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# Antidiabetic effect of *Punica granatum* flowers: Effect on hyperlipidemia, pancreatic cells lipid peroxidation and antioxidant enzymes in experimental diabetes

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ABSTRACT

The present study investigated the effects of *Punica granatum* aqueous extract (PgAq) on streptozotocin (STZ) induced diabetic rats by measuring fasting blood glucose, lipid profiles (atherogenic index), lipid peroxidation (LPO) and activities of both non-enzymatic and enzymatic antioxidants. Diabetes was induced by single intraperitoneal injection of STZ (60 mg/kg) to albino Wistar rats. The increase in blood glucose level, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), very low density lipoprotein (VLDL), LPO level with decrease in high density lipoprotein cholesterol (HDL-C), reduced glutathione (GSH) content and antioxidant enzymes namely, glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-*S*-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) were the salient features observed in diabetic rats. On the other hand, oral administration of PgAq at doses of 250 mg/kg and 500 mg/kg for 21 days resulted in a significant reduction in fasting blood glucose, TC, TG, LDL-C, VLDL-C and tissue LPO levels coupled with elevation of HDL-C, GSH content and antioxidant enzymes in comparison with diabetic control group.

The results suggest that PG could be used, as a dietary supplement, in the treatment of chronic diseases characterized by atherogenous lipoprotein profile, aggravated antioxidant status and impaired glucose metabolism and also in their prevention.



## 1. Introduction

Free radical may play a pivotal role in the pathogenesis of a number of diseases including diabetes mellitus (DM) (Feillet-Coudray et al., 1999). DM is a prevalent systemic disease affecting a significant proportion of the population worldwide. The effects of diabetes are devastating and well documented (Duckworth, 2001). STZ is frequently used to induce DM in experimental animals through its toxic effects on pancreatic β-cells (Kim et al., 2003). The cytotoxic action of STZ is associated with the generation of reactive oxygen species causing oxidative damage (Szkudelski, 2001). There is increasing evidence that in certain pathologic states, especially chronic diseases, the increased production and/or ineffective scavenging of reactive oxygen species (ROS) may play a critical role. High reactivity of ROS exerts toxic effects on the pancreatic acinar cells. Sato et al. (1979) reported that plasma TBARS levels increased in diabetic patients due to vascular lesions induced by hyperglycemia. Diabetes manifested by experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, which thereby depletes the activity of antioxidative enzymes and thus promote free radical generation (Bayness and Thorpe, 1996). Disturbances of antioxidants defence system in diabetes involves: alteration in antioxidant enzymes (Strain, 1991), impaired glutathione metabolism (McLennan et al., 1991), and decreased ascorbic acid levels (Jennings et al., 1987). Dietary supplements contribute to the prevention of diabetic complications by decreasing LPO and improving antioxidant status.

*Punica granatum* Linn. (Punicaceae), commonly called pomegranate, is a large shrub or small tree grows well in the warm valleys and outer hills of the Himalayas and is cultivated throughout the India (Anonymous, 1969).

Pomegranate is a rich source of bioactive compounds and is used in folk medicine for the treatment of various diseases. The ripe fruit is tonic, astringent, laxative, diuretic, used in brain diseases, chest troubles, bronchitis and earache. Bark and fruit rind are administered orally to prevent dysentery, diarrhoea, piles, bronchitis, biliousness and as an anthelmintic. The powdered flower buds are given internally to relieve bronchitis, diarrhoea and dysentery of children. A decoction of the flowers is gargled to reduced oral and

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throat inflammation. In Unani medicine, the flowers of PG are efficacious to treat diabetes, either as a single drug or in polyherbal formulations (Nandkarni, 1976). The biological activities viz. antibacterial (Chopra et al., 1960), antifungal (Charya et al., 1979), anthelmintic (Singhal, 1983), antifertility (Gujral et al., 1960), antioxidant (Yasuko et al., 2002), antidiabetic (Jafri et al., 2000), and antiulcer (Ajaikumar et al., 2005) of the various extracts of different parts of this plant have already been documented.

Flavonoids are one of the most numerous and widespread group of phenolics found in vegetables and fruits (Schinella et al., 2002; Tepe et al., 2005). Some of them, due to their phenolic structure, are known to be involved in the healing process of free radical-mediated diseases including diabetes (Czinner et al., 2000). Several alkaloids, flavonoids, polyphenolic compounds (such as delphinidin, cyanidin and pelargonidin) and hydrolyzable tannins (such as punicalin, pedunculagin, punicalagin, gallic and ellagic acid esters of glucose) which possesses strong antioxidant properties have been reported from PG (Du et al., 1975).

The presence of strong antioxidant principles of PG thus prompted us to design the present study to investigate whether management with PG has any protective effect on serum lipid profile, pancreatic lipid peroxidation and activities of both enzymatic and non-enzymatic antioxidant status in diabetic rat.

## 2. Materials and methods

### 2.1. Animals

Male Albino Wistar rats (150–200 g) were used for this study. They were housed in macrolon cages under standard laboratory conditions (12 h light/12 h darkness, 21 ± 2 °C). The animals were given standard pellets diet (Lipton rat feed, Ltd., Pune) and water *ad libitum* throughout the experimental period. The experimental study was approved by the Institutional Animal Ethical Committee of Jamia Hamdard, New Delhi.

### 2.2. Preparation of plant extract

*P. granatum* flowers were procured from the Khari Baoli, local market of Delhi, India. The plant was identified by the Dr. M.P. Sharma, Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi. A voucher specimen was deposited in the Pharmacognosy and Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi. The air-dried powdered flowers (500 g) were extracted with water in soxhlet apparatus for 6 h. The extract was evaporated to dryness under reduced pressure to give solid residues. The residue was stored at 0–4 °C for subsequent experiments.

### 2.3. Chemicals

Streptozotocin was obtained from Sigma chemicals (USA). All the other chemicals used were of analytical grade.

### 2.4. Induction of diabetes

The animals were fasted for 16 h prior to the induction of diabetes. STZ freshly prepared in citrate buffer (0.1 M, pH 4.5) was administered intraperitoneally (i.p.) at a single dose of 60 mg/kg. Development of diabetes was confirmed by polydipsia, polyuria and by measuring blood glucose concentrations 72 h after injection of STZ. Rats with blood glucose level of 250 mg/dl or higher were considered to be diabetic.

### 2.5. Experimental design

The rats were randomized into four groups comprising of six animals in each groups as given below. PG (250 mg/kg and 500 mg/kg) were administered orally in aqueous solution (3% v/v tween 80 in water) once per day.

- Group I: normal control rats, received citrate buffer (pH 4.5) (1 ml/kg, i.p.).
- Group II: diabetic control rats, received STZ in single dose (60 mg/kg, i.p.).
- Group III: PgAq treated diabetic rats, received PgAq extract (250 mg/kg/day, p.o.) 3 days after STZ treatment and continued for 21 days.
- Group IV: PgAq treated diabetic rats, received PgAq extract (500 mg/kg/day, p.o.) 3 days after STZ treatment and continued for 21 days.

On the last day of experiment, blood samples were collected for biochemical estimations. Later the animals were sacrificed and pancreas was removed, cleaned and washed in ice-cold normal saline for biochemical study.

### 2.6. Post-mitochondrial supernatant preparation (PMS)

Pancreas was removed quickly, perfused immediately with ice cold normal saline and homogenized in chilled phosphate buffer (0.1 M, pH 7.4) containing potassium chloride (1.17% w/v), using a Potter Elvehjem homogenizer. The homogenate was centrifuged at 800g for 5 min at 4 °C in a refrigerated centrifuge to separate the nuclear debris. The supernatent so obtained was centrifuged at 10, 500g for 20 min at 4 °C to get the PMS which was used to assay GPx, GR, GST, SOD and CAT activity.

### 2.7. Analytical procedures

Blood glucose was estimated by Ames One Touch Glucometer (Accu Check Roche, Germany). Serum TC (Demacker et al., 1980), TG (Foster and Dunn, 1973), LDL-C, VLDL-C (Friedword et al., 1972) and HDL-C (Burnstein et al., 1970) were estimated by using standard enzymatic colorimetric kits (Span diagnostics Ltd. Surat, India). LPO was estimated by thiobarbituric acid (TBA) reaction with malondialdehyde (MDA), a product formed due to the peroxidation of membrane lipids (Ohkawa et al., 1979). Protein was estimated by the method of Lowry et al. (1951) using bovine serum albumin as standard. GSH content was determined according to the method of Ellman (1959). GPx and GR activities were measured as described by Mohandas et al. (1984). GST activity was estimated according to the method of Habig et al. (1974). SOD activity was assessed according to the method of Marklund and Marklund, 1974. CAT activity was assayed following the method of Claiborne, 1985.

### 2.8. Statistical analysis

Data were expressed as the mean ± S.D. For statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA) followed by Tukey post hoc test for multiple comparison. $P < 0.05$ was considered to be statistically significant.

## 3. Results

### 3.1. Effect of PgAq on hyperglycemia

Table 1 summarizes the levels of glucose in normal and diabetic animals. In all the groups prior to STZ administration, the basal levels of blood glucose of the rats were not significantly different. However, there was a significant elevation in glucose 72 h after administration of STZ. Although a significant antihyperglycemic effect was evident from the first week onwards, the decrease in blood glucose was maximum on third week in group receiving PgAq. After STZ administration, significant ($P < 0.001$) increase in

**Table 1**
Effect of *Punica granatum* flowers on blood glucose level in diabetic rats.

| Groups | Blood glucose ($mg\,dl^{-1}$) | | | | |
|---|---|---|---|---|---|
| | 0 day | 3 days after STZ | First week | Second week | Third week |
| Normal control | 85.16 ± 12.18 | — | 83.5 ± 10.05 | 89.5 ± 9.16 | 82.33 ± 9.18 |
| Diabetic control | 78.50 ± 9.48 | 318.66 ± 15.48 | 325.16 ± 5.30[#] | 343.83 ± 9.21[#] | 352 ± 7.12[#] |
| Diabetic + PgAq (250 mg/kg) | 83.83 ± 4.95 | 308.16 ± 8.72 | 156.34 ± 8.81[**] | 132.67 ± 7.87[**] | 112. 84 ± 7.39[**] |
| Diabetic + PgAq (500 mg/kg) | 77.16 ± 11.70 | 332.83 ± 6.86 | 140.67 ± 9.86[**] | 119.34 ± 10.56[**] | 84.67 ± 10.17[**] |

The data are expressed in mean ± S.D.; $n = 6$ in each group.
[#] $P < 0.001$ compared with the corresponding value for normal control animals.
[**] $P < 0.001$ compared with the corresponding value for diabetic control animals.