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Antihyperglycemic, antioxidant and hypolipidemic effect of *Punica granatum* L flower extract in streptozotocin induced diabetic rats

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ABSTRACT

Objective: To investigate the antihyperglycemic, hypolipidemic and antioxidant effects of aqueous and ethanolic flower extracts of *Punica granatum* (*P. granatum*) in streptozotocin (STZ) induced diabetes mellitus in wistar rats. **Methods:** Antihyperglycemic, hypolipidemic and antioxidant effect of *P. granatum* aqueous (PGFAet) and ethanolic extracts (PGFEet) at doses of 200 and 400 mg/kg bw was evaluated in streptozotocin induced diabetic rats. Glycosylated hemoglobin, plasma insulin, cholesterol and lipoprotein levels, lipid peroxide levels and the antioxidant enzyme levels were determined. **Results:** The administration of the extracts markedly reduced blood glucose and glycosylated hemoglobin increased the levels of plasma insulin and liver glycogen. The extract also had a hypolipidemic activity decreasing the levels of total cholesterol, LDL-cholesterol, VLDL-cholesterol and triglycerides. The levels of lipid peroxides in terms of thiobarbituric acid reactive substances (TBARS) were remarkably reduced and the activities of the enzymes SOD, CAT and GPx and GSH were increased. We also saw an increase in the activity of the enzyme glucokinase and decrease in glucose-6-phosphatase. It can be concluded that the flower extracts of *P. granatum* (PGFAet and PGFEet) has significant restorative effect on the blood glucose, hyperlipidemia and oxidative stress. **Conclusions:** It can be concluded from the studies that the ethanolic extracts of the flowers of *P. granatum* at dosage 400 mg/kg/day exhibit significant effect in lowering blood sugar and lipid levels and increasing the antioxidant enzymes when compared to the aqueous extract. Glycosylated hemoglobin levels were reduced and the extract exhibited a stimulatory effect on insulin.

1. Introduction

Diabetes mellitus is the most common endocrine disorder, characterized by a defective or deficient insulin secretory process, glucose underutilization and increased blood sugar (hyperglycemia). During diabetes mellitus persistent hyperglycemia causes an increased production of reactive oxygen species (ROS) via auto-oxidation of glucose and non-enzymatic protein glycation which may lead to disruption of cellular functions and oxidative damage to membranes. An increase in ROS is an impairment of antioxidant defence system or an insufficient capacity to repair oxidative damage[1].

Baynes reported that plasma thiobarbituric acid reactive substance (TBARS) levels increased in diabetic patients due

to vascular lesions induced by hyperglycemia[2]. Circulating lipid peroxides were shown to be capable of initiating the secondary complication of diabetes. The concentration of the ROS are modulated by the antioxidants both enzymic and non-enzymic. Enzymes viz, superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) and non-enzymic like reduced glutathione (GSH). In diabetes, oxidative stress has been found to be mainly due to an increased production of reactive oxygen species and a sharp reduction of antioxidant defenses[3].

The management of diabetes without any side effects is still a challenge to the medical system. Recent years has witnessed a renewed interest in plants as pharmaceuticals because they synthesize a variety of secondary metabolites with antioxidant potential which can play a major role in protection against molecular damage induced by reactive oxygen species. Hence, compounds with both hypoglycemic and antioxidative properties would be useful antidiabetic agents.

Pomegranate (*Punica granatum* L.) (*P. granatum*) belongs

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to the Punicacea family. It is one of the important and commercial horticultural fruits which are generally very well adapted to the Mediterranean climate. It is native to India, Pakistan and Afghanistan and also cultivated in some parts of USA (California), China, Japan and Russia. Pomegranate fruits are consumed fresh or processes as juice, jellies and syrup for industrial production^[4,5]. Different parts of its tree (leaves, fruits and bark skin) have been used traditionally for their medicinal properties and for other purposes such as in tanning. It is proved to have high antioxidant activity^[6] and good potency for cancer prevention^[7].

However, not much research has been done on the usefulness of *P. granatum* flower extract in the treatment of diabetes mellitus, the present study aimed at evaluating the ameliorative potential of the flower extract on hyperglycemia-mediated oxidative stress in streptozotocin-induced diabetes mellitus.

2. Materials and methods

2.1. Plant material

The flowers of *P. granatum* was collected in and around Trichy, Tamil nadu, India. The plant was taxonomically identified and authenticated by Dr. S. Kalavathy, Professor of Botany, Bishop Heber College, Trichy and a voucher specimen is deposited in the Department of Botany, Bishop Heber College, Trichy.

2.2. Preparation of plant extracts

2.2.1. Ethanolic extract (PGFEet)

Flowers of *P. granatum* (500 g) were shade dried, powdered and then soaked in 1500 mL of 95% ethanol overnight. After filtration, the residue obtained was resuspended in 95% ethanol for 48 h and filtered. The filtrates obtained were combined and the solvent was evaporated in a rotavapour at 40–50 °C under reduced pressure. A semisolid brown powder of the extract was obtained (20% w/w) and stored until further use. A known amount of the residual extract was suspended in distilled water and was orally administered during the experiment.

2.2.2. Aqueous extract (PGFAet)

Fresh flowers (100 g) of *P. granatum* was shade dried, powdered and then soaked in 250 mL of water for 2 h and then heated at 60–65 °C and 45–55% of relative humidity with 12:12 h light and dark cycles. Rats were fed with pellet diet and water ad libitum. All the animal experimentations were premeditated and executed in compliance with the ethical norm approved by ministry of Social Justice and Empowerment, Government of India and Institutional Ethics Committee Guidelines (743/03/CPSEA dt. 3.3.03).

2.3. Induction of diabetes

A freshly prepared solution of streptozotocin (45 mg/kg bw) in 0.1 M citrate buffer^[8], pH 4.5 was injected intraperitoneally

to overnight fasted rats. After 3 days, blood was collected in vials from the tail vein of overnight fasted rats and was allowed to clot to separate serum. It is then centrifuged at 4000 rpm for 10 min to obtain clear serum. FBG level was estimated and PPG was checked regularly up to stable hyperglycemia, usually 1 week after streptozotocin injection. Animals having marked hyperglycemia (FBG>250 mg/dL) were selected for the study.

2.4. Experimental design

The animals were randomly divided into 7 groups of six rats each:

Group I: control animals (normal, non-diabetic animals);

Group II: STZ-diabetic animals;

Group III: STZ-diabetic rats treated with PGFEet (200 mg/kg bw);

Group IV: STZ-diabetic rats treated with PGFEet (400 mg/kg bw);

Group V: STZ-diabetic rats treated with PGFAet (200 mg/kg bw);

Group VI: STZ-diabetic rats treated with PGFAet (400 mg/kg bw);

Group VII: STZ-diabetic rats treated tolbutamide (250 mg/kg bw) used as reference standard drug.

After 30 days of treatment the animals were euthanized. Blood was collected and liver samples were dissected out and washed immediately with ice cold saline to remove as much as blood as possible and immediately stored at –20 °C until analysis.

2.5. Biochemical assay

Estimation of blood glucose was carried out by glucose oxidase method^[9] and glycosylated hemoglobin^[10]. Plasma insulin levels were measured using RIA kit (Diasorin, Italy). Glycogen was estimated by the method of Seifter *et al*^[11]. The extraction of serum lipids was carried out by the method of Folch *et al*^[12] and cholesterol and the lipoprotein (HDL, LDL and VLDL) were fractionated by a dual precipitation^[13] and cholesterol was estimated by the method of Parekh and Jung^[14] and TG was estimated by the method of Rice^[15]. Glucokinase and glucose-6-phosphatase were assayed by the method of Brandstrup *et al*^[16] and Baginsky *et al*^[17]. The activities of enzymatic antioxidants such as glutathione peroxidase^[18], catalase^[19] and superoxide dismutase^[20], reduced glutathione^[21] and TBARS^[22] were also determined.

2.6. Statistical analysis

Data were evaluated with SPSS/10 software hypothesis testing methods that include one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. P values of less than 0.05 were considered to show statistical significance. All the results were expressed as mean±SEM for six animals in each group.

3. Results

3.1. Body weight

The body weight of the control and experimental rats are given in Table 1. The total body weight decreased during diabetes when compared with the control rats. These changes were alleviated to some extent by the administration with the PGFAet and PGFEet.

Table 1

Effect of the flower extracts of *P. granatum* on the body weight of control and experimental animals.

| Groups | Body weight | |
|-----------------------------------------------|-------------|-------------|
| | Initial | Final |
| I Control | 195.2±16.6 | 219.5±18.6* |
| II Diabetic control | 198.5±18.7 | 161.4±15.3* |
| III Diabetic control+ PgFAet (200 mg/kg) | 190.3±17.5 | 193.2±16.4* |
| IV Diabetic control+ PgFAet (400 mg/kg) | 191.2±16.7 | 193.7±5.4* |
| V Diabetic control + PgFEet (200 mg/kg) | 190.5±17.6 | 194.3±5.3* |
| VI Diabetic control + PgFEet (400 mg/kg) | 192.4±16.5 | 196.8±6.7* |
| VII Diabetic control + Tolbutamide(250 mg/kg) | 192.4±17.1 | 196.5±17.4* |

Values are expressed as mean±SEM of six animals *P<0.05, as compared to STZ-diabetic rats

PgFAet– *Punica granatum* flower aqueous extract; PgFEet– *Punica granatum* flower ethanolic extract

3.2. Serum glucose, insulin and glycogen levels

Table 2

Effect of flower extracts of *P. granatum* on FBG, plasma insulin, glycosylated Hb and liver glycogen in control and experimental animals.

| Groups | Fasting blood glucose (mg/dL) | Plasma insulin(μ U/mL) | Glycosylated hemoglobin (HbA1c%) |
|----------------------------------------------|-------------------------------|-----------------------------|----------------------------------|
| I Control | 89.8±7.67 | 14.3±1.15 | 3.41 + 0.22 |
| II Diabetic control | 315.7±27.7 | 7.69±0.59 | 7.29 + 0.46 |
| III Diabeticcontrol+ PgFAet(200 mg/kg) | 104.9±10.3* | 9.7±0.79* | 3.92±0.45* |
| IV Diabeticcontrol+ PgFAet(400 mg/kg) | 102.5±9.4* | 10.1±0.74* | 3.83 + 0.31* |
| V Diabetic control+ PgFEet (200 mg/kg) | 103.9±10.8* | 9.6±0.81* | 3.90±0.28* |
| VI Diabetic control+ PgFEet (400 mg/kg) | 101.2±9.4* | 10.2±0.74* | 3.78 + 0.33* |
| VII Diabetic control+ Tolbutamide(250 mg/dl) | 100.6±8.9* | 13.01±0.83* | 3.81 + 0.34* |

Values are expressed as mean±SEM of six animals

*P<0.05, as compared to STZ-diabetic rats

PgFAet– *Punica granatum* flower aqueous extract; PgFEet– *P. granatum* flower ethanolic extract.

Table 3

Effect of flower extracts of *Punica granatum* on liver glycogen, hexokinase and glucose 6– phosphatase

| Groups | Glycogen(mg glucose/g tissues) | Hexokinase(U1/g protein) | Glucose–6–phosphatase(U2/mg of protein) |
|------------------------------------------------|--------------------------------|--------------------------|-----------------------------------------|
| I Control | 3.41±0.22 | 139.95±11.44 | 3.14±0.27 |
| II Diabetic control | 7.29±0.46 | 98.07±6.73 | 5.11±0.21 |
| III Diabetic control+ PgFAet (200 mg/kg) | 3.83±0.31* | 126.12±10.77* | 3.59±0.30* |
| IV Diabetic control+ PgFAet (400 mg/kg) | 3.75±0.35* | 130.5±8.5* | 3.89±0.35* |
| V Diabetic control + PgFEet (200 mg/kg) | 3.85±0.33* | 123.25±10.88* | 3.53±0.28* |
| VI Diabetic control + PgFEet (400 mg/kg) | 3.69±0.40* | 132.8±10.7* | 4.01±0.26* |
| VII Diabetic control + Tolbutamide (250 mg/dl) | 3.81±0.34* | 125.25±10.52* | 3.57±0.31* |

Values are expressed as mean±SEM of six animals

*P<0.05, as compared to STZ-diabetic rats

1 – μ moles of glucose phosphorylated/min.

2 – μ moles Pi liberated/min.

PgFAet– *Punica granatum* flower aqueous extract; PgFEet– *P. granatum* flower ethanolic extract.

The extracts PGFAet and PGFEet significantly reduced the glucose concentration in the diabetic rats. The oral administration of aqueous extract of *P. granatum* reduced the blood sugar from 315.7±27.77 to 102.5±9.4, while the ethanolic extract this initial level to 101.2±9.4, respectively (p<0.05). It can be seen from the results that the ethanolic extract is able to reduce the blood glucose little better than the aqueous extract at 400 mg/kg bw (Table 2).

A significant decrease in plasma insulin levels in STZ induced diabetic rats as compared with control rats was observed. Administration of PGF extracts and tolbutamide tended to bring plasma insulin towards near normal levels.

A remarkable decrease in liver glycogen was shown by the administration of the extract in all the groups when compared to the diabetic rats.

Enzyme markers

Table 3 represents the activities of glucokinase and glucose –6–phosphatase in liver of control and STZ–diabetic rats. The activity of glucokinase decreased while the activity of glucose–6–phosphatase was found to be increased in the liver. Oral administration of PGFAet and PGFEet for 30 days reversed the levels comparable tot that of tolbutamide treated diabetic rats.

3.3 Serum lipid profile

Table 4Effect of flower extracts of *P. granatum* on lipid profiles in control and experimental animals.

| Groups | Cholesterol (mg/dL) | Triglyceride (mg/dL) | HDL (mg/dL) | LDL (mg/dL) | VLDL (mg/dL) |
|------------------------------------------------|---------------------|----------------------|--------------|-------------|--------------|
| I Control | 84.0±0.38 | 93.0±0.38 | 26.68±0.06 | 37.85±0.04 | 19.20±0.03 |
| II Diabetic control | 247.5±0.15 | 178.3±0.49 | 14.32±0.002 | 195.25±0.05 | 35.47±0.31 |
| III Diabetic control+ PgFAet (200 mg/kg) | 176.5±*0.24 | 120.2±*0.45 | 17.25±*0.01 | 78.9±*0.08 | 29.4±0.23* |
| IV Diabetic control+ PgFAet (400 mg/kg) | 107.5±0.35* | 105.2±0.35* | 21.43±0.002* | 66.2±0.05* | 20.41±0.14* |
| V Diabetic control + PgFEet (200 mg/kg) | 175.5±0.35* | 119.5±0.25* | 17.2±0.004* | 78.5±0.04* | 28.6±0.22* |
| VI Diabetic control + PgFEet (400 mg/kg) | 100.2±0.36* | 103.5±0.42* | 22.2±0.004* | 64.4±0.04* | 20.5±0.15* |
| VII Diabetic control + Tolbutamide (250 mg/dl) | 98.2±0.12* | 102.5±0.41* | 23.2±0.005* | 54.2±0.05* | 20.02±0.12* |

Values are expressed as mean±SEM of six animals.

* $P<0.05$, as compared to STZ–diabetic rats.PgFAet– *P. granatum* flower aqueous extract; PgFEet– *P. granatum* flower ethanolic extract.**Table 5**Effect of flower extracts of *P. granatum* on lipid peroxidation and antioxidant enzymes in control and experimental animals

| Groups | TBARS (mmol/100g tissues) | GSH (mg/100mg tissues) | GPx (U/ mg protein) | SOD (U2/mg protein) | CAT (U3/ mg protein) |
|------------------------------------------------|------------------------------|---------------------------|------------------------|------------------------|-------------------------|
| I Control | 0.75±0.05 | 45.2±3.7 | 5.09±0.40 | 3.15±0.29 | 75.82±5.24 |
| II Diabetic control | 1.89±0.08 | 18.6±4.05 | 3.02±0.29 | 1.98±0.18 | 39.5±3.81 |
| III Diabetic control+ PgFAet (200 mg/kg) | 1.03±0.06* | 30.4±3.5* | 4.05±0.32* | 2.12±0.35* | 55.4±3.87* |
| IV Diabetic control+ PgFAet (400 mg/kg) | 0.83±0.05* | 39.6±3.5* | 4.49±0.43* | 2.75±0.21* | 67.72±5.20* |
| V Diabetic control + PgFEet (200 mg/kg) | 1.05±0.08* | 29.8±3.98* | 4.10±0.42* | 2.20±0.35* | 60.2±4.15* |
| VI Diabetic control + PgFEet (400 mg/kg) | 0.85±0.08* | 38.8±4.0* | 4.56±0.41* | 2.71±0.25* | 69.27±4.14* |
| VII Diabetic control + Tolbutamide (250 mg/dl) | 0.82±*0.07 | 40.6±*3.6 | 4.53±*0.39 | 2.69±*0.27 | 68.9±4.91* |

Values are expressed as mean±SEM of six animals. * $P<0.05$, as compared to STZ– diabetic rats.1 – μ moles of glutathione utilized/minute;

2 – The amount of enzyme required to inhibit 50% NBT reduction;

3 – μ moles of H_2O_2 utilized/second.PgFAet– *P. granatum* flower aqueous extract; PgFEet– *P. granatum* flower ethanolic extract.

Table 4 shows the total cholesterol and the lipoprotein fractions of all groups of animals. Serum cholesterol levels were significantly increased in the diabetic rats when compared with control rats. However, administration of both PGFEet and PGFAet significantly reduced serum cholesterol the ethanolic extract exhibited better activity. In diabetic rats, a significant increase of serum LDL–cholesterol and VLDL–cholesterol was observed in control rats. But a marked decrease in HDL–cholesterol and VLDL–cholesterol was observed in control rats. But a marked decrease in HDL–cholesterol was observed. The treatment with the extract of *P. granatum* a decrease in LDL cholesterol ($P<0.05$) and VLDL–cholesterol and an increase in HDL–cholesterol were observed. Triglyceride levels in serum were increased significantly in diabetic rats compared to normal rats. The administration of *P. granatum* ethanolic flower extract markedly reduced triglyceride levels.

3.4 TBARS and antioxidant levels

The lipid peroxide levels as thiobarbituric acid reactive substance (TBARS) and the antioxidant levels are presented in Table 5. A marked increase in the TBARS levels and a concomitant decrease in the antioxidant levels were noted. However, administration of PGFEet and PGFAet reduced the

TBARS and increased the levels of the enzymic antioxidant and reduced glutathione. We observed that an administration of 400 mg/kg of PGFEet was better when compared to PGFAet. The activities of SOD, CAT and GPx in liver were significantly ($P<0.05$) lower in diabetic rats compared to control rats. Treatment with PGF and tolbutamide showed a significant increase in SOD, CAT and GPx in the diabetic rats. The level of GSH was also significantly elevated in the diabetic rats.

4. Discussion

Streptozotocin (STZ)–induced hyperglycemia in animals is considered to be a good model for the preliminary screening of agents active against type II diabetes[23]. Streptozotocin is a potent DNA methylating agent and acts as a nitric oxide donor in pancreatic cells. β cells are particularly sensitive to damage by nitric oxide and free radicals because of their low levels of free radical scavenging enzymes. Administration of STZ results in increased blood glucose levels and decreased plasma insulin levels.

Plasma insulin level of diabetic animals treated with the extracts of PGFAet and PGFEet increased when compared to the diabetic control animals. Administration of the

extracts increased the plasma insulin levels in STZ-induced diabetic rats suggesting its possible action by increasing insulin release. This result is in agreement with other studies reported on *Gymnema sylvestre*[23] and *Momordica charantia*[24].

Glycosylated hemoglobin is an excellent marker of glycemic control as it is formed progressively over a period of time and it is stable. In our study the diabetic rats had higher levels of glycosylated hemoglobin, the significant decrease in glycosylated hemoglobin in diabetic rats after the supplementation of *P. granatum* flower extract indicates that the overall blood glucose level is controlled which must be due to improvement in insulin secretion.

Glycogen content of liver markedly decreased in diabetes. The decrease in glycogen may be due to lack of insulin in the diabetic state. An increase in the glycogen levels were observed after the administration of the extract which again suggests the possibility of insulin release[25].

Administration of PGFAet and PGFEet to diabetic animals increased the activity of glucokinase in liver. The extract induced decrease in concentration of blood glucose in STZ diabetic rats may be the result of increased glycolysis. This is in agreement with previous studies on *Gymnema sylvestre*[26], while the activity of glucogenic enzyme glucose-6-phosphatase is increased during diabetes. The administration of the flower extract not only reduced the blood glucose levels but also increased the liver glycogen levels. This may be due to the mobilization of blood glucose towards glycogen reserves. The activity of glucose-6-phosphatase was almost inhibited after administration of the extracts suggesting that glucose-6-phosphate is not utilized for the synthesis of glucose in the glycogenic pathway, but may be used as a substrate for glycogenesis[27].

Hyperlipidemia is recognized consequence of diabetes mellitus. Chronic administration of different tests extracts normalized serum lipid profile. Diabetes induced hyperlipidemia is attributable to excess mobilization of fat from adipose due to the under utilization of glucose caused by acute insulin deficiency. This results in an increased production of LDL-cholesterol particles[28].

The plasma levels of TC, LDL-cholesterol, VLDL-cholesterol and TG increased, while the HDL level declines. HDL functions in the transport of cholesterol away from the peripheral tissues to the liver, thus preventing the genesis of atherosclerosis. *P. granatum* administration resulted in lowering the plasma levels of TC, LDL-cholesterol, VLDL-cholesterol and TG with elevation of HDL-cholesterol. It is reported that administration of insulin to diabetic subjects not only elevates lipoprotein lipase activity, but also lowers the plasma TG concentration[29]. In the present study we observed a decrease in lipid profile following the administration of PGF extracts suggesting that the extract's potential is possibly due to the elevation of insulin level. The observed significant increase in the level of HDL, further points to the cardio-protective activity of the extracts.

Hyperglycemia generates reactive oxygen species which in turn causes lipid peroxidation. Free radicals induce lipid peroxidation, results in reactive molecules such as TBARS. Treatment with *P. granatum* flower extracts for 30 days was observed to significantly reduce plasma TBARS level indicating a protective role of the extract. This may be attributed to the presence of phytochemicals such as phenol and flavonoids. This is further supported by evidence indicating the use of natural extracts from plant source in reducing the risk of oxidative stress due to their rich source of phytochemicals[30].

Reduced activities of SOD, GPx and CAT in liver have been observed during diabetes. A reduction in the activities of these enzymes results in the accumulation of superoxide anion and hydrogen peroxides which would have otherwise been effectively scavenged by these enzymes. GSH is an important biomolecule effective against the reactive oxygen species which is also reduced in diabetes. The results of the present study are in line with that of the previous studies. Administration of the PGFAet and PGFEet significantly improved the levels of the antioxidant enzymes and GSH and a concomitant decrease in TBARS was also seen.

Thus, it can be concluded that both the flower extracts of PG possess significant hypoglycemic and hypolipidemic activity, with the ethanolic extract exhibiting better activity than the aqueous extract. The antihyperglycemic effect of these extracts may be partly due to their stimulatory action on insulin release. Both effects may in part be due to its antioxidant activity. Further studies on molecular level are required to determine the exact mechanism.

Conflict of interest

We declare that we no conflict of interest.

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