Hypolipidemic effect of methanolic extract of Dolichos biflorus Linn. in high fat diet fed rats

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Received 5 August 2004; revised 9 February 2005

High fat diet fed rats showed significant increased levels of plasma and tissue total cholesterol, triglycerides, free fatty acids, phospholipids, plasma LDL cholesterol and decreased level of plasma HDL cholesterol. Methanolic extract of D. biflorus administration to high fat diet fed rats showed near to normal levels of the above lipids in plasma and tissues. Higher dose of the extract (400 mg/kg body weight) showed comparable results with standard drug atorvastatin. It is concluded that the methanolic extract of D. biflorus possesses hypolipidemic activity in high fat diet fed rats.

Keywords: Dolichos biflorus, Hypolipidemic effect, Methanolic extract, Rat

Research on medicinal plants has increased recently all over the world. Medicinal plants have been used in various traditional systems, as they have immune potential against numerous diseases. One such plant Dolichos biflorus Linn. (Fabaceae), commonly known as Kollu in Tamil and horse gram in English is chosen in this investigation. Branches of D. biflorus are sub erect or twining, glabrescent, leaflets are 2.5 to 5 cm. broadly lanceolate, stipules subulate, seeds 5-6, compressed reniform, grey or reddish brown in colour1. Dolichos biflorus has antitumor activity2, the root of the plant is used as expectorant in China3, the entire dried plant is used in abortion in India4, it is also used in menstrual problems5. The plant has been traditionally used as a lipid lowering agent. Therefore hypolipidemic effect of this plant is screened systematically in this study.

Materials and Methods

Whole plants of D. biflorus were collected from Sankaran coil, Tirunelveli district of Tamilnadu, India. Taxonomic identification was made from Botanical Survey of Medicinal Plant Unit, Siddha, Government of India, Palayamkottai, Tamilnadu. Four months old whole plants were dried under shade, segregated and pulverized by a mechanical grinder and passed through a 40 mesh sieve. The powdered materials were successively extracted by hot continuous percolation method in soxhlet apparatus6. The extracts were suspended in 2% tween 807. Wistar male rats of 16-19 weeks age, weighing 150-175 g were procured from the Central Animal House, Rajah Muthiah Medical College, Annamalai University. The animals were kept in plastic cages, 2 per cage, with 12:12 hr light and dark cycle at 25° ± 2°C. The animals were maintained on their respective diets and water ad libitum. Animal were divided into following 5 groups of 6 animals each:

Group I: Standard chow diet (Control):
Group II: High fat diet
Group III: High fat diet plus methanolic extract of D. biflorus (dose 1-200 mg/kg body weight)
Group IV: High fat diet plus methanolic extract of D. biflorus (dose 11-400 mg/kg body weight)
Group V: High fat diet plus standard drug atorvastatin (1.2 mg/kg body weight)

The compositions of the two diets were as follows:

Control diet: Wheat flour 22.5%, roasted bengal gram powder 60%, skimmed milk powder 5%, casein 4%, refined oil 4%, salt mixture with starch 4% and vitamin & choline mixture 0.5%.

High fat diet: Wheat flour 20.5%, roasted bengal gram 52.6%, skimmed milk powder 5%, casein 4%, refined oil 4%, coconut oil 9%, salt mixture with...
starch 4% and vitamin and choline mixture 0.5%, cholesterol 0.4%.

Rats of groups III and IV were orally fed the methanolic extracts of *D. biflorus* dose I and dose II respectively and rats of group V were fed standard drug atorvastatin. Both, the *D. biflorus* extracts and atorvastatin were suspended in 2% tween 80 and fed to their respective rats in addition to their respective diets. At the end of 9 weeks all the animals were sacrificed by cervical decapitation after overnight fasting. Animals were given enough care as per the Animal Ethical Committee's recommendations.

Plasma total cholesterol, triglyceride, phospholipids, free fatty acids. LDL cholesterol and HDL cholesterol were estimated using Boehringer Mannheim kits by Erba Smart Lab analyzer, USA. Ester cholesterol\(^8\) and free cholesterol\(^6\) were analysed using digitonin. Portions of liver, heart and aorta tissues were blotted, weighed and homogenized with methanol (3 volumes) and the lipid extract was obtained by the method of Folch et al.\(^9\) and were used for the estimation of ester cholesterol, free cholesterol, triglyceride\(^8\), phospholipids\(^1\).

Results were expressed as mean ± SE of 6 rats in each group. Student's t test was used to determine the statistical significance between each control group and its test groups. Significance level was fixed at 0.05.

### Results and Discussion

Results are presented in Tables 1-3.

The average food intake per rat per day was found to be 19.6±1.2g. Results clearly show that feeding HFD increased plasma and tissue lipids and lipoprotein levels. Earlier studies have also shown a significant elevation in the plasma and tissue lipid parameters in response to high fat diet\(^12,13\).

Both plasma and tissue cholesterol as well as phospholipids were reduced remarkably on treating the HFD rats with methanolic extract of *D. biflorus*. This lipid lowering effect may be due to the inhibition of hepatic cholesterolgenesis or due to the increased excretion of fecal sterols. Like many species *D.biflorus* may stimulate hepatic microsomal cytochrome P450 dependent aryl hydroxylase activity which is believed to be involved in the hydroxylation of endogenous steroids such as cholesterol\(^14\) and thereby increases the catabolic conversion of cholesterol to bile acids in liver\(^15\).

Concentration of phospholipids decreased on treatment with *D.biflorus* extract in the HFD fed rats.
Table 2 — Effect of methanol extract of *D. biflorus* on tissue cholesterol level in HFD rats

[Values are mean ± SE of 6 rats]

<table>
<thead>
<tr>
<th>Group</th>
<th>Free cholesterol (mg/g tissue)</th>
<th>Ester cholesterol (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Heart</td>
</tr>
<tr>
<td>Group I</td>
<td>0.74±0.01</td>
<td>0.67±0.011</td>
</tr>
<tr>
<td>Group II</td>
<td>1.18±0.01*</td>
<td>0.94±0.01*</td>
</tr>
<tr>
<td>Group III</td>
<td>0.98±0.02*</td>
<td>0.87±0.01*</td>
</tr>
<tr>
<td>Group IV</td>
<td>0.81±0.01*</td>
<td>0.67±0.01*</td>
</tr>
<tr>
<td>Group V</td>
<td>0.88±0.01*</td>
<td>0.73±0.01*</td>
</tr>
</tbody>
</table>

P values:
* P < 0.001, * P < 0.01, * P < 0.02, * P < 0.05
NS: Non Significant

Details of groups I – V are same as in Table 1.

Marked reduction in cholesterol and phospholipids levels may be attributed to the significantly higher doses (400 mg/kg) of *D. biflorus* which is comparable to the standard drug atorvastatin.

Increased levels of plasma free fatty acids were observed in HFD rats as compared to the control rats. This significant increase of free fatty acids may be due to the breakdown of membrane phospholipids by the action of oxygen derived free radicals induced during hyperlipidemia or may be due to the increased activity of phospholipase A2. Treatment with *D. biflorus* extract decreased the free fatty acids concentration.

Supplementatin of *D. biflorus* extract lowered the concentration of triglyceride level significantly in HFD fed rats. HFD fed rats showed decrease activity of lipoprotein lipase in adipose tissue. Stimulation of the activities of skeletal muscle lipoprotein lipase and adipose tissue hormone sensitive lipase may be responsible for the increased uptake of triglycerides from plasma by skeletal muscle and adipose tissue.

Increased concentration of VLDL and LDL were observed in the plasma of HFD treated rats when compared with the control. Treatment with *D. biflorus* extract reduced VLDL and LDL levels significantly. Dose II of the plant extract was found to have the effect to the extent as that of the standard drug atorvastatin. VLDL is highly rich in triglycerides and is involved in the transport of triglycerides from liver to extrahepatic tissues whereas LDL is mainly formed from VLDL in presence of heparin releasable lipoprotein lipase, an enzyme present in the endothelial cells of the blood vessel walls. Studies show that both LDL and VLDL have a positive role in atherogenesis. Dose II of *D. biflorus* was found to be more effective than dose I in reducing the concentration of LDL in plasma. Reduced levels of LDL in *D. biflorus* extract on HFD fed rats may be possibly due to increase with catabolism of LDL.

Table 3 — Effect of methanol extract of *D. biflorus* on tissue lipid content in HFD rats

[Values are mean ± SE of 6 rats]

<table>
<thead>
<tr>
<th>Group</th>
<th>Phospholipid (mg/g tissue)</th>
<th>Triglyceride (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Heart</td>
</tr>
<tr>
<td>Group I</td>
<td>17.57±0.37</td>
<td>23.90±0.58</td>
</tr>
<tr>
<td>Group II</td>
<td>27.52±0.37*</td>
<td>36.22±0.54*</td>
</tr>
<tr>
<td>Group III</td>
<td>23.71±0.58*</td>
<td>36.40±0.64*</td>
</tr>
<tr>
<td>Group IV</td>
<td>19.31±0.54*</td>
<td>26.51±0.37*</td>
</tr>
<tr>
<td>Group V</td>
<td>20.96±0.51*</td>
<td>27.27±0.35*</td>
</tr>
</tbody>
</table>

P values:
* P < 0.001, * P < 0.01, * P < 0.02, * P < 0.05
NS: Non-Significant

Details of groups I – V are same as in Table 1.
HDL is synthesized mainly in intestine and liver. It has a high phospholipids content and is involved in reverse cholesterol transport. HDL is considered to be a beneficial lipoprotein as it has an inhibitory effect in the pathogenesis of atherosclerosis. HDL concentration in plasma was significantly increased on *D. biflorus* treatment in this present investigation which shows that *D. biflorus* has a preventive role in the development of atherogenesis.

Atherogenic index is used as a marker to assess the susceptibility of atherogenesis. It was markedly increased on feeding HFD to rats. It was significantly decreased on feeding experimental plant extract, *D. biflorus*, thus emphasizing the protective role of *D. biflorus* against atherogenesis. Further investigation is needed to explore the exact mechanism of action of the plant extract.

References