Short Communication

Antihyperlipidemic effects of alcoholic extract of *Pongamia pinnata* Linn. leaves on high fat diet fed rats

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SUMMARY

The objective of the study was to investigate the antihyperlipidemic activity of alcoholic extract of *Pongamia pinnata* (PPAE) leaves in rats fed with high fat diet (HFD). PPAE was administered orally in the divided doses of 250 and 500 mg/kg/day for 30 days in HFD fed rats. Body weights were observed and the analysis of serum lipid profile was carried out on day 30. Marked decrease in the body weight, total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) whereas significant increase in the levels of high density lipoprotein (HDL) were observed after treatment with PPAE. However, PPAE in a dose of 250 mg/kg did not show significant (*P* < 0.05) increase in HDL levels. The PPAE also lowered TC: HDL-c and LDL: HDL-c ratios significantly suggesting it’s antihyperlipidemic and cardioprotective potential. The present work reveals that PPAE at the dose of 500 mg/kg/day exhibited significant (*P* < 0.01) antihyperlipidemic effects.

Key words: *Pongamia pinnata*; Hyperlipidemia; High fat diet; Body weight; Lipid profile

INTRODUCTION

Increased plasma lipid levels, mainly total cholesterol (TC), triglyceride (TG) and low density lipoproteins (LDL) along with decrease in high density lipoprotein (HDL) are known to cause hyperlipidemia which is core in initiation and progression of atherosclerotic impasse (Harrison *et al.*, 2003). The beneficial effect of lowering elevated cholesterol level in the prevention of coronary heart disease is well established (Simons, 2002). Therefore, prime consideration in the therapy for hyperlipidemia and atherosclerosis is to enervate the elevated plasma levels of TC, TG and LDL along with increase in HDL lipids levels (Brown and Goldstein, 1990).

The screening and development of drugs for their antihyperlipidemic activity is still in progress and there is much hope of finding antihyperlipidemic drugs from indigenous medicinal plants. This can be focused on plants used in traditional medicine because of leads provided by natural products that may offer better treatment than currently used drugs. *Pongamia pinnata* (Linn.) Pierre (Leguminoseae, Papilionaceae; synonym- *Pongamia glabra* Vent.)
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popularly known as ‘Karanj’ or ‘karanja’ in Hindi, is a medium sized glabrous tree, found throughout India and further distributed eastwards, mainly in the regions of South Eastern Asia and Australia (Satyavati et al., 1987). Different parts of this plant have been recommended as a remedy for various ailments, and have been used in traditional medicines for bronchitis, whooping cough, rheumatic joints and to quench dipsia in diabetes (Kirtikar and Basu, 1995). Different extracts of roots and seeds of *P. pinnata* have been reported to possess anti-inflammatory activity (Shrinivasan et al., 2001). A hot infusion of leaves is used as a medicated bath for relieving rheumatic pains and for cleaning ulcer in ganorrhea and scrofulous enlargement. The leaves are hot, digestive, laxative, anthelmintic and cure piles, wounds and other inflammatory disorders (Kirtikar and Basu, 1995). Seed oil has been used for treating various infectious diseases such as leucoderma, leprosy and muscular and articular rheumatism. Phytochemical examination of *P. pinnata* revealed the presence of furanoflavones, furanoflavonols, chromenaflavones, flavones, furanodiketones and flavonoid glycosides (Talapatra et al., 1980; Ahmad et al., 2004).

However, despite such interesting health virtue of *P. pinnata* leaves, a persual of literature reveals that no scientific study has been carried out to screen its antihyperlipidemic effects. Therefore, an attempt was made to elucidate the possible effects of alcoholic extract of *P. pinnata* leaves (PPAE) in high fat diet (HFD) fed rats by measuring different biochemical parameters.

**MATERIALS AND METHODS**

**Plant material collection and extraction**

The fresh leaves of *Pongamia pinnata* Linn. were collected from local area of Wardha district, Maharashtra, India during August and September 2005 and were authenticated by the authority of Botany Department, Nagpur University, Nagpur, India where a voucher specimen number 9015 was deposited in the Botany Department of Nagpur University, Nagpur for the future reference. The shade dried and finely powdered (sieve no. 40) leaves of *P. pinnata* were cold macerated with 70% v/v ethanol and evaporated upto dryness under reduced pressure (40 °C). The obtained yield of the crude extract was found to be 20% w/w of the plant material on the dry weight basis. The extract was subjected for preliminary phytochemical screening to detect the presence of various classes of phytoconstituents by different chemical tests.

**Animals**

Male albino rats (Wistar strain) weighing between 140 - 200 g were maintained at 25 ± 2 °C and kept in well ventilated animal house under natural photoperiodic condition in large polypropylene cages and were freed to the access of water ad libitum. The animal experiment was approved by Animal Ethical Committee of the Institute (535/02/a/CPCSEA/Jan 2004).

**Preparation of high fat diet**

The high fat diet were prepared by mixing calculated amount of whole wheat (50.0 g), yellow corn (50.0 g), barley (25.0 g), Anik spray (37.5 g), bone meal (2.5 g), calcium chloride (2.5 g), salt (2.5 g), oil (25.0 g), butter (25.0 g) and Vitamin B12 1 tablet. Twelve grams of diet of above composition was supplied to each animal everyday.

**Experimental protocol**

Thirty male Albino rats of Wistar strain were employed in the study. The rats were divided into five groups of six rats each. Group I served as normal control and received the standard diet throughout the experimental period. Group II served as hyperlipidemic control and received HFD. Group III received HFD and PPAE at a dose 250 mg/kg/day by oral administration. Group IV received HFD and PPAE at dose 500 mg/kg/day by oral administration. Group V was treated with
HFD and Lovastatin (5 mg/kg/day; p.o.) as a reference standard for comparison of results. The treatment period for all these groups was of 30 days.

Biochemical evaluation
On day 30 of treatment protocol, the blood was withdrawn from the orbital sinus of rats under light ether anaesthesia and centrifuged at 2,000 rpm for 30 min to obtain the blood serum sample. Serum total cholesterol (TC), triglyceride (TG) was estimated by the method of CHOD-PAP, and high density cholesterol (HDL-c) by the method of GPO-PAP using Span Diagnostic Kits. The level of low density lipoprotein cholesterol and very low density lipoprotein cholesterol was calculated by Friedwald formula as given below:

$$LDL-c = TC \times (HDL-c + VLDL-c); \quad VLDL-c = TG/5$$

The ratios of TC: HDL-c and LDL: HDL-c was also calculated.

Statistical analysis
Statistical analysis was performed by GraphPad InStat Software. Data are given as means ± S.D. Statistical comparisons were made by using one way analysis of variance (ANOVA) followed by Dunnett’s t-test. A P value less than 0.05 was considered as significant.

Table 1. Effect of PPAE on body weight and serum lipid parameters in high fat diet fed rat

<table>
<thead>
<tr>
<th>Group</th>
<th>Change in body weight (g)</th>
<th>Serum lipid parameters (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Normal</td>
<td>178.08</td>
<td>188.12</td>
</tr>
<tr>
<td>HFD Control</td>
<td>180.21</td>
<td>203.06</td>
</tr>
<tr>
<td>PPAE (250 mg/kg; p.o.)</td>
<td>178.22</td>
<td>194.33</td>
</tr>
<tr>
<td>PPAE (500 mg/kg; p.o.)</td>
<td>179.25</td>
<td>189.61</td>
</tr>
<tr>
<td>Lovastatin (5 mg/kg; p.o.)</td>
<td>180.22</td>
<td>187.69</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. (n = 6). One way ANOVA followed by Dunnet’s t-test. $P < 0.05$, $P < 0.01$ considered as significant.

RESULTS AND DISCUSSION
Rats fed with high fat diet for 30 days displayed increase in their body weights as compared to normal rats. Treatment with PPAE at the doses of 250 and 500 mg/kg/day showed significant ($P < 0.05$) decrease in body weight to 194.33 ± 7.24 and 189.61 ± 8.77 respectively, as compared to control group (203.06 ± 8.35). PPAE (500 mg/kg/day; p.o.) showed marked reduction in body weight which is near about the same level, as Lovastatin (5 mg/kg/day; p.o.) treatment (187.69 ± 9.22). Reduction of body weight suggests the effectiveness of PPAE against hyperlipidemia (Table 1).

There was marked increase in the level of serum TC and LDL-c and decrease in the level of good cholesterol carrier i.e. HDL in the animals fed with high fat diet. Elevated level of blood cholesterol especially LDL-c is the major risk factor for coronary heart diseases (CHD) and HDL is a cardioprotective lipoprotein (Babu and Gangadevi, 2002; Basarkar and Nath, 1984). Treatment with PPAE at two different doses significantly decreased the level of TC (86.22 ± 3.26 and 82.44 ± 2.96 mg/dl) and LDL-c (41.23 ± 0.80 and 6.21 ± 0.47 mg/dl) as compared to control.

Several studies showed that an increase in HDL cholesterol is associated with a decrease in coronary risk and most of the drugs that decrease TC also decrease the HDL cholesterol (Wilson, 1990). In the
present study, the administration of PPAE at a dose of 250 mg/kg/day did not show significant changes in HDL level whereas 500 mg/kg/day dose significantly \((P < 0.05)\) increased the level of HDL (31.92 ± 2.13 mg/dl) as compared to HFD control (28.42 ± 1.11) (Table 2). This effect of PPAE may be due to increase in the activity of lecithin: Cholesterol Acetyl Transferase which incorporates free cholesterol from LDL into HDL and transfers back to VLDL and intermediate density lipoprotein (Joshep Witztum, 1996). Elevated level of HDL is considered as beneficial as it carry cholesterol from cells back to liver where it is converted to bile ready for excretion (Treasure \textit{et al.}, 1995).

Recent studies showed that TG is also directly or indirectly related to CHD (Bainton \textit{et al.}, 1992) and in the present study PPAE decreased the level of TG significantly. Treatment with PPAE at the doses of 250 and 500 mg/kg/day showed marked reduction in TG level to 78.34 ± 1.69 and 71.55 ± 1.83 mg/dl respectively as compared to HFD control (83.16 ± 2.16) (Table 2). This effect might be due to increase in the activity of endothelium bound lipoprotein lipase that causes hydrolysis of triglyceride into fatty acid or may be due to inhibition of lipolysis so that fatty acids do not get converted into triglycerides (Joshep and Witztum, 1996).

TC: HDL-c ratio, LDL: HDL-c ratio is the effective predictor of coronary risk (Dhuley \textit{et al.}, 1999). Study revealed the significant reduction in TC: HDL-c ratio, LDL: HDL-c ratio after treatment of rats with PPAE (250 and 500 mg/kg/day, p.o.) (Table 2).

Preliminary phytochemical screening revealed the presence of phenolic compounds i.e. tannins and flavonoids, saponins and carbohydrates in the PPAE. Several studies show that plant saponins are known to possess both hypolipidemic and antihyperlipidemic activities (Sidhu and Oakenful, 1986; Inoue \textit{et al.}, 1999). Flavonoids and other polyphenolics have also suggested for their hypocholesterolemic and hypolipidemic effects (Chan \textit{et al.}, 1999; Guimaraes \textit{et al.}, 2000).

### REFERENCES


Guimaraes PR, Galavao AMP, Batista CM, Azovedo

### Table 2. Effect of PPAE on lipid ratio in high fat diet fed rat

<table>
<thead>
<tr>
<th>Group</th>
<th>TC: HDL</th>
<th>LDL: HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2.18 ± 1.51</td>
<td>0.79 ± 0.28</td>
</tr>
<tr>
<td>HFD Control</td>
<td>3.45 ± 2.37</td>
<td>1.87 ± 1.00</td>
</tr>
<tr>
<td>PPAE (250 mg/kg)</td>
<td>2.93 ± 1.53</td>
<td>1.40 ± 0.37</td>
</tr>
<tr>
<td>PPAE (500 mg/kg)</td>
<td>2.58 ± 1.38</td>
<td>1.13 ± 0.22</td>
</tr>
<tr>
<td>Lovastatin (5 mg/kg)</td>
<td>2.34 ± 1.75</td>
<td>0.93 ± 0.48</td>
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</tbody>
</table>

Values are expressed as mean ± S.D. (n = 6).
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