Antihyperlipidemic Activity of Flowers of *Punica granatum* in Poloxamer-407 Induced Hyperlipidemic Mice Model

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**ABSTRACT**

*Introduction:* The methanolic partitionate of pet ether extract of flowers of *Punica granatum* (Family: Punicaceae) was evaluated for antihyperlipidemic activity in poloxamer 407 induced hyperlipidemic mice. *Methods:* Hyperlipidemia was induced in mice by i.p. injection of poloxamer 407 (30% w/w in distilled cool water; 600 mg/kg) and 2 hours after the administration of P-407, the mice of reference group were administered with atorvastatin (50 mg/kg p.o.), while test group received the methanolic partitionate of pet ether extract of flowers of *Punica granatum* (500 mg/kg p.o). After 15 and 24 hour of treatment, serum lipid profiles were investigated using commercially available kits. *Results:* The administration of the flower extracts significantly (*p* < 0.05) reduced the serum levels of triglycerides (TG) and very low density lipoprotein (VLDL) as well as the atherogenic index (A.I.) and significantly increased the serum high density lipoprotein (HDL) level compared to the P-407 induced hyperlipidemic control mice after 15 h of treatment at a single dose of 500 mg/kg p.o. After 24 h of treatment, the extract induced a significant reduction (*p* < 0.05) in serum total cholesterol (TC), VLDL, low density lipoprotein (LDL) as well as the atherogenic index and significant increase in HDL levels, when compared to P-407 control group. All these effects were comparable to those of the reference standard, atorvastatin. *Conclusions:* The results of the investigation demonstrated that the flower extract of *Punica granatum* has potential antihyperlipidemic activity and might be used for the prevention of hyperlipidemia associated disorders.

**Key words:** atherogenic index; hyperlipidemia; lipid profile; methanolic partitionate; pet ether extract; serum

**INTRODUCTION**

*Punica granatum* Linn. (Punicaceae), commonly called pomegranate, is an attractive large shrub or small tree native from Iran to the Himalayas in northern India and has been cultivated since ancient times throughout the Mediterranean region of Asia, Africa and Europe.¹ The most important growing regions are Egypt, China, Afghanistan, Pakistan, Bangladesh, Iran, Iraq, India, Burma and Saudi Arabia.

Pomegranate is an important medicinal plant containing versatile bioactive compounds and traditionally was used in the treatment of different diseases. The ripe fruit is tonic, astringent, laxative and diuretic, used in brain diseases, chest troubles, bronchitis and earache. Bark and fruit rind are administered orally to prevent dysentery, diarrhea, piles, bronchitis, biliousness and as an anthelmintic.² The dried flowers, known as Gulnar, are efficaceous to treat hematuria, hemoptysis, diarrhea, dysentery, bronchitis, nasal hemorrhage.³ Pomegranate flowers have been prescribed in Unani and Ayurvedic medicines for the treatment of diabetes.⁴ It has been demonstrated that flower extract shows hypoglycemic activity in normal and alloxan-induced diabetic animals.⁵ Flower juice is recommended as a gargle for sore throat, oral and throat inflammation, in leucorrhea, hemorrhage and ulcers of the uterus and rectus.⁶

Some studies have reported that both the pomegranate flowers and fruit extracts exhibited high activity on lowering
circulation lipid and modifying heart disease risk factors in diabetic animals and humans with hyperlipidemia.\textsuperscript{[6-8]} Lei et al.\textsuperscript{[9]} reported that the pomegranate leaf extract containing abundant tannins, had a strong lipid-lowering action in hyperlipidemic animals after a long-term of oral administration. Huang et al.\textsuperscript{[9]} investigated the effects of flower extract of \textit{Punica granatum} extract on abnormal cardiac lipid metabolism both in vivo and in vitro and reported that it reduced cardiac TG content, accompanied by a decrease in plasma levels of TG and total cholesterol in Zucker diabetic fatty (ZDF) rats. Priyanka et al.\textsuperscript{[9]} demonstrated that oral administration of aqueous extract of flower of \textit{Punica granatum} significantly reduced fasting blood glucose and serum lipid profile in streptozotocin induced diabetic rats.

As the dried flowers of \textit{Punica granatum} have been prescribed in Unani and Ayurvedic medicines for the treatment of diabetes and as all the previous investigations reported the lipid lowering activity of flowers of \textit{Punica granatum} in diabetic rat model, our present study was designed to investigate the antihyperlipidemic activity of methanolic fraction of petroleum ether extract of flowers of \textit{Punica granatum} in Poloxamer-407 induced hyperlipidemic mice model.

**MATERIALS and METHODS**

**Chemicals and reagents**

Poloxamer 407, also called Pluronic RF-127, was donated by BASF Bangladesh Ltd. Atorvastatin tablet at a strength of 40 mg was collected from Beximco Pharmaceuticals Ltd. Bangladesh. Total cholesterol (TC), total triglyceride, (TG), high density lipoprotein cholesterol (HDL) measuring kits were purchased from Linear Chemicals S.L. (Barcelona, Spain). Dimethylsulfoxide and Tween-80 were purchased from Sigma-Aldrich. All other reagents and chemicals were of BDH and E-Merck analytical grade.

**Preparation of plant extract**

The flower of the plant \textit{Punica granatum} was collected from Naogaon in February, 2010 and authentication of the sample was confirmed by the taxonomist of the National Herbarium of Bangladesh, Mirpur, Dhaka. A voucher specimen was deposited (accession number: DACB 35050) in the Herbarium for further reference. The collected flowers were washed, cut into small pieces and dried in the sun for about a week. After drying, the plant materials were kept in an oven at 40 °C to ensure complete drying. The dried samples were then ground in coarse powder using high capacity grinding machine. The coarse powder was then stored in air-tight container with marking for identification and kept in cool, dark and dry place for future use. About 500 g of powdered flower material was taken in clean, round bottomed flask (5 liters) and macerated with 2 liters of petroleum ether at room temperature for 7 days with occasional shaking. The whole mixture was then filtered through cotton plug followed by Whatman No.1 filter paper and the filtrate thus obtained was concentrated at 40 °C under reduced pressure with a Heidolph rotary evaporator. The concentrated extract was then air dried to solid residue. The weight of the crude pet-ether extract was 70.5 g. Solvent–solvent partitioning was done using the protocol designed by Kupchan\textsuperscript{[10]} and modified version of Wagenen et al.\textsuperscript{[11]} The crude extract (5 gm) was dissolved in 10% aqueous methanol which was subsequently extracted with petroleum ether, dichloromethane and methanol. All the three partitioning (pet ether fraction, dichloromethane fraction and methanolic fraction) fractions were evaporated to dryness by using rotary evaporator and kept in air tight containers for further analysis. The methanolic partitionate and standard drug atorvastatin were suspended in normal saline using 0.1% tween-80.

**Experimental animals**

Swiss albino mice of either sex, weighing 35-40 g were purchased from the animal resource branch of International Center for Diarrheal Diseases and Research, Bangladesh (ICDDR,B). The animals were kept in standard environmental conditions (temperature: 23 ± 2°C, relative humidity: 55 ± 10 % and 12 hours light/dark cycle). The animals were fed with standard pellets diet (ICDDR,B formulated) and water \textit{ad libitum} and acclimatized to laboratory conditions for 7 days before the experimentation. The design and performance of research study involving mice have been approved by the Ethical Review Committee, Faculty of Biological Science, University of Dhaka through the submission of a research protocol before the study.

**Experimental procedures**

\textit{Poloxamer-407-induced acute hyperlipidemia in mice}

The antihyperlipidemic activity was evaluated according to the method described by Hitesh et al.\textsuperscript{[12]} Twenty swiss albino mice of either sex were divided into four groups of five mice each (Table I). To render the mice hyperlipidemic, the animals were kept in fasting condition for 6 hours before the experimentation. Based on the reported method\textsuperscript{[13]} the mice of Group II, III and IV were made hyperlipidemic by an intraperitoneal injection of 600 mg/kg of P-407 that had been prepared at a final concentration of 30% (w/w) by dissolving the powder in distilled cool water and the solution was then kept refrigerated overnight to facilitate its dissolution.\textsuperscript{[14]} Two hours after the administration of P-407, the mice of Group III (reference group) were administered with atorvastatin at a dose of 50 mg/kg p.o., while group IV (test group) received the methanolic partitionate of pet ether extract of flowers of \textit{Punica granatum} and standard drug atorvastatin were suspended in normal saline using 0.1% tween-80.
at a dose of 500 mg/kg p.o. On the other hand, the normal control group (Group I) received the vehicles (1% Tween-80 in normal saline) at a dose of 10 ml/kg p.o.

**Blood sampling**

After 15 and 24 h of treatment, blood samples were collected from the retro-orbital plexus of each mouse after they have anesthetized with diethyl ether and the samples were incubated at room temperature for 30 minutes. The blood samples were then centrifuged at 4000 rpm at 4 ºC for 10 min; the serum was separated and stored at –40 ºC until to be used for biochemical tests.

**Biochemical estimations of lipid profile**

The levels of triglycerides (TG), total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) in the serum were estimated by enzymatic colorimetric methods using commercial Kits (Linear Chemicals Ltd, Spain) according to manufacturer’s instructions.[15-18] Very low–density lipoprotein cholesterol (VLDL-C) was calculated as TG/5. LDL- cholesterol (LDL-C) levels were calculated using Friedewald’s formula:[19]:

\[ \text{LDL-C} = \text{TC} - \text{HDL-C} - \frac{\text{VLDL-C}}{5} \]

The atherogenic index (A.I) was calculated using the formula:

\[ \text{Atherogenic index (A.I)} = \frac{(\text{VLDL-C} + \text{LDL-C})}{\text{HDL-C}} \]

**Statistical analysis**

The results were expressed as the mean ± SEM (Standard error mean). The data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett’s t test to determine the level of significance. A value of \( p < 0.05 \) was considered to be significant. The statistical analysis was carried out using the SPSS program (version 17.0).

**RESULTS**

The serum TC, TG, LDL-C and VLDL-C levels were significantly (\( p < 0.001 \)) increased in the hyperlipidemic P-407 control group at 15 h (Table I) and 24 h (Table II), when compared with the normal control group. But the methanolic partitionate of pet ether extract of flowers of P. granatum (MPPG) was found to be effective in significantly reducing serum TG and VLDL (\( p < 0.001 \)) levels when compared to the P-407 induced hyperlipidemic control

### Table I: Effect of methanolic partitionate of flowers of Punica granatum (MPPG) on serum lipid profile 15 h after poloxamer 407 -induced acute hyperlipidemia in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>TC (mmol/L)</th>
<th>TG (mmol/L)</th>
<th>HDLb (mmol/L)</th>
<th>LDL (mmol/L)</th>
<th>VLDL (mmol/L)</th>
<th>A.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I (Normal control)</td>
<td>3.62 ± 0.42</td>
<td>3.19 ± 0.16</td>
<td>1.13 ± 0.09</td>
<td>2.05 ± 0.39</td>
<td>0.63 ± 0.03</td>
<td>2.42 ± 0.39</td>
</tr>
<tr>
<td>Group-II (P-407 control)</td>
<td>18.39 ± 0.63*</td>
<td>16.93 ± 0.75*</td>
<td>5.04 ± 0.20*</td>
<td>9.9 ± 0.67*</td>
<td>3.38 ± 0.08*</td>
<td>2.67 ± 0.27*</td>
</tr>
<tr>
<td>Group-III (Atorvastatin)</td>
<td>13.47 ± 0.51**</td>
<td>9.45 ± 0.40**</td>
<td>7.68 ± 0.29**</td>
<td>3.9 ± 0.56**</td>
<td>1.89 ± 0.08**</td>
<td>0.76 ± 0.08**</td>
</tr>
<tr>
<td>Group-IV (MPPG)</td>
<td>16.9 ± 0.60</td>
<td>10.57 ± 1.17***</td>
<td>6.23 ± 0.39***</td>
<td>8.56 ± 0.62</td>
<td>2.11 ± 0.23***</td>
<td>1.74 ± 0.16***</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± S.E.M. (\( n = 5 \)). One way ANOVA followed by Dunnett’s t test. *compared to normal control group (\( p < 0.001 \)); **compared to P-407 control group (\( p < 0.001 \)); ***compared to P-407 control (\( p < 0.05 \)). Figures in parentheses are the percentage reduction compared to P-407 control group. *TC: total cholesterol, TG: triglyceride, HDL: High-density lipoprotein, LDL: low-density lipoprotein, VLDL: very low-density lipoprotein, A.I.: atherogenic index.

### Table II: Effect of methanolic partitionate of flowers of Punica granatum (MPPG) on serum lipid profile 24 h after poloxamer 407 -induced acute hyperlipidemia in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>TC (mmol/L)</th>
<th>TG (mmol/L)</th>
<th>HDLb (mmol/L)</th>
<th>LDL (mmol/L)</th>
<th>VLDL (mmol/L)</th>
<th>A.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I (Normal control)</td>
<td>4.21 ± 0.51</td>
<td>3.43 ± 0.13</td>
<td>1.18 ± 0.07</td>
<td>2.46 ± 0.44</td>
<td>0.68 ± 0.02</td>
<td>2.64 ± 0.32</td>
</tr>
<tr>
<td>Group-II (P-407 control)</td>
<td>24.28 ± 0.89*</td>
<td>17.66 ± 0.46*</td>
<td>5.05 ± 0.17*</td>
<td>15.7 ± 0.80*</td>
<td>3.52 ± 0.09*</td>
<td>3.82 ± 0.20*</td>
</tr>
<tr>
<td>Group-III (Atorvastatin)</td>
<td>14.6 ± 0.44**</td>
<td>13.61 ± 0.24**</td>
<td>7.00 ± 0.30**</td>
<td>4.87 ± 0.69**</td>
<td>2.72 ± 0.049**</td>
<td>1.1 ± 0.12**</td>
</tr>
<tr>
<td>Group-IV (MPPG)</td>
<td>19.72 ± 0.67***</td>
<td>14.56 ± 0.46**</td>
<td>6.06 ± 0.29***</td>
<td>10.74 ± 0.95***</td>
<td>2.91 ± 0.09**</td>
<td>2.3 ± 0.26**</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± S.E.M. (\( n = 5 \)). One way ANOVA followed by Dunnett’s t test. *compared to normal control group (\( p < 0.001 \)); **compared to P-407 control group (\( p < 0.001 \)); ***compared to P-407 control (\( p < 0.05 \)). Figures in parentheses are the percentage reduction compared to P-407 control group. *TC: total cholesterol, TG: triglyceride, HDL: High-density lipoprotein, LDL: low-density lipoprotein, VLDL: very low-density lipoprotein, A.I.: atherogenic index.
mice, after 15 and 24 h of treatment at a dose of 500 mg/kg p.o. (Table I and Table II). After 15 h of treatment, MPPG reduced serum TC and LDL-C levels but the results were not found to be significant but after 24 h of treatment, both the parameters were reduced significantly ($p < 0.05$). MPPG also increased the serum HDL-C levels significantly ($p < 0.05$) after 15 and 24 h of treatment, when compared to the P-407 control group. The most useful finding was that MPPG significantly lowered the atherogenic index (A.I.) after 15 h ($p < 0.05$) and 24 h ($p < 0.001$) of treatment. All these effects were comparable to those of the reference standard, atorvastatin.

**DISCUSSION**

Hyperlipidemia characterized by abnormally elevated serum triacylglycerol (TG), total cholesterol (TC), LDL-C and VLDL-C, is an established risk factor for the development of coronary artery disease (CAD). In the present study, the effects of methanolic partitionate of pet-ether extract of flowers of *P. granatum* on serum lipid levels was evaluated in hyperlipidemic mice induced by poloxamer 407. P-407 has been utilized in the hyperlipidemic model due to its convenience, reproducibility and the lack of undesirable underlying pathological conditions. Increased serum total cholesterol (TC), triglycerides (TG) and low density lipoprotein cholesterol (LDL-C) levels are important risk factors for atherosclerosis development.

On the other hand, elevated levels of HDL-C exert an antiatherogenic effect by counteracting LDL-C oxidation and facilitating the translocation of cholesterol from peripheral tissue such as arterial walls to the liver for catabolism. The A.I., the ratio of LDL to HDL, is commonly used as an index for atherosclerosis. Treatment of mice with methanolic partitionate of pet-ether extract of *Punica granatum* significantly reduced the serum TC, TG, VLDL and LDL levels as well as the A.I and significantly increased the serum HDL-C levels when compared to hyperlipidemic control mice (Table I and II). The results imply that oral administration of methanolic extract of flowers of *P. granatum* have potential ability to reduce the risk of atherosclerosis.

The serum TG lowering activities of methanolic partitionate of pet-ether extract of *Punica granatum* flower can be attributed to the ability of the extract to increase the lipoprotein lipase activities since Johnston and Plamer and Johnston have demonstrated that the increase in triglycerides (TG) mediated by P-407 ip. injection to rats results primarily from an inhibition of TG degradation, where P-407 directly inhibits capillary lipoprotein lipase (LPL) responsible for plasma TG hydrolysis. The cholesterol lowering effects of the extracts of the plant might be due to the inhibition of hepatic HMG CoA reductase, the rate-limiting enzyme in the biosynthesis of cholesterol since atorvastatin which was used as positive control in this study is a HMG-CoA reductase inhibitor and since Johnston demonstrated that the elevation of serum cholesterol levels following i.p. injection of poloxamer 407 solution to rats was due to stimulation of 3-hydroxy-3-methylglutaryl-Co-enzyme A (HMG-CoA) reductase activity in the liver by the poloxamer vehicle.

Flowers of the *P. granatum* contains different tannins such as ellagic acid and gallic acid, 1, 2-di-O-galloyl-4, 6-O-(S)-hexahydroxydiphenyl β-D-glucopyranoside, pomegranate as well as different flavonoids such as apigenin, punicaclavone etc. Besides, the flower also contains different triterpenoids: ursoic acid, maslinic acid and asiatic acid. The observed antihyperlipidemic activity of the flower extract of *Punica granatum* may be due to the presence of the above mentioned flavonoids, tannins and triterpenoids as previously it has been reported that flavonoids, tannins and terpenoids possess antihyperlipidemic activity.

In conclusion, the present study demonstrated that the methanolic fraction of pet ether extract of flowers of *Punica granatum* possesses potential antihyperlipidemic activity. The results imply that the flower extracts of the plant may be used for the prevention and/or treatment of atherosclerosis which leads to different coronary artery diseases (CAD). Further investigations are required to elucidate the mechanism of action of the extracts.

**REFERENCES**