ANTI-HYPERLIPIDEMIC ACTIVITY OF BAUHINIA VARIEGATE ROOT EXTRACTS.

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Abstract
In athreogenic diet induced hyperlipedimia model, the rats receiving treatments with the aqueous and ethanolic extracts of the root of Bauhinia variegata shows significant reduction in Total cholesterol, Triglycerides, Very low density lipoprotein & elevated in High density lipoprotein cholesterol. The ethanolic extract of B. variegata was found to posses significant hypolipidemic activity. The results also suggest that B. variegata root extract at 200 and 400mg/kg b. wt. concentration is an excellent lipid lowering agent.

Keywords: Cholesterol, Hypolipidemic, Athreogenic diet, Bauhinia variegate.

Introduction
Cardiovascular diseases cause death approximately 50% in develop countries1. The disease burden contributed by cardiovascular disease has been increasing in the developing world also. The world health organizations (WHO) estimate that 12 million people’s word wide die by cardiovascular diseases, with most of them from developing world. Most people benefit from lowering their B.P and cholesterol level. The underlying primary cause of cardiovascular disease is believed to be arthrosclerosis, a progressive multifactorial disease of arterial wall. Central to the pathogenesis, arthrosclerosis is deposition of cholesterol in arterial wall, therefore primary consideration in therapy for hyperlipidemia and arthrosclerosis is to enervate the elevated plasma level of TC, TG and LDL along with increase in HDL lipid level2.

The searches for effective herbal drugs for treatment of hyperlipedemia still continue and have yielded invaluable herbal remedies. To prove the ethnomedical use of such folklor traditional medicines we selected Bauhinia Variegata. Linn., a plant used in traditional system of medicine as anti-hyperlipedemic remedy for treatment of hyperlipedemia. Bauhinia variegata Linn. is a species of flowering plant belongs to Leguminosae family and native of southeastern Asia, southern China and west India. Common names include Orchid tree and Mountain-ebony. The bark is a used in salivation, sore throat, cough, bleeding piles, haematuria,
menorrhagia, enlargement of glands of the neck and tumors. Decoction of bark is a useful in ulcers and skin diseases and a remedy for diarrhea. Dried buds are useful in diarrhea, worms, piles, dysentery. Decoction of the root is given in dyspepsia and flatulence, it is also an anti fat remedy. Flowers contain sugar and have gentle laxative activity. A preparation known as kachnara guggula is useful in scrofulous tumors, ulcers, skin diseases, dropsy etc.\(^3\)

**Materials and Methods**

Collection and authentication of plant material: The roots of *Bauhinia variegata* were collected in the month of July (2007) from Tirunelveli district, Tamil Nadu and were authenticated by V. Chelladurai, Research officer Botany C.C.R.A.S, Govt of India. The voucher specimen was deposited in the department of Pharmacognosy, C.L Baid Metha College of pharmacy, Chennai.

**Preparation of alcoholic plant extract:**
The roots of *Bauhinia variegata* were dried in shade, powdered and extracted with ethanol (55\(^{0}\)C to 65\(^{0}\)C) in a Soxhlet apparatus. Before and after every extraction the marc was completely dried and weighed. The extract was evaporated under reduced pressure by a rotary vacuum evaporator until all the solvent had been removed to give an extract with a yield of 8.83% w/w.

**Preparation of aqueous plant extract:**
The aqueous extract was prepared by using fresh powder by maceration process i.e. 200g of the powdered drug was taken in 2000ml round bottom flask and to which 1000ml of distill water was added. The extraction process was carried out for a week with occasional stirring the result obtained so after filtration at the end of seven day was subjected for drying at 40\(^{0}\)C to get a solid mass. The resulted yield was 12% w/w.

**Experimental animals:** All the experiments were carried out using colony inbreed strains of wistar female rats (150-180g). Animals were housed at a temperature 22 ± 1\(^{0}\)C. A 12:12 light day cycle was followed. All animals were allowed to free access to water & fed normal pellet diet and water *ad libitum*. The composition of athreogenic diet used during study is given in Table. 1.

The experimental procedure and protocol use in this study were viewed by the Institutional Animal Ethical Committee (Ref. no. IAEC/XIII/13/CLBMCP/2007-2008) and were in accordance with the guidelines of the CPCSEA.
Table 1. athreogenic diet used during study

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Composition</th>
<th>Normal diet (%)</th>
<th>Athreogenic diet (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Protein (milk powder)</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrates (wheat powder)</td>
<td>71</td>
<td>61</td>
</tr>
<tr>
<td>3</td>
<td>Sugar</td>
<td>05</td>
<td>05</td>
</tr>
<tr>
<td>4</td>
<td>Fat (butter)</td>
<td>05</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>Salt</td>
<td>04</td>
<td>04</td>
</tr>
<tr>
<td>6</td>
<td>Vitamin</td>
<td>01</td>
<td>02</td>
</tr>
<tr>
<td>7</td>
<td>Fibers</td>
<td>02</td>
<td>01</td>
</tr>
<tr>
<td>8</td>
<td>Cholesterol</td>
<td>-</td>
<td>01</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>100 gm</td>
<td>100 gm</td>
</tr>
</tbody>
</table>

Chemicals: All the chemicals used in the study were of analytical grade.

Preliminary phytochemical analysis: Primary Phytochemical analyses were carried out with both alcoholic and aqueous extracts adopting standard procedures4,5.

Pharmacological studies

Induction of hyperlipedemia: In order to induce hyperlipedemia the method reported by Bopanna et al6 was followed. Female wistar rats (150-180g) were given atherogenic diet for 45 days. Forty two rats were randomly divided into 7 groups of six animals each.

Treatment Protocol:
The following schedules of dose & diet administration in experimental groups were followed:

Group: I - Normal diet
Group: II - Atherogenic diet containing cholesterol.
Group: III - Atherogenic diet + AEBV (aqueous extract of Bauhinia variegate) (200mg/kg/p.o) suspended in 0.9% saline.
Group: IV - Atherogenic diet + AEBV (400mg/kg/p.o) suspended in 0.9% saline.
Group: V - Atherogenic diet + EEBV (ethanolic extract of Bauhinia variegate) (200mg/kg p.o) suspended in 0.9% saline.
Group: VI - Atherogenic diet + EEBV (400mg/kg/p.o) suspended in 0.9% saline.
Group: VII - Atherogenic diet + atorvastatin (2.7 mg/kg/p.o) suspended in 0.9% saline.

The above mentioned treatment schedule was followed for the respective group of animals for 45 days. Blood was withdrawn using the heparanised capillaries from the retro-orbital sinus in the over night fasted animals. The serum was obtained after centrifuging the blood, which was used to estimate the concentration of biochemical parameters TC, HDL, TG, LDL, VLDL using the semi
autoanalyser and relevant lipid profile kits (Agappe Diagnostic’s Pvt.Ltd)

**Statistical analysis**
The data were statistically analyzed and all values were expressed as mean ± SEM. The data were also analyzed by one way ANOVA followed by Dunnet’s t-test [11]. ‘P’ value less than 0.05 is considered significant.

**Results**

**Preliminary Phytochemical screening:**
Phytochemical investigation revealed the presence of alkaloids, proteins, carbohydrates, tannins, sterols, glycosides, flavanoids, saponins, fixed oils in both alcoholic and aqueous extracts while proteins, flavones, fixed oils were found absent in aqueous extract.

**Biochemical estimation**

**Total cholesterol:** Group II animals fed with HFD exhibited significant (p<0.01) increase in total cholesterol when compared with group I animals.

Group III to group VI animals exhibited a significant (p<0.01) decrease in total cholesterol when compared with group II animals.

**HDL:** Group II animals exhibited significant (p<0.01) reduction in HDL cholesterol when compared with group I animals. Group III animals (AEBV 200 mg/ kg/p.o) when compared to group II animals did not exhibit any significant changes. Group IV with group VI animals exhibited significant (p<0.01) increase HDL when compared to Group II animals.

**Triglycerides:** Group II animals exhibited significant (p<0.01) increase in triglycerides when compared with group I animals. Group III exhibited significant (p<0.05) decrease in triglycerides when compared with group II animals. Group IV to Group VI caused significant (p<0.01) decrease in triglycerides when compared with group II animals.

**LDL:** Group II animals when compared with group I animals exhibited significant (p<0.01) increase in LDL levels. Group III exhibited significant (p<0.05) decrease when compared to group II animals. Group IV to group VI animals compared with group II animals exhibited significant (p<0.01) decrease LDL levels.

**VLDL:** Group II animals when compared with group I animals exhibited significant (p<0.01) increase VLDL level. Group III animals when compared with group II exhibited significant (p<0.05) decrease in VLDL levels. Group IV to Group VI when compared with group II animals exhibited significant (p<0.01) decrease in VLDL levels.

Results are shown in Table: 2.

**Atherogenic index and percentage protection:** There was decrease in atherogenic index in all the treated groups.
Percentage protection for Group III (14.31%), Group IV (20.50%) Group V (19.5%) and Group VI (33.64%) were shown in Table 3.

Discussion
Cholesterol feeding elevates the level of serum cholesterol, phospholipids and triglycerides leads to development of atherosclerosis and hyperlipidemia. Both cholesterol as well as HDL was reduced remarkably on treating the high fat diet with methanolic extract of leaves of Bauhinia varirgata. This lipid lowering effect may be due to the inhibition of hepatic cholesterogenesis and catabolic conversion of cholesterol to bile acids in the liver. Marked reduction in cholesterol, triglycerides and phospholipids levels may be attributed to higher dose of drug of Bauhinia varirgata which is comparable to the standard drug atorvastatin. Statins competitively inhibit conversion of 3-hydroxy 3-methyl glutaryl coenzyme A (HMG-CoA) to mevalonate (rate limiting step in cholesterol synthesis) by the enzyme HMG-CoA reductase. This results in compensatory increase in LDL receptor expression on liver cells increased receptor mediated uptake and catabolism of IDL and LDL. Over long term, feed back induction of HMG-CoA reductase tend to increase cholesterol synthesis, but a steady state is attained with a dose dependent lowering of LDL cholesterol levels. Statins lower LDL cholesterol by 20-55%, raise HDL cholesterol levels by 5-15%, and lower triglycerides by 10-35%. Increased concentration of LDL was observed in the serum of high fat fed treated rats when compared with the control. Treatment with Bauhinia varirgata extract reduced LDL levels significantly. HDL is synthesized mainly in intestine and liver. It has 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 was significantly increased on Bauhinia varirgata treatment in this present investigation. In conclusion our results showed that the orally administered Bauhinia varirgata administered effective in suppressing high fat diet induced hyperlipidemia and suggests its beneficial use as an antiatherogenic drug.

References


<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Total cholesterol (mg/dl)</th>
<th>HDL-C(mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>LDL-C(mg/dl)</th>
<th>VLDL-C(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Control (Normal saline)</td>
<td>66.37±1.58</td>
<td>43.17±0.94</td>
<td>173±2.21</td>
<td>25.48±0.51</td>
<td>34.61±0.44</td>
</tr>
<tr>
<td>Group II</td>
<td>Diet Control</td>
<td>154.08±2.35**</td>
<td>32.44±1.11**</td>
<td>250.2±4.61**</td>
<td>43.12±0.90**</td>
<td>50.08±0.91**</td>
</tr>
<tr>
<td>Group III</td>
<td>AEBV (200mg)</td>
<td>125.82±1.79**</td>
<td>34.47±0.36**</td>
<td>237.6±1.96*</td>
<td>40.64±0.40*</td>
<td>47.53±0.39*</td>
</tr>
<tr>
<td>Group IV</td>
<td>AEBV (400mg)</td>
<td>113.92±1.23**</td>
<td>37.37±0.42**</td>
<td>229.2±2.56**</td>
<td>38.35±0.62**</td>
<td>45.83±0.51**</td>
</tr>
<tr>
<td>Group V</td>
<td>EEBV (200mg)</td>
<td>104.37±0.92**</td>
<td>37.27±0.42**</td>
<td>223.7±219**</td>
<td>37.29±0.47**</td>
<td>44.74±0.43**</td>
</tr>
<tr>
<td>Group VI</td>
<td>EEBV (400mg)</td>
<td>94.47±0.71**</td>
<td>40.39±0.62**</td>
<td>208.8±2.67**</td>
<td>33.68±0.53**</td>
<td>41.76±0.53**</td>
</tr>
<tr>
<td>Group VII</td>
<td>Sibutramine (5mg)</td>
<td>77.36±1.45**</td>
<td>45.18±0.41**</td>
<td>192.2±1.92**</td>
<td>29.37±0.39**</td>
<td>38.46±0.39**</td>
</tr>
</tbody>
</table>

Table no 1, 2 and 3 values are expressed in mean ± SEM of 6 animals
Statistical significance test for comparisons was done by ANOVA, followed by Dunnet’s test. Comparisons were made between: a) Group I vs Group II
b) Group III, IV, V, VI, VII vs Group II

** p value< 0.01
Table- 3
Atherogenic index and percentage protection in various groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Atherogenic index</th>
<th>Percentage protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Control (Normal saline)</td>
<td>4.00</td>
<td>-</td>
</tr>
<tr>
<td>Group II</td>
<td>Diet Control</td>
<td>7.71</td>
<td>-</td>
</tr>
<tr>
<td>Group III</td>
<td>AEBV (200mg)</td>
<td>6.61</td>
<td>14.31</td>
</tr>
<tr>
<td>Group IV</td>
<td>AEBV (400mg)</td>
<td>6.13</td>
<td>20.50</td>
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<td>Group V</td>
<td>EEBV (200mg)</td>
<td>6.23</td>
<td>19.15</td>
</tr>
<tr>
<td>Group VI</td>
<td>EEBV (400mg)</td>
<td>5.12</td>
<td>33.64</td>
</tr>
<tr>
<td>Group VII</td>
<td>Sibutramine (5mg)</td>
<td>4.25</td>
<td>44.90</td>
</tr>
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</table>