1.22 | 397.1±11.15[a,c] | 289.7±13.29[a,c] | 276.7±14.23[a,c] |
| EP-100 | 319.7±5.31 | 273.5±12.24[a,b,c,d] | 253.5±12.21[a,b,c,d] | 161.5±11.51[a,b,c,d] |
| EP-200 | 316.5±5.41 | 243.7±10.45[a,b,d,e] | 216.6±10.73[a,b,d,e] | 120.6±11.23[a,b,d,e] |

*All values are Mean±SEM. [a]P<0.05 compared to control, [b]P<0.05 compared to diabetes, [c]P<0.05 compared to GL, [d]P<0.05 compared to EP-50 and [e]P<0.05 compared to EP-100 (One-way ANOVA followed by Student Newmann keuls test).*

Further, statistical analysis at 7 day showed that there was significant difference among the groups [F (5, 30) = 3.64, P<0.05]. Post-hoc test revealed that DM, GL and EP (50, 100 and 200 mg/kg) showed significant increase in the plasma sugar level compared to control. Further, GL and EP (100 and 200 mg/kg) groups showed significant decrease in the blood glucose level compared to DM. The EP (50 and 100 mg/kg) groups showed significant increase in sugar level compared to GL. Further, EP (100 and 200 mg/kg) groups showed significant difference compared to EP (50 mg/kg). In addition, EP (200 mg/kg) group showed significant decrease in blood sugar level compared to EP (100 mg/kg).

2.14. **Effect of EP (50, 100 and 200 mg/kg) on body weight, food and fluid intake of diabetic rats:**

**Fig. 1.**

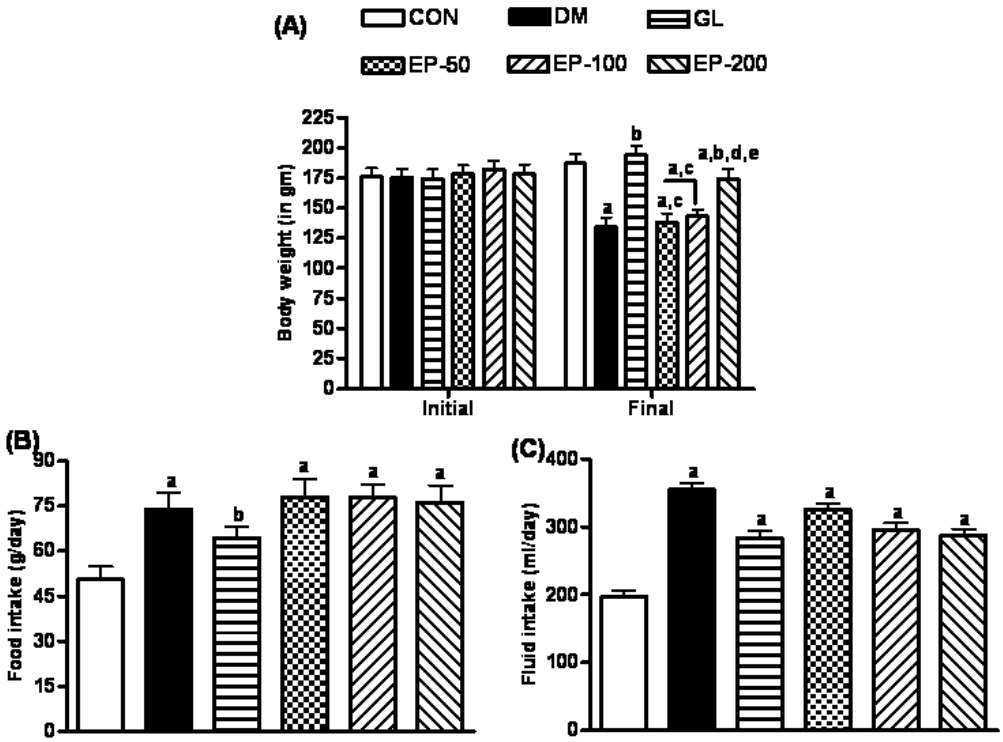


**Fig-1. Effect of EP (50, 100 and 200 mg/kg) on the body weight (A), food (B) and fluid (C) intake. All values are Mean±SEM. [a]P<0.05 compared to control, [b]P<0.05 compared to diabetes, [c]P<0.05 compared to GL, [d]P<0.05 compared to EP-50 and [e]P<0.05 compared to EP-100 (One-way ANOVA followed by Student Newmann keuls test).**

The effect of EP (50, 100 and 200 mg/kg) on initial and final body weight is illustrated in figure-1(A). Statistical analysis by One-way ANOVA revealed that there was no significant difference among the groups during initial body weight estimation [F (5, 30) = 0.61, P>0.05]. Further, statistical analysis revealed that there was significant difference among the groups during final body weight estimation [F (5, 30) = 1.73, P<0.05]. Post-hoc test revealed that DM and EP (50, 100 and 200 mg/kg) showed significant decrease in body weight compared to control. GL and EP (100 and 200 mg/kg) groups showed significant increase in body weight compared to DM. EP (50 and 100 mg/kg) groups showed significant decrease in body weight compared to GL. The body weight was significantly increased in EP (200 mg/kg) compared to EP (50 and 100 mg/kg).

The effect of EP (50, 100 and 200 mg/kg) on food intake of diabetic rats are illustrated in figure-1 (B). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 4.41, P<0.05]. Post-hoc test revealed that all the groups except GL group showed significant increase in food intake compared to control. However, GL showed significant reduce in food intake compared to DM group. Figure-1 (C) depicts the effect of EP (50, 100 and 200 mg/kg) on fluid intake of diabetic animals. Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 71.40,

P<0.05]. Post-hoc test revealed that all the groups showed significant increase in fluid intake compared to control. However, all the treated groups did not show any significant change in fluid intake among themselves.

2.15. **Effect of EP (50, 100 and 200 mg/kg) on plasma lipid profile:**

The effect of EP (50, 100 and 200 mg/kg) on TC, TG, LDL and HDL is depicted in figure-2. Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 8.36, P<0.05]. Post-hoc test revealed that DM and EP (50 and 100 mg/kg) showed significant increase in TC level compared to control. The TC levels were decreased significantly in GL and EP (200 mg/kg) compared to DM. The groups EP (50 and 100 mg/kg) showed significant increased in TC levels compared to GL. Further, EP (200 mg/kg) showed significant decrease in TC levels compared to EP (50 and 100 mg/kg).

Furthermore, statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 10.36, P<0.05]. Post-hoc test revealed that the TG levels were significantly elevated in all the groups compared to control. Further, EP (200 mg/kg) showed significant decrease in TG levels compared to DM, GL, and EP (50 and 100 mg/kg) groups.

**Fig. 2.**

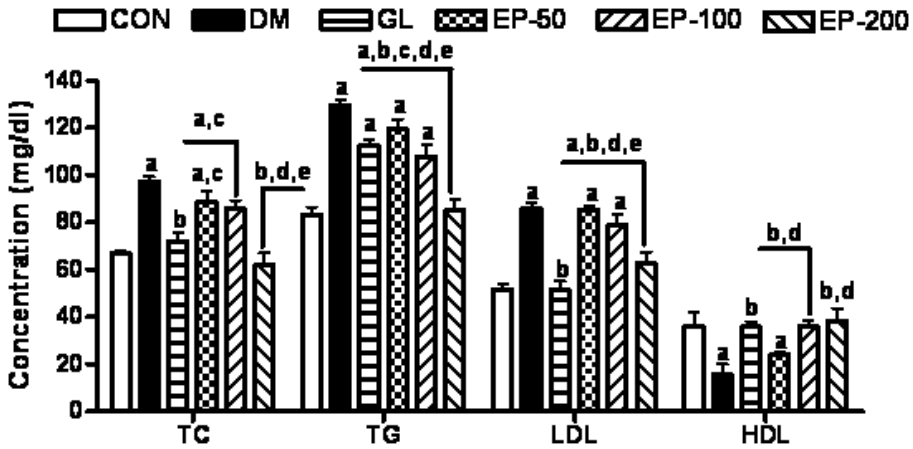


**Fig-2. Effect of EP (50, 100 and 200 mg/kg) on plasma TC (A), TG (B), LDL (C) and HDL (D) levels. All values are Mean±SEM. [a]P<0.05 compared to control, [b]P<0.05 compared to diabetes, [c]P<0.05 compared to GL, [d]P<0.05 compared to EP-50 and [e]P<0.05 compared to EP-100 (One-way ANOVA followed by Student Newmann keuls test).**

Similarly, statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 11.20, P<0.05]. Post-hoc test revealed that all the groups except GL showed significant increase in LDL levels compared to control. Further, GL and EP (200 mg/kg) showed significant decrease in LDL levels compared to DM. EP (200 mg/kg) showed significant decrease in LDL levels compared to EP (50 and 100 mg/kg) groups.

Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 16.38, P<0.05]. Post-hoc test revealed that DM and EP (50 mg/kg) showed significant decrease in HDL levels, however there was no change in HDL levels of GL and EP (50 and 100 mg/kg) compared to control. Further, GL and EP (100 and 200 mg/kg)

showed significant increase in HDL levels compared to DM. EP (100 and 200 mg/kg) showed significant increase in HDL levels compared to EP (50 mg/kg).

### 2.16. Effect of EP (50, 100 and 200 mg/kg) on liver function and plasma antioxidant profile:

The effect of EP (50, 100 and 200 mg/kg) on SGOT is depicted in figure-3 (A). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 17.77, P<0.05]. Post-hoc test revealed that all the groups showed significant elevation in SGOT levels compared to control. GL and EP (200 mg/kg) showed significant decrease in SGOT levels compared to DM. EP (50 mg/kg) showed significant increase in SGOT levels compared to GL. EP (200 mg/kg) showed significant decrease in SGOT levels compared to EP (50 mg/kg) group.

The effect of EP (50, 100 and 200 mg/kg) on SGPT is depicted in figure-3 (B). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 19.81, P<0.05]. Post-hoc test revealed that all the groups showed significant increase in SGPT levels compared to control. GL and EP (100 and 200 mg/kg) showed significant decrease in SGPT levels compared to DM. EP (50 mg/kg) showed significant increase in SGPT levels compared to GL. EP (200 mg/kg) showed significant decrease in SGOT levels compared to EP (50 mg/kg) group.

**Fig. 3.**

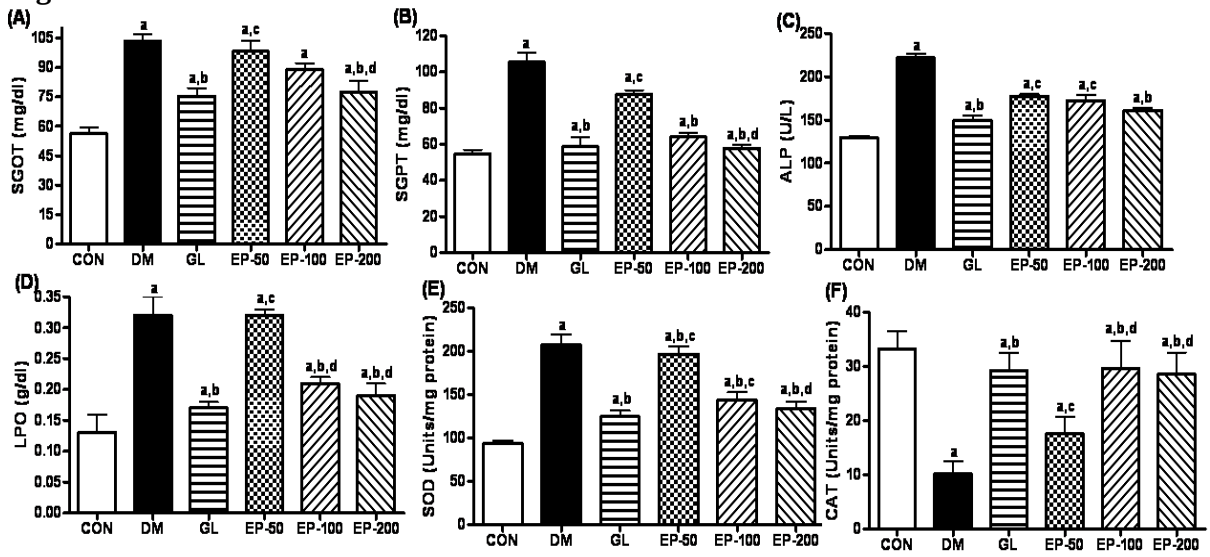


**Fig-3. Effect of EP (50, 100 and 200 mg/kg) on the levels of SGOT (A), SGPT (B), ALP (C), LPO (D), SOD (E) and CAT (F). All values are Mean±SEM. [a]P<0.05 compared to control, [b]P<0.05 compared to diabetes, [c]P<0.05 compared to GL, [d]P<0.05 compared to EP-50 and [e]P<0.05 compared to EP-100 (One-way ANOVA followed by Student Newmann keuls test).**

The effect of EP (50, 100 and 200 mg/kg) on ALP is depicted in figure-3 (C). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 82.16, P<0.05]. Post-hoc test revealed that all the groups showed significant elevation in ALP levels compared to control. GL and EP (200 mg/kg) showed significant decrease in ALP levels

compared to DM. EP (50 and 100 mg/kg) showed significant increase in SGOT levels compared to GL.

The effect on LPO of EP (50, 100 and 200 mg/kg) is depicted in figure-3 (D). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 19.37, P<0.05]. Post-hoc test revealed that all the groups showed significant elevation in LPO levels compared to control. GL and EP (100 and 200 mg/kg) showed significant decrease in LPO levels compared to DM. EP (50 mg/kg) showed significant increase in LPO levels compared to GL. EP (100 and 200 mg/kg) showed significant decrease in LPO levels compared to EP (50 mg/kg) group.

The effect of EP (50, 100 and 200 mg/kg) on SOD is depicted in figure-3 (E). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 23.15, P<0.05]. Post-hoc test revealed that all the groups showed significant elevation in SGOT levels compared to control. GL and EP (50, 100 and 200 mg/kg) showed significant decrease in SOD levels compared to DM. EP (50 and 100 mg/kg) showed significant increase in SOD levels compared to GL. EP (200 mg/kg) showed significant decrease in SOD levels compared to EP (50 mg/kg).

The effect of EP (50, 100 and 200 mg/kg) on CAT is depicted in figure-3 (F). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 22.11, P<0.05]. Post-hoc test revealed that all the groups showed significant reduction in CAT levels compared to control. GL and EP (100 and 200 mg/kg) showed significant increase in CAT levels compared to DM. EP (50 mg/kg) showed significant decrease in CAT levels compared to GL. EP (100 and 200 mg/kg) showed significant decrease in CAT levels compared to EP (50 mg/kg).

### 2.17. Effect of EP (50, 100 and 200 mg/kg) on Total Hb, HbA1c, plasma insulin, urea, creatinine and albumin:

The effect of EP (50, 100 and 200 mg/kg) on total Hb is depicted in figure-4 (A). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 0.81, P<0.05]. Post-hoc test revealed that DM showed significant reduction and no other groups did not show any change in total Hb levels compared to control. The GL and EP (50, 100 and 200 mg/kg) groups showed significant increase in total Hb levels compared to DM.

The effect of EP (50, 100 and 200 mg/kg) on HbA1c is depicted in figure-4 (B). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 17.19, P<0.05]. Post-hoc test revealed that DM, GL and EP (50, 100 and 200 mg/kg) showed significant elevation in HbA1c levels compared to control. GL and EP (50, 100 and 200 mg/kg) showed significant decrease in HbA1c levels compared to DM. EP (50 and 100 mg/kg) showed significant increase in HbA1c levels compared to GL.

**Fig. 4.**

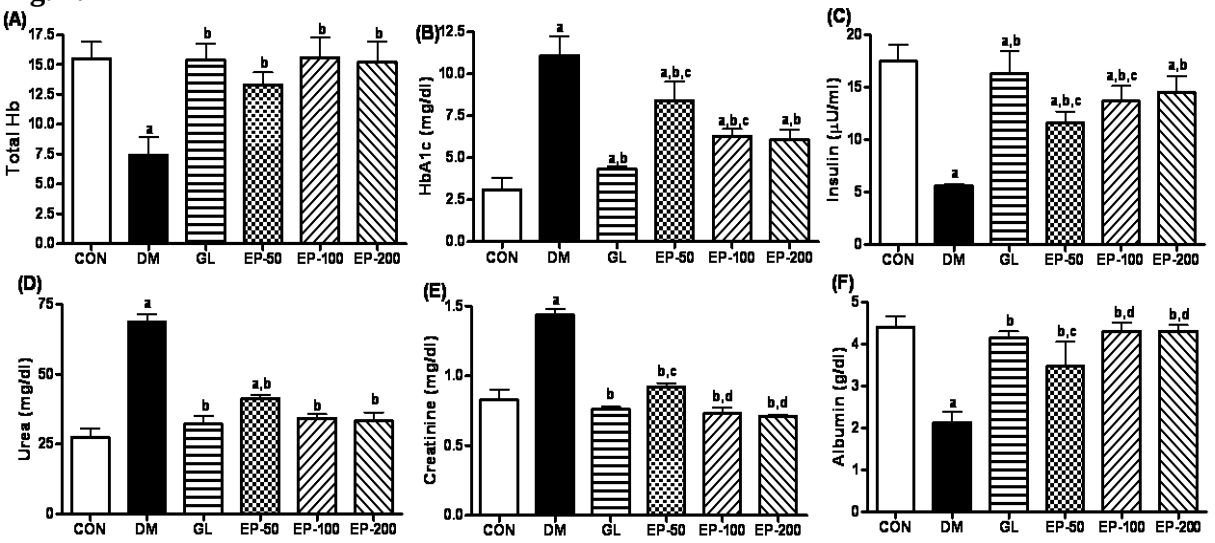


**Fig-4. Effect of EP (50, 100 and 200 mg/kg) on the levels of Total Hb (A), HbA1c (B), Insulin (C), Urea (D), Creatinine (E) and Albumin (F). All values are Mean±SEM. [a]P<0.05 compared to control, [b]P<0.05 compared to diabetes, [c]P<0.05 compared to GL, [d]P<0.05 compared to EP-50 and [e]P<0.05 compared to EP-100 (One-way ANOVA followed by Student Newmann keuls test).**

The effect of EP (50, 100 and 200 mg/kg) on plasma insulin is depicted in figure-4 (C). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 35.69, P<0.05]. Post-hoc test revealed that DM, GL and EP (50, 100 and 200 mg/kg) showed significant reduction in plasma insulin levels compared to control. GL and EP (50, 100 and 200 mg/kg) showed significant increase in plasma insulin levels compared to DM. EP (50 and 100 mg/kg) showed significant decrease in insulin levels compared to GL.

The effect of EP (50, 100 and 200 mg/kg) on plasma urea levels is depicted in figure-4 (D). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 41.39, P<0.05]. Post-hoc test revealed that DM and EP (50 mg/kg) showed significant increase in plasma urea levels compared to control. The GL and EP (50, 100 and 200 mg/kg) groups showed significant decrease in plasma urea levels compared to DM.

The effect of EP (50, 100 and 200 mg/kg) on plasma creatinine is depicted in figure-4 (E). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 76.91, P<0.05]. Post-hoc test revealed that DM showed significant increase in plasma creatinine levels compared to control. GL and EP (50, 100 and 200 mg/kg) showed significant decrease in plasma creatinine levels compared to DM. EP (50 mg/kg) showed significant increase in creatinine levels compared to GL. EP (100 and 200 mg/kg) showed significant decrease in creatinine levels compared to EP (50 mg/kg).

The effect of EP (50, 100 and 200 mg/kg) on plasma albumin is depicted in figure-4 (F). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 15.09, P<0.05]. Post-hoc test revealed that DM showed significant decrease in plasma albumin levels compared to control. GL and EP (50, 100 and 200 mg/kg) showed significant increase in plasma albumin levels compared to DM. EP (50 mg/kg) showed significant decrease in albumin levels compared to GL. EP (100 and 200 mg/kg) showed significant increase in albumin levels compared to EP (50 mg/kg).

## DISCUSSION

In the present experiment, we for the first time have reported that the routine post-treatment of EP (50, 100 and 200 mg/kg) for 21 days showed potential hypoglycemic activity in OGTT and normoglycemic rats and anti-hyperglycemic activity in alloxan-induced rats. Further, EP showed good hypoglycemic effect by dose dependant manner.

Preliminary phytochemical screening revealed that EP showed positive response to alkaloids, saponins and triterpenes, tannins, flavonoids, carbohydrates and sterols. Further, the flavoniod content of EP was found to be 28.1±0.7 mg in quercetin equivalent/ 1g extract. Further, it has been suggested that this plant contains furanoflavones, furanoflavonols, chromenoflavones, and flavones. furanodiketones and flavonoid glycosides [17,18]. It has been well-documented that most medicinal plants are enriched with phenolic compounds and bioflavonoids that have excellent antioxidant and antidiabetic properties. It has been suggested that diabetes is of two categories out of which one is genetically based and other as a result of dietary indiscretion [28]. In the present experiment we have found that only the EP (200 mg/kg) significantly reduced the blood sugar level in hyperglycemic rats in dose-dependent manner. Moreover, EP (200 mg/kg) showed similar significant lowering of blood glucose level in OGTT and normoglycemic rats. Therefore, the present study is indicating that the EP (200 mg/kg) would be a potential candidate to treat both categories of diabetes which could be due to presence of flavonoids.

The final body weight decreased instead of no change in initial body weight and increased food and fluid intake in diabetic animals had been observed in the current study. Further, there was significant decrease in total Hb, plasma insulin and albumin levels, however there was significant increase in HbA1c, urea and creatinine levels. The above findings are found to be similar with the previous published results [29]. It has been reported that protein glycation during hyperglycemia mostly leads to production of HbA1c. Hence, HbA1c level is used as the most reliable indicator for assessment of retrospective glycemic control in the management of diabetes [30]. The reduction of HbA1c levels in diabetic animals indicated that there was less protein glycation, possibly resulting from the decrease in blood glucose levels observed in these animals. In continuation with the above findings EP (200 mg/kg) showed significant mitigating effect in HbA1c levels.

It has been documented that the levels of serum lipids are usually elevated in diabetes mellitus, representing a high risk factor for cardiovascular disorders [31]. This abnormal high level of lipids is mainly due to the absence of inhibitory actions of lipolytic hormones on the fat depots. It has been observed earlier that the hypercholesterolemia and hypertriglyceridemia occurs in

diabetic animals. Under normal conditions, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides [32]. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridemia. Since lipid abnormalities accompanying with premature atherosclerosis, is the major cause of cardiovascular diseases in diabetic patient therefore ideal treatment for diabetes, in addition to glycemic control, should have a favorable effect on lipid profile. It has been systematically reported that cardiovascular diseases are listed as the cause of death in 65% people suffering from diabetes [33]. The EP (200 mg/kg) not only lowered the TC, TG and LDL levels but also enhanced the cardio protective lipid HDL in diabetic rats, respectively. It has been evidenced that an increase in HDL levels is associated with a decrease in coronary risk levels [34]. In the present study the EP (200 mg/kg) not only decreased the TC levels but also enhances the HDL levels significantly. High levels of TC and more importantly LDL levels are major coronary risk factors [35]. In the present study it has been found that the EP (200 mg/kg) mitigated the elevated TC and LDL levels in diabetic rats. Further, it has been suggested that TG itself is independently related to coronary heart disorders [36] and in the present investigation the EP (200 mg/kg) lowered TG levels in alloxan-induced animals.

Routine post-treatment of the EP (200 mg/kg) for 21 days to diabetic animals improved the liver function by decreasing the serum SGOT, SGPT and ALP levels. It is reported that the increase in levels of SGOT and SGPT will increase the incidence of heart and liver diseases. SGOT is an enzyme found primarily in the cells of the liver, heart, skeletal muscles, kidneys, and pancreas and to a lesser extent in red blood cells. Its serum concentration is directly proportional to the amount of cellular leakage or damage. It is released into serum in larger quantities when any one of these tissues is damaged. The increased levels of these enzymes are usually associated with heart attacks or liver disease. The EP (200 mg/kg) decreased the SGOT level, which is an indication of the protective effect on liver and heart. It is known that SGPT, an enzyme found primarily in the liver, the enhanced release of which into the bloodstream is the result of liver aberration. It therefore serves as a fairly specific indicator of liver status and its elevated levels in serum indicate liver damage. It has been reported that the increased levels of ALP indicates bone disease, liver disease or bile tract blockage. The EP (200 mg/kg) reduced the ALP levels, indicating its protective effect on liver function.

It has been suggested that oxidative stress plays a central role in the pathogenesis of diabetes [37]. Oxidative stress is the result of excessive free radical production and lipid peroxidation due to aberrant protection by the enzymatic and nonenzymatic anti-oxidative defense system. Oxidative stress is thought to be a direct concequence of increased production of reactive oxygen species (ROS). Degradation of lipid peroxides leads to the accumulation of toxic aldehydes, one of the most toxic being malondialdehyde (MDA), as an index of lipid peroxidation. In the present study we measured MDA because this parameter is now considered to be one of the indicative of *in vivo* lipid peroxidation [38]. Further, it has been studied that lipid peroxidation is augmented in diabetes [39]. Oxidative stress, arising from an increase in ROS coupled with impaired antioxidant protection, has been suggested to be an important etiological factor for diabetes [40]. During stress LPO and SOD were significantly increased and CAT level was significantly decreased. The increase in SOD was due to increased ROS generation. This led to increased generation of $H_2O_2^-$ and its accumulation due to decreased CAT level [41]. In the

present study it has been found that LPO and SOD levels were increased, and CAT levels were decreased in diabetic rats compared to control rats and was reversed by the EP (200 mg/kg) significantly, indicating improved in oxidative damage and antioxidant profile.

In conclusion, the EP (200 mg/kg) found to be potential antidiabetic extract in alloxan-induced diabetic animals. Further, isolation and establishment of exact mechanism of action of specific compound from EP is to be carried out in the future.

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