EVALUATION OF ANTIHYPERLIPIDEMIC ACTIVITY OF METHANOLIC EXTRACT OF LEAVES OF PRUNUS PERSICA

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ABSTRACT

The aim of the present study was to evaluate the Antihyperlipedemic activity of methanolic extracts of leaves of Prunus persica using high fat diet-induced hyperlipidemia and TritonX-100 induced hyperlipidemia models in Wistar albino rats. Hyperlipidemia is a disorder of lipid metabolism manifested by elevation of plasma concentrations of the various lipid and lipoprotein fractions, which is the key risk factor for cardiovascular disorders (CVD). Prunus persica extract was administered at different dose of 100mg/kg, 200mg/kg, 400mg/kg to hyperlipidemic rats. Atorvastatin is used as reference standard. The statistical analysis were carried out using one way ANOVA followed by Dunnet’s multiple comparison test. The serumTotal cholesterol, Triglyceride, HDL, VLDL, LDL levels and atherogenic index were analyzed. There is also significant improvement in atherogenic index in 400mg/kg extract treated animals. Methanolic leaf extract of prunus persica exhibited significant (p<0.01) effect in reducing the serum cholesterol, lipid levels and atherogenic index. The present study clearly demonstrated the antihyperlipedemic activity of Prunus persica supporting the traditional claim.

KEY WORDS: Hyperlipidemia, Prunus persica, high fat diet, triton x 100, atorvastatin.

INTRODUCTION

Hyperlipidemia is a heterogeneous group of disorders characterized by elevation of plasma concentrations of the various lipids and lipoprotein fractions, which is the key risk factor for cardiovascular disorders (CVD)¹. These lipids include cholesterol, cholesterol esters,
phospholipids, and triglycerides. Lipids are transported in the blood as large 'lipoproteins' and has been reported as the most common cause of death in developed as well as developing nations.\textsuperscript{[2,3]} Hyperlipidemia associated lipid disorders are considered to cause the atherosclerotic cardiovascular disease\textsuperscript{[4]} . The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular disease like atherosclerosis or cerebrovascular disease.\textsuperscript{[5]} Currently available drugs have been associated with number of side effects.\textsuperscript{[6]} Currently the use of complementary/ alternative medicines and especially the consumption of phytochemicals have been rapidly increased worldwide. As herbal medicines are less damaging than synthethic drugs and they have better compatibility thus improving patients tolerance even on long term use.\textsuperscript{[7]} \textit{Prunus persica} is an orchard tree native to China that bears a juicy edible peach. It belongs to the \textit{Rosaceae} family. It is highly useful in treating inflammatory disorders.\textsuperscript{[8]} The leaves or powdered bark are excellent for inflammatory bowel disease and gastritis. Both the leaves and bark are still employed for their curative powers. They have demulcent, sedative, diuretic and expectorant action. It is used in the treatment of constipation.\textsuperscript{[9]} Considering the traditional uses of \textit{Pruus persica} the present study was undertaken to investigate the antihyperlipedemic activity of methanolic extract of leaves of \textit{Pruus persica} against high fat diet induced hyperlipedemia in rats

**MATERIALS AND METHODS**

**Plant material**

Plant material used in this study consisted of the leaves of \textit{Prunus persica}, collected and authentified by Prof. V. Chelladurai, Ph.D., Research officer – Botany, Tirunelveli. A specimen was deposited in the Hindu College of Pharmacy, Guntur.

**Preparation of extract**

The shade dried leaf powdered material was subjected to batch extraction in Soxhlet apparatus. The solvent used was Methanol. The powdered material leaves of \textit{Prunus persica} was evenly packed in Soxhlet extractor for extraction with solvent. The temperature was maintained on an electric heating mantle with thermostat control. Appearance of colorless solvent in the siphon tube was taken as the termination of extraction. The extract was then concentrated by Helidolph rotary vacuum evaporator and percentage yield was calculated. Hence forth the Methanolic extract of \textit{Prunus persica} will be called as MEPP.
Animals
Wistar male albino rats (150-180 g) were selected for the present study. The animals had free access to standard rat pellet, with water supplied *ad libitum* under strict hygienic conditions. The experimental protocol was approved by IAEC (Institutional Animal Ethics Committee) of HCOP (Hindu college of pharmacy). The study followed all the rules of (CPCSEA) Committee for the Purpose of Control and Supervision of Experiments on Animals.

Chemicals and Reagents
Triton X-100, Atorvastatin, Cholesterol, Cholic acid was obtained from LOBA Chemie Pvt Ltd, Mumbai. All other chemicals were of analytical grade and obtained locally from National Scientific Products, Guntur.

Antihyperlipedemic Activity
High fat diet induced hyperlipidemic model Preparation of feed
Method of Blank et al. (1963) with modification was used to produce high fat diet induced hyperlipidemia. Normal animal food pellets were crushed in mortar and pestle to crush into small pieces and then grinded into fine powder in mixer grinder. The other ingredients i.e. cholesterol 1.2%, Cholic acid 1%, sucrose 40%, and coconut oil 10% were added in the mixer grinder in an ascending order of their quantity and mixed well. This dried powder was then mixed with same quantity of water every time to make small balls of feed and later this was stored in self sealing plastic covers in refrigerator at 2°C to 8°C. The feed for normal group was prepared similarly by grinding only the normal food pellets and then mixing with water without the other excipients. This preparation of feed was done once in three days for all the animals. The animals were fed with the high fat diet for 30 days. Check the serum blood cholesterol levels

Study design
Wistar rats weighing 150-180 gm, were divided into 6 groups of 6 animals each.

**Group I**- served as normal control and were given only vehicle (distilled water)

**Group II**- received high fat diet served as hyperlipedemic control

**Group III**- received 100mg/kg MEPP

**Group IV**- received 200mg/kg MEPP

**Group V**- received 400mg/kg MEPP

**Group VI**- received atorvastatin 10mg/kg served as standard drug
After treatment for fourteen days with the test drug and on 15th day the rats are kept fasting and the blood was collected by retro orbital sinus puncture, under mild ether anaesthesia. The collected samples were centrifuged for 10 minutes at 2000 r.p.m. and serum samples so collected were used for various biochemical tests.

**Triton X-100 induced Hyperlipidemia**

Thirty six Wistar rats were randomly divided into 6 groups of 6 each. The first group was given standard pellet diet, water and orally administered with 5% CMC. The II,III,IV,V,VI group animals were injected i.p. with 10% aqueous solution of Triton 100mg /kg body weight. After 72 hours of triton injection, the second group received a daily dose of 5% CMC (p.o) for 7 days. The third, fourth and fifth group was administered a daily dose of *Prunus persica* [PP] 100,200 and 400 mg/kg suspended in 5%CMC,p.o., for 7 days, after inducing hyperlipidemia. sixth group was administered with the standard Atorvastatin 10mg/kg, p.o. for 7 days. Food was withdrawn 10h prior to the blood sampling. The control group animals received the vehicle in the same volume orally.

- **Group 1**: Administered vehicle and served as normal control.
- **Group 2**: Administered Triton X 100 (TR) and served as hyperlipidemic control.
- **Group 3**: Administered PP (100mg/kg),p.o.,
- **Group 4**: Administered PP (200mg/kg), p.o.
- **Group 5**: Administered PP (400mg/kg), p.o.
- **Group 6**: Administered Atorvastatin(10mg/kg), p.o.

On the 8th day, blood was collected by reteroorbital sinus puncture, under mild ether anaesthesia. The collected samples were centrifuged for 15 minutes at 2500rpm. Then serum samples were collected and analyzed for serum Total Cholesterol,Triglycerides, High Density Lipoprotein Cholesterol,Low Density Lipoprotein Cholesterol and Very Density Lipoprotein Cholesterol. Atherogenix index is calcucated. Triglycerides, Cholesterol, HDL-cholesterol were measured with enzymatic kits. The LDL, VLDL –Cholesterol was calaculated by friedewalds formula.

\[
\text{VLDL Cholesterol} = \frac{\text{TG}}{5}
\]

\[
\text{LDL Cholesterol} = \text{TC} - (\text{HDL-Cholesterol} + \text{VLDL-Cholesterol})
\]

Atherogenic index was calculated by using the formula of Schulpis.

\[
\text{Atherogenic index (AI)} = \frac{\text{Total cholesterol} - \text{HDL-Cholesterol}}{\text{HDL-Cholesterol}}
\]
Statistical Analysis
The data expressed as the mean± SD. Data were analyzed by one-way ANOVA followed by using Dunnetts T test. Instat® (Graph Pad software, U.S.A).

RESULTS
Effect of methanolic extract of Prunus persica on lipid profile in high fat diet induced model
In high fat diet induce model, oral administration of methanolic extract of leaves of Prunus persica (100mg/kg, 200 mg/kg and 400mg/kg, p.o.) significantly reduced the serum total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C), VLDL-cholesterol levels but significantly increased serum HDL-cholesterol level as compared with positive control group. This study shows serum lipid parameters in animals were significantly reduced (p<0.001,) by fourteen days treatment with MEPP at dose levels 100, 200 mg/kg and 400 mg/kg, when compared with control group. 400 mg/kg of MEPP group animals has shown very significant (p<0.001) compared with control group

Effect on atherogenic index
Decrease in atherogenic index was observed in all treated groups with MEPP and Atorvastatin when compared to high fat diet treated alone rats (positive control)

Effect of methanolic extract of Prunus persica on lipid profile in Triton induced hyperlipidemia
In triton induced study results shows serum lipid parameters in animals were significantly reduced (p<0.01,) by seven days treatment with MEPP at dose levels 100, 200 mg/kg and 400 mg/kg, when compared with control group 400 mg/kg of MEPP group animals has shown significant (p<0.001) compared with control group. At this time, an increased level of HDL was also observed.

Effect on Atherogenic index
Decrease in atherogenic index was observed in all treated groups with MEPP and Atorvastatin when compared to TritonX-100 treated group
Table 1: Effect of MEPP on serum lipid parameter levels in high fat diet induced Hyperlipidemic rats

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TC (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>TGL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>82.3±3.12</td>
<td>42.31±1.34</td>
<td>68.99±2.12</td>
<td>13.79±0.42</td>
<td>26.69±3.55</td>
<td>0.95</td>
</tr>
<tr>
<td>Positive control</td>
<td>147.72±2.61</td>
<td>34.57±2.82</td>
<td>144.18±3.80</td>
<td>28.83±0.75</td>
<td>84.31±2.82</td>
<td>3.27</td>
</tr>
<tr>
<td>400mg/kg MEPP</td>
<td>92.035±1.6**</td>
<td>43.59±2.18***</td>
<td>101.06±2.89</td>
<td>20.2±0.58</td>
<td>28.24±2.02**</td>
<td>1.11</td>
</tr>
<tr>
<td>200mg/kg MEPP</td>
<td>108.82±2.65</td>
<td>40.91±2.40</td>
<td>115.99±2.10**</td>
<td>23.2±0.42</td>
<td>44.71±2.33</td>
<td>1.66</td>
</tr>
<tr>
<td>100mg/kg MEPP</td>
<td>119.25±1.79</td>
<td>36.36±2.40</td>
<td>119.28±1.1*</td>
<td>23.85±0.22</td>
<td>59.04±3.5*</td>
<td>2.27</td>
</tr>
<tr>
<td>Atorvastatin 10mg/kg</td>
<td>87.12±2.73</td>
<td>46.34±2.02</td>
<td>98.2±2.35***</td>
<td>19.53±0.64</td>
<td>21.24±2.42**</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Values were mean ±sd (n=6). Values are statistically significant at *P<0.05 and more significant at **P<0.01,***P<0.001 Vs hyperlipidemic control using one way ANOVA followed by Dunnet’s test

Table 2: Effect of MEPP on serum lipid parameter levels in triton induced Hyperlipidemic rats

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TC (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>TGL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>82.63±0.80</td>
<td>42.9±0.69</td>
<td>67.51±0.77</td>
<td>14.04±0.50</td>
<td>26.09±0.95</td>
<td>0.93</td>
</tr>
<tr>
<td>Positive control</td>
<td>185.76±2.20</td>
<td>33.04±2.13</td>
<td>127.1±2.01</td>
<td>27.11±0.99</td>
<td>67.93±1.93</td>
<td>4.62</td>
</tr>
<tr>
<td>400mg/kg MEPP</td>
<td>91.46±1.72***</td>
<td>42.89±0.98**</td>
<td>82.08±0.86</td>
<td>20.5±0.92</td>
<td>25.09±0.76**</td>
<td>1.13</td>
</tr>
<tr>
<td>200mg/kg MEPP</td>
<td>108.76±0.97</td>
<td>39.73±0.72</td>
<td>96.2±0.62*</td>
<td>23.98±0.54</td>
<td>38.33±0.61</td>
<td>1.74</td>
</tr>
<tr>
<td>100mg/kg MEPP</td>
<td>123.8±1.30*</td>
<td>36.12±0.71</td>
<td>104.53±0.56</td>
<td>24.58±0.49</td>
<td>46.63±0.32*</td>
<td>2.43</td>
</tr>
<tr>
<td>Atorvastatin 10mg/kg</td>
<td>86.35±0.71***</td>
<td>45.36±0.65</td>
<td>76.55±0.54</td>
<td>18.68±0.33**</td>
<td>22.78±0.59</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Values were mean ±sd (n=6). Values are statistically significant at *P<0.05 and more significant at **P<0.01,***P<0.001 Vs hyperlipidemic control using one way ANOVA followed by Dunnet’s test

Fig1: Effect of MEPP on serum lipid parameter levels in high fat diet induced Hyperlipidemic rats
Fig 1.2 Effect of MEPP on atherogenic index in high fat diet induced Hyperlipidemic rats

Fig 2 : Effect of MEPP on serum lipid parameter levels in triton induced Hyperlipidemic rats
DISCUSSION

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases. It is not a disease but a metabolic derangement that can be secondary to many diseases and can contribute to many forms of disease, most notably cardiovascular disease. Hyperlipidemia associated lipid disorders are considered to cause the atherosclerotic cardiovascular disease. There is significant decrease in the serum total cholesterol, triglycerides, LDL, VLDL and a significant increase in the HDL levels was observed with 400mg/kg MEPP, 200mg/kg and 100mg/kg when compared with the hyperlipedemic control. Atherosclerotic index (A.I) is believed to be an important risk factor for diagnosis of atherosclerosis. The methanolic extract of our Prunus persica reduced atherogenic index which is one of the most important risk factors of atherosclerotic plaques. Naringenin ability to inhibit the secretion of very-low-density lipoprotein by the cells. Naringenin seems to protect LDLR-deficient mice from the obesity effects of a high-fat diet. Naringenin lowers the plasma and hepatic cholesterol concentrations by suppressing HMG-CoA reductase and ACAT in rats fed a high-cholesterol diet. Peach leaves rich in naringenin,Quinic Acid,Lycopene,Tannins and Glycoside. As peach leaves are rich in naringenin, it is the chemical constituent that may be responsible for reducing the serum lipid levels.

CONCLUSION

Thus it can be concluded that methanolic extract of leaves of Prunus persica at the dose of 100mg/kg,200mg/kg and 400mg/kg; p.o. showed good anti-hyperlipidemic action in both High fat diet induced hyperlipidemia and triton induced hyperlipidemia models. The
probable mechanism of action of the extract may be due to the presence of naringenin that lowers the plasma and hepatic cholesterol concentrations by suppressing HMG-CoA reductase. A further study on the exact mechanism of action and isolation of the active constituents is needed.

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