**Protective Activity of Aqueous Pericarp Extract of *Punica granatum* Against Hyperglycaemia Induced by streptozotocin in Rats**

Swathi Putta and Eswar Kumar Kilari

Pharmacology Division, A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India.

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Diabetes mellitus is a chronic metabolic disorder characterized by raise in blood glucose levels known as hyperglycemia. In the present study, the aqueous pericarp extract of *Punica granatum* (APPG) was evaluated for hypoglycemia and its antihyperglycemic activity in streptozotocin-induced diabetic rats. The % inhibition of blood glucose levels in hypoglycemic activity of APPG 50 mg/kg and 100 mg/kg were found to be 26.91% (3hr) & 34.67% (6h) and 22.51% (3hr) & 25.24%(6 hr) hypoglycaemic normal rats. The antihyperglycemic activity of APPG with 150 mg/kg and 300 mg/kg in STZ induced diabetic rats were found to be 31.38% and 35.42% at 6hr respectively. The biphasic reduction was observed in both in normal and diabetic rats might be due to biphasic absorption or biliary secretion of the active principle present in APPG. At the end of 12 weeks study period the serum parameters were found to be increased in diabetic rats and treatment with APPG there is a significant reduction in all the parameters. In conclusion, the APPG found to have significant antihyperglycemic, antihyperlipidemia activity and significant protection against the damage to kidney. Which might be due to antioxidant potentials like flavonoids, polyphenols and tannins present in the APPG.

**Key words:** Diabetes mellitus, antihyperglycemic activity; *Punica granatum*

Diabetes mellitus the most common endocrine disorder of carbohydrate metabolism is affecting approximately 8.3% of the population worldwide (IDF Diabetes Atlas, 2013). In 1675 Thomas Willis added the word ‘mellitus’ to the disease, a word from Latin meaning ‘Honey’ a reference to the sweet taste of the urine (Chopra RN et al., 1956). Diabetes mellitus is not a single disease entity, but rather a group of metabolic disorders sharing a common underlying feature of hyperglycemia. Hyperglycemia in diabetes, results from defects in insulin secretion, insulin action or most commonly both (Robbins et al., 2004). Chronic elevation of blood glucose level leads to damage of blood vessels (angiopathy). The endothelial cells lining the blood vessels take in more glucose than normal, since they don’t depend on insulin. They then form more surface glycoproteins than normal, and cause the basement membrane to grow thicker and weaker. In diabetes, the resulting problems are grouped under “microvascular disease” (due to damage to small blood vessels) like retinopathy, nephropathy & neuropathy and “macrovascular disease” (due to damage to the arteries) like cardiomyopathy.

Different groups of oral hypoglycemic agents are currently available with characteristic profiles of side effects (Prout 1974; Holman and Turner 1991; Williams and Pickup 1991; Kameswara, 1997). The search for antidiabetic
agents with little or no side effects is continuous processes. The plant kingdom is a wide field to look for effective oral hypoglycemic agents. More than 300 species have been reported to display hypoglycemic activity (Rahman and Zaman, 1989) but only few of them have been investigated despite the World Health Organization (WHO) recommendation that traditional plant remedy for diabetics warrant further evaluation (WHO, 1980).

Effective control of blood glucose level is a key step in preventing or reverses diabetic complications and improving the quality of life in both type I and type II diabetic patients (Xie JT et al., 2005). In the history of Unani, Ayurveda, Siddha or Homeopathic has been well documented that illness can be managed purely by herbal preparations, thus the diabetic individual could lead a healthy life as non-diabetics. Experiments and clinical trials conducted worldwide have provided dependable evidences on the effects of various herbal formulations in the maintenance of normal blood sugar level. These invaluable findings are now conclusively processed in the backdrop of Ayurveda, Unani and Siddha system (Ameen Syed M. M et al., 2005).

Punica granatum L. (Punicaceae), commonly known as pomegranate. Pomagranate pericarp is a rich source of tannins, flavanoids, polyphenols and some anthocyanins. The potential therapeutic properties of Punica granatum are treatment and prevention for diabetes (Middha et al., 2012), cancer (Dikmen M et al., 2011; Hong MY., 2008), cardiovascular disease (Jurenka J et al., 2008), dental conditions (Viuda-Martos M et al., 2010), and erectile dysfunction (Kanatta S R et al., 2010), protection from ultraviolet (UV) radiation (Kanatta S R et al., 2010) and antimicrobial (Eswar Kumar et al., 2013). Other potential applications include infant brain ischemia, Alzheimer’s disease (Middha et al., 2012), male infertility, arthritis (Kanatta S R et al., 2010), dermal wounds (Hayouni EA et al., 2011) and obesity (Kanatta S R et al., 2010).

**MATERIALS AND METHODS**

**Chemicals**

Streptozotocin was purchased from SIGMA Aldrich, St. LOUIS, MO, USA. All other chemicals used for this study were analytical grade.

**Plant Materials**

The ripened pomegranate (*Punica granatum*) were obtained from local market. The pericarps were manually separated and shade dried. The pericarps were powdered in a grinder to get 40-mesh size powder. The moisture content of pericarp powder was found to be 13.5%. The powder was suspended in 2% gum acacia and used in the experimental studies.

**Animals**

Animals were obtained from the Tina laboratories, Hyderabad. Albino Wistar rats (180-200 g) of male were used in the present study. The animals were housed under standard environmental conditions (23±1ºC) with relative humidity of 50±10% and maintain 12:12 dark and light cycle, maintained with free access to water and *ad libitum* standard laboratory diet (70% carbohydrates, 25% proteins, 5% lipids (Hindustan liver Bangalore). After randomization before the experiment, the rats were acclimatized for a period of two weeks. The animal housing and handling were in accordance with CPSCEA guidelines. Our college was approved by CPCSEA for conducting animal experiments with the registration No. 516/01/A/CPCSEA. The prior permission for the study was obtained from our Institutional Animal Ethics Committee (IAEC).

**Induction of Diabetes**

The rats were fasted for 18 h prior to the experiment with water *ad libitum*. The rats were injected intraperitoneally with nicotinamide 100mg/kg. After 15 minutes streptozotocin (STZ) were administered. STZ dissolved in citrate buffer at a dose of 55 mg/kg body weight. Animals were treated with 10% glucose to combat the early phase of hypoglycemia. Blood samples were collected after 72 hours of STZ treatment and the induction of diabetes mellitus was confirmed by estimation of fasting blood glucose levels (FBG). Only those rats with blood glucose levels <250 mg/dl were included in the study (Day 0).

**EXPERIMENTAL**

**Procedures**

Control group was administered with distilled water, aqueous pericarp extract of *Punica granatum* (APPG) at 50mg/kg and 100 mg/kg of rat body weight to group-I, and group-II respectively.
Blood samples were withdrawn at 0, 1, 2, 3, 4, 6, 8, 10 & 12h intervals by retro-orbital puncture method and were analyzed for blood glucose by GOD/POD method using SCREEN MASTER 3000 autoanalyser.

After the induction of diabetes, the rats were grouped in to eleven different groups of each containing six animals. Group 1 contains control rats received distilled water and fed on normal diet, group 2 as diabetic control received vehicle only, group 3 contains diabetic rats treated with gliclazide at a dose of 1 mg/kg body weight, group 4 contains diabetic rats received APPG at a dose of 50 mg/kg body weight, group 5 contains diabetic rats received APPG at a dose of 100 mg/kg body weight for 12 weeks. Treatment with drugs was started after 72 hours of STZ treatment (i.e. Day 1) and was continued for 12 weeks. All drugs were given orally as a single oral dose. Blood glucose was measured before starting the treatment (day0) and 4 weekly thereafter up to the end of the treatment and estimated fasting blood glucose by glucose-oxidase-peroxidase (GOD-POD) method. All the treatment groups were compared with diabetic control group.

Biochemical assays

At the end of the 12 week study period, rats were fasted overnight and blood samples were withdrawn through the retroorbital plexus using glass capillary. Blood was allowed to clot and serum was separated by centrifugation at 4000rpm for 10 min. Serum glucose levels were estimated at 0, 1, 2, 3, 4, 6 and 8 hours intervals. Serum glycosylated haemoglobin, triglycerides, total cholesterol, HDL, LDL, VLDL, bilirubin, creatinine, albumin, total protein, urea, uric acid and BUN levels were estimated. Serum glucose levels were estimated by GOD/POD method. Triglyceride, total cholesterol, HDL was measured by commercially available kits (Bucolo G et al., 1973) (Nader R et al., 2001). Bilirubin (Jendrassik, 1938), creatinine (Bowers L D, 1980), total protein (Tietz, N.W 1996), albumin (Doumas BT et al., 1972), uric acid (Tomas L et al., 1998)), urea (Fossati P et al., 1980). At the end of the study all the rats were dissected and pancreas was used for histopathological studies.

Statistical analysis

All the data were expressed as mean SEM. Statistical analysis was carried out using one way ANOVA followed by Dunnet's multiple comparison test.

RESULTS AND DISCUSSION

The WHO Expert Committee recommended the importance to investigate and explore hypoglycemic agents from plant origin because plants used in the traditional medicine have fewer side effects than synthetic drugs (Alarcon-Aguilara F J et al., 1998). So in the present study discussed about the hypoglycemic and antihyperglycemic effects of APPG. The doses of selected fruit pericarp extracts were fixed basing on their acute toxicity study in mice and preliminary hypoglycemic studies in Wistar albino rats. The doses that produce optimal and dose dependent reduction in blood glucose levels were selected for hypoglycemic and antihyperglycemic studies. Some medicinal plants with hypoglycemic properties are known to increase circulating insulin level (pancreatic mechanism) in normoglycemic rats (Lamela et al., 1985). Another possible mechanism of action is that the extracts might stimulate residual pancreatic mechanism (extra pancreatic), probably increasing peripheral utilization of glucose as postulated by Erah et al., 1996. The APPG were shown significant hypoglycaemic activity with biphasic effect. The biphasic effect might be due to biphasic absorption or enterohepatic recirculation. We hypothesized that APPG could have a sulfonylurea-like mechanism since they significantly decreased the blood glucose levels in normoglycaemic rats. Sulfonylurea compounds lower blood glucose in normal and in diabetic animals by stimulating insulin release from pancreatic β cells and by peripheral utilization of glucose (Swathi p et al., 2014). The APPG shown to have better hypoglycemic activity compared to standard Gliclazide in normal rats. Streptozotocin (STZ) is used to induce diabetes mellitus in albino Wistar rats, a poly ADP ribose inhibitor, nicotinamide was administered before 15 minutes of STZ administration to offer partial protection against the action of STZ in rats. So in the present study we used the Streptozotocin – nicotinamide model to prevent the excessive damage to the pancreas of diabetic rats. In this study, treatment with the APPG significantly reduced the elevated plasma glucose levels in STZ induced diabetic rats. The APPG shown delayed absorption in diabetic animals compared to normoglycemic animals, this might be due to delayed gastric absorption and
motility in diabetic condition as diabetes affecting the digestive processes, the motility and nervous control of the entire system of gastrointestinal tract. The effect of diabetes on digestive system can also cause malabsorption (Bener et al., 2012; Bernstein, 2000). The APPG shown to have better antihyperglycemic activity compared to standard gliclazide in STZ induced diabetic rats.

Streptozotocin (STZ) induced diabetic rats enhanced the level of glycated hemoglobin (HbA1c) due to raise in levels of glucose in blood which further react with hemoglobin and produce the glycated hemoglobin formation (Pari L et al., 2004). The APPG significantly lowered the blood glucose, which lead to the decrease in the levels of glycated hemoglobin.

The levels of serum lipids are usually elevated in diabetes mellitus (Pushparaj P et al., 2000). This abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots. It is reported that hypercholesterolemia (increased levels of total cholesterol) and hypertriglyceridemia (increased levels of triglycerides) occurs in STZ-induced diabetic rats (Pushparaj P et al., 2000; Pushparaj N P et al., 2001; Swathi P et al., 2014). Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides (Taskinen M R 1987). However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridemia. The APPG significantly reduced the levels of total cholesterol, triglycerides, LDL and VLDL and increased the levels of HDL. The APPG shown to have better antihyperlipidemic activity compared to standard gliclazide in STZ induced diabetic rats (table 3).

The serum bilirubin levels were found to be increased in STZ induced diabetic rats. Ranaet al., 1996 reported that the increase in serum bilirubin (hyper-bilirubinemia) in STZ induced diabetic rats, may be resulted from the decrease of liver uptake, conjugation or increase total bilirubin, direct bilirubin production from hemolysis. The elevation in serum bilirubin indicates liver damage (Swathi P et al., 2012). The APPG shown to have better hepatoprotective activity than standard gliclazide. The estimation of total protein is useful for measuring gross changes in protein levels caused by various disease states. In diabetic conditions

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Control</th>
<th>Standard</th>
<th>APPG (50mg/kg)</th>
<th>APPG (100mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
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<tr>
<td>1</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>-0.54±2.02</td>
<td>-0.54±2.02</td>
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<tr>
<td>2</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
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<td>0.00±0.00</td>
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<td>0.00±0.00</td>
<td>0.00±0.00</td>
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<tr>
<td>12</td>
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<td>0.00±0.00</td>
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</tr>
</tbody>
</table>
Table 2. Effect of APPG on antihyperglycemic activity in STZ induced diabetic rats

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.00±0.00 ns</td>
<td>0.96±0.35 ns</td>
<td>0.09±0.83 ns</td>
<td>0.95±0.28 ns</td>
<td>0.39±0.28 ns</td>
<td>0.52±0.30* ns</td>
</tr>
<tr>
<td>D.Control</td>
<td>0.00±0.00 ns</td>
<td>0.28±0.07 ns</td>
<td>0.36±0.12 ns</td>
<td>0.69±0.06 ns</td>
<td>0.80±0.07 ns</td>
<td>1.27±0.05 ns</td>
</tr>
<tr>
<td>Standard</td>
<td>0.00±0.00 ns</td>
<td>2.39±1.42 ns</td>
<td>13.22±2.01 ns</td>
<td>32.00±1.03 ns</td>
<td>25.02±0.73 ns</td>
<td>20.59±0.94 ns</td>
</tr>
<tr>
<td>APPG (50mg/kg)</td>
<td>0.00±0.00 ns</td>
<td>0.04±2.09 ns</td>
<td>9.9±1.45 ns</td>
<td>16.8±1.75 ns</td>
<td>34.52±1.08 ns</td>
<td>22.93±1.35 ns</td>
</tr>
<tr>
<td>APPG (100mg/kg)</td>
<td>0.00±0.00 ns</td>
<td>0.75±1.69 ns</td>
<td>20.24±2.35 ns</td>
<td>41.58±1.20 ns</td>
<td>25.97±0.84 ns</td>
<td>12.58±1.58 ns</td>
</tr>
</tbody>
</table>

p>0.05 ns, p<0.05*, p<0.01*, p<0.001$ Significance followed by one way ANOVA followed by Dunnet's multiple comparison test when compared with disease control group.

Table 3. Effect of APPG on lipid profile of STZ induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Triglycerides (mg/dl)</th>
<th>Total cholesterol (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>80.3±1.8*</td>
<td>127.8±2.0*</td>
<td>62.0±1.9*</td>
<td>49.5±2.9*</td>
<td>16.0±0.3*</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>132.5±3.0</td>
<td>241.8±4.6</td>
<td>36.3±1.3</td>
<td>179.0±4.9</td>
<td>26.5±0.6</td>
</tr>
<tr>
<td>Standard</td>
<td>82.5±2.6*</td>
<td>130.6±3.1*</td>
<td>58.0±2.0*</td>
<td>56.16±4.9*</td>
<td>16.5±0.5*</td>
</tr>
<tr>
<td>APPG (50mg/kg)</td>
<td>92.1±2.3*</td>
<td>135.8±1.9*</td>
<td>60.5±2.1*</td>
<td>56.9±2.3*</td>
<td>18.4±0.4*</td>
</tr>
<tr>
<td>APPG (100mg/kg)</td>
<td>83.8±2.7*</td>
<td>133.0±1.9*</td>
<td>57.1±1.9*</td>
<td>59.2±2.8*</td>
<td>16.5±0.5*</td>
</tr>
</tbody>
</table>

P>0.05 ns, P>0.001*, P>0.01*, P>0.05 $ two way ANOVA followed by bonferroni post test when compared with toxicant group.

The circulating protein binds with free reducing sugars leads to formation amadori products. The APPG able to increase the protein levels may be by breaking the link between the reducing sugars and amino acids of proteins. Urea is the major nitrogen containing metabolic product of protein metabolism, uric acid is the major product of purine nucleotides, adenosine and guanosine; creatinine is endogenously produced and released into body fluids and its clearance measured as an indicator of glomerular filtration rate (Burtis CA et al., 1996). The metabolism of protein is found to be increased in the diabetic rats as indicated by increase in the levels of serum urea, uric acid and decreased levels of proteins as explained above. The APPG shown to decrease the levels of urea and uric acid probably by decreasing the metabolism of proteins.

The treatment with the APPG found to be useful in reducing the damage caused due to hyperglycaemia induced by STZ. The APPG was shown to have better and comparable antihyperglycemic activity with standard gliclazide. Serum creatinine and serum BUN levels measurement is taken as an index of altered GFR in diabetic nephropathy (Sugimoto H et al., 1999). Our results showed that the level of serum

Fig. 1. Effect of control on histopathological studies on rat pancreas

Fig. 2. Effect of disease control on histopathological studies on rat pancreas
creatinine and BUN levels was significantly elevated in diabetic animals. The treatment with APPG for 12 weeks shown significant reduction in the creatinine and BUN.

The light microscopic examination of pancreatic section of control group revealed that the normal structure of the exocrine and endocrine parts of the pancreas. Previous studies reported similar findings and added that the pancreas had a rich capillary network essential for the secretary process (Junqueira LC et al., 2005). The light microscopic examination of endocrine part of pancreas of disease control group revealed the altered structure of both the exocrine and endocrine portions with significant decrease in the number

![Fig. 3. Effect of standard on histopathological studies on rat pancreas](image1)

![Fig. 4. Effect of APPG (50mg/kg) on histopathological studies on rat pancreas](image2)

![Fig. 5. Effect of APPG (100mg/kg) on histopathological studies on rat pancreas](image3)
of secretary cells. The treatment with the APPG extract and Gliclazide for 12 weeks found to prevent the degenerative changes in STZ induced diabetic rats.

CONCLUSIONS

It is concluded that, The *Punica granatum* showed better hypoglycemic and antihyperglycemic activity against STZ induced diabetic rats. All the activities might be due to high levels of flavanoids, tannins and polyphenols present in aqueous pericarp extract of *Punica granatum*.

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