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Effect of *Punica granatum* Flowers Extract on Hypercholesterolemic and Alloxan Induced Diabetic Rats

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Abstract: Effect of *Punica granatum* on blood glucose and cholesterol levels in normal and alloxan induced diabetic rats was investigated. In this study 40 rats were included and divided into five groups of eight rats in each group. One group was normal (A), second group was control diabetic(B), third groupwas diabetic(C), fourth was control hypercholesterolemic (D) and fifth group was hypercholesterolemic (E). Two groups B and C were made diabetic by intraperitonial injection of alloxan. Groups D and E were made hypercholesterolemic by oral administration of cholesterol. The blood samples from all the rats were collected and analyzed for blood glucose and cholesterol level by using enzymatic kits. The blood glucose level reduced from 170.6±6.67 (mg/dL) to 133±6.399 (mg/dL) and cholesterol levels of treated groups of rats showed significant reduction from 228.8±5.986 (mg/dL) to 199.37±4.307 (mg/dL) after 7 weeks of treatment with *Punica granatum*. It was concluded that *punica granatum* flowers extract has hypoglycemic and hypocholesterolemic effect so it can be used to overcome hypoglycemic and hypercholesterolemic conditions.

Key words: *Punica granatum* · Hyperglycemia · Hypercholesterolemia · Alloxan

INTRODUCTION

Diabetes mellitus is a syndrome of disordered metabolism usually due to a combination of hereditary and environmental causes, resulting in abnormally high blood sugar levels [1]. It is characterized by depleted insulin secretion, hyperglycemia and altered metabolism of lipids, carbohydrates and proteins in addition to damage to the β-cells of pancreas and an increased risk of complications of vascular disease [2].

Diabetes mellitus is a major endocrine disorder of carbohydrate disturbed metabolism and growing health problem in the world [3]. Recently, it was suggested that formation of free radicals is involved in the pathogenesis of diabetes and the development of diabetic complications because a prolonged exposure to hyperglycemia increases the generation of free radicals and reduces capacities of the antioxidant defense system [4].

Hypercholesterolemia is the presence of high level of cholesterol in blood. It is not a disease, but a metabolic derangement that can be secondary to many diseases and can contribute too many forms of disease, most notably cardiovascular disease. This may be related to diet, genetic factors and the presence of other diseases such as diabetes and under active thyroid [5].

More than 400 traditional plants treatments for *diabetes mellitus* Type-II have been recorded, but only a small number of these have received scientific and medical evaluation to assess their therapeutic efficacy [6] *Punica granatum* (Punicaceae) is a shrub or small tree and considered to be a native of Iran and Afghanistan. It is also found growing wild in the warm valleys and outer hills of the Himalayas and is cultivated throughout India. Gulnar (flower of *P. granatum*) has been known for a long time in Unani literature as an astringent, haemostatic and as a remedy for diabetes [7]. So it was considered worthwhile to investigate the effect of the flowers of *P. granatum* on blood glucose levels of glucose fed hyperglycemic rats [8].

MATERIALS AND METHOD

Venue: The whole experimental work was conducted at the institute of Molecular Biology and Biotechnology, The University of Lahore.

***Punica Granatum* Flowers:** The flowers of *P. granatum* were purchased under the Unani name "Gulnar farsi" from local market.

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Extract Preparation of Plant Material: Air-dried and ground spomegrante flowers (500 g) were extracted at room temperature three times with 5 vol of methanol (w/v). The solvent was evaporated under reduced pressure below 50°C to give a methanolic extract of pomegranate flowers [9].

Alloxan: Alloxan induced hyperglycemia has been described as a useful experimental model to evaluate the activity of hypoglycemic agents 14. Diabetes was induced by a single intra-peritoneal injection of alloxan prepared in 0.1mol/L citrate buffers at a dose of 100 mg/Kg body weight. Diabetes was confirmed in the alloxan treated rats by measuring the fasting blood glucose concentration 8-10 days post-injection [10].

Animals: Forty adult male albino rats (*Ratis norvegicus*) weighing between 200 to 250g were used and housed in stainless steel cages with wire mash floor. Rats were randomized into five groups of 8 animals in each.

Induction of Diabetes: The animals were divided into five groups and each group consisted of eight rats. *Diabetes mellitus* was induced in overnight fasted animals by a single subcutaneous injection of alloxan in a dose of 125mg/kg body weight dissolved in 1 ml distilled water prepared immediately before use. After 7 days of alloxan injection, the level of glucose was measured [11]. Rats with serum glucose level, ranging between 150 mg/dl or above were considered as diabetic.

InductionofHypercholesterolemia: Hypercholesterolemia was induced in rats by feeding (1g/Kg diet) cholesterol orally for 10 days. The rats with cholesterol level above 200 mg/dl were considered as hypercholesterolemic [12].

Samples Collection and Analysis: Blood (1ml) was collected from coccygial vein of each rat and was transferred into sample tubes for analysis. The serum was obtained by centrifuging each blood sample at 3000 rpm for 10 minutes. The specific enzymatic kit was used to assess the serum glucose levels of rats using spectrophotometer [13].

Estimation of Blood Glucose: Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as an indicator.

Procedure: Total 42 test tubes were taken. 40 tubes were labeled as 1, 2, 3 ... 40 for each sample of rat's serum from each group i.e., normal + normal feed, normal + *Punica granatum* extract, diabetic + normal feed and diabetic + *Punica granatum* extract. The remaining two tubes were labeled as blank and standard. 2ml reagent was taken in all the tubes by pipette. 20µl of distilled water was added to the test tube labeled as blank, 20µl of standard solution from the kit was added to the test tube labeled as standard and 20µl of each serum sample was taken in tubes labeled as 1,2,3 ..... 40. Then the contents of all tubes were mixed and incubated at 37°C for 10 minutes. Then absorbance of standard (Abs Std.) and sample (AbsS) were measured at 546nm against the blank (AbsRB).

Calculation:

Glucose concentration (mg/dl) = (Abs test/ Abs Standard) × 100 [14].

Statistical Analysis: The obtained data was subjected to statistical analysis for the determination of significance by using ANOVA ($P<0.05$) was considered as significant [15].

RESULTS

In the control group, blood glucose levels have almost similar levels through out the 42 days of study. The serum glucose level was slightly changed to 107.5 mg/dl at 21st and 42nd day of the experiment, respectively while they were not treated with *punica granatum* flowers extract as shown in table 1.

In diabetic rats (group B) a gradual increase in blood glucose level was observed. The average serum glucose level in rats were recorded as 167 mg/dl at 1st day which raised to 171.8mg/dl and 176.6mg/dl at 21st and 42nd day of the experiment, respectively while they were not treated with *punica granatum* flowers extract.

Diabetic rats (group C) who fed *punica granatum* flowers extract (500mg/kg) showed a significant decrease in serum glucose levels as presented in Table 2. The average blood glucose level of rats was recorded as 170.6mg/dl at 1st day of experiment before the treatment with *punica granatum* flowers extract. The blood glucose level after the treatment with *punica granatum* flowers extract reduced to 153.7mg/dl and further to 133mg/dl at 21st and 42nd day of the experiment, respectively (Table 1).

Table 1: Descriptive statistics of mean values of glucose level (mg/dL) with standard deviation in different rat groups

Groups	Glucose Level (mg/dl)				
Means±S.D	Day 1	Day 21	Day 42	F	p
Group A:					
Normal Control	107±4.72	107±5.04	107.5±4.98	304.764*	.000*
Group B:					
Diabetic Control	167±5.81	171.8±5.03	176.6±5.73	318.848*	.000*
Group C:Diabetic with					
N.feed+*P.granatum* extract	170.6±6.6	153.7±5.5	133±6.39	297.570*	.000*

*= Significant as P< 0.05

Table 2: Descriptive statistics of mean values of cholesterol level (mg/dL) with standard deviation in different rat groups

Groups	Cholesterol Level (mg/dl)				
Means±S.D	Day 1	Day 21	Day 42	F	p
Group A: Control	176.7±4.3	176.6±3.6	176.3±3.7	206.090*	.000*
Group D: Control +					
Hypercholesterolemic	214.8±5.5	217±5.8	219.1±5.9	171.211*	.000*
Group E: Hypercholesterolemic +					
P.G Flowers Extract	228.8±5.9	217.5±5.5	199.37±4.3	162.720*	.000*

*= Significant as P< 0.05

In hypercholesterolimic rats (group D) a gradual increase in blood cholesterol level was observed. The average cholesterol levels were recorded as 214.8 mg/dl at 1st day of experiment. The blood cholesterol level raised to 217mg/dl and 219.1mg/dl at 21st and 42nd day of the experiment, respectively (Table 2) while they were not treated with *punica granatum* flowers extract.

Hypercholessterolimic rats (grpup E) who received *punica granatum* flowers extract (250mg/kg) showed a significant reduction in blood cholesterol levels. The average blood cholesterol levels were recorded as 228.8mg/dl at 1st day of experiment before feeding with *punica granatum* flowers extract. The blood cholesterol levels for this group were reduced to 217.5mg/dl and 199.3mg/dl at 21st and 42nd day of the experiment, respectively after the treatment with *punica granatum* flowers extract. The overall decrease in blood cholesterol levels was found for this group which was significant (P<0.05) as shown in Table 2.

DISCUSSION

Significant decrease in blood glucose and blood cholesterol level was observed in groups treated with *P.grantum* flower extract as compared to control (P<0.05) which was in agreement with the work of Bagri *et al.* [16] where they observed the hypoglcaemic and hypocholesreolimic effect *P.grantum* on blood glucose, total cholesterol, triglyceride (TG), low density lipoprotein (LDL-C), very low density lipoprotein (VLDL). This effect could, possibly due to increase peripheral glucose utilization. Inhibition of the proximal tubular reabsorption mechanism for glucose in the kidney also contributes towards blood lowering effect [17].

The testplant extract also improve oral glucose tolerance in normal rats. At the present juncture it is not possible to pinpoint the exact mechanism of antihyperglycaemic action of extract of *P.grantum.* However based on earlier studies some suggestions can be made for possible mechanism. It is reported that an infusion of epicarps of *P.grantum* inhibit the intestinal absorption of glucose in rats. Thus a possibility exists that intestinal glucose absorption could be partly responsible for inhibition of hyperglycemia [18]. *P.grantum* is also beneficial in control of h ypercholesterolemia. This effect could be due to the activity of SOD and CAT, which in turn cause reduction in ROS such as superoxid, hydrogen peroxide (H_2O_2) and hydroxal redical (OH) that reduces the activity of these enzymes which leads to accumulation of lipids [19].

As compared to the group B (Diabetic control), the group C treated with *P.grantum* flower extract show significant decrease in blood glucose level (P<0.05) [20]. Significant decrease in blood cholesterol level was also observed in group E treated with *P.grantum* as compared to group D (hypercholesterolemic control) [21].

It was concluded from the present study that *punica granatum* flowers extract has hypoglycemic and hypocholesterolemic effect so it can be used to overcome hypoglycemic and hypercholesterolemic conditions.

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