Evaluation of Antihyperlipidemic Activity on Ethanolic extract of *Caesalpinia sappan* L in hyperlipidemic rats

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**ABSTRACT**

The anti-hyperlipidemic effect of ethanolic extract of heart wood of *caesalpinia sappan* L (CSP) was tested in Triton X 100 and High fat diet induced hyperlipidemic rat models. Here, Acute hyperlipidemia was induced by administration of single dose of Triton X 100 (100 mg/kg,i.p) and Chronic hyperlipidemia was induced by feeding fat diet for 21 days to rats. Treatment with CSP (200 and 400 mg/kg, p.o ) significantly reduced the hyperlipidemia i.e., decreased levels of serum total Cholesterol, triglycerides, low density lipoprotein Cholesterol (LDL-C), very low density lipoprotein Cholesterol (VLDL-C) , and increase of serum high density lipoprotein Cholesterol (HDL-C) when compared to vehicle control and hyperlipidemic (diseased) groups. The results demonstrated that ethanolic extract of heart wood of *caesalpinia sappan* L possessed significant antihyperlipidemic activity.

**Keywords:** Triton, fat diet, hyperlipidemia, *caesalpinia sappan* L, heartwood, rats, Atrovastatin.

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**1. Introduction**

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases. Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of
death [1,2]. Hyperlipidemia is characterized by elevated serum total cholesterol, low density lipoprotein, very low density lipoprotein and decreased high density lipoprotein levels. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease. Among these hypercholesterolemia & hypertriglyceridemia are closely related to ischemic heart disease. 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. Currently available drugs have been associated with number of side effects [3,4,5].

caesalpinia sappan L is a herbaceous plant and belongs to the family Caesalpiniaeae. It has been used especially in south India to treat diabetes mellitus. The caesalpinia sappan L is valued mainly for analgesic, adaptogenic, antiulcer, antihelmintic, antibacterial, insecticidal, antifungal, anti-inflammatory, antipyretic, antioxidant, antiproliferative, antiviral, immunomodulatory, and immunosuppressive activities [6,7].

2. Materials and Methods

Chemicals

Triton X-100 was obtained from H.Chandanmal & CO., Suppliers of chemicals, Chennai. Atorvastatin was obtained from Alembic pharmaceuticals Ltd Vadodhara, gujarat. All other chemicals were of analytical grade and obtained locally.

Experimental Animals

Wistar albino adult male rats weighing 200-250g were grouped and housed in polypropene cages (38x 23x 10cm) with not more than five animals per cage and maintained under standard laboratory conditions. They were allowed free access to standard dry pellet diet and water. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) constituted under CPCSEA. All this procedure were carried as per the CPCSEA norms.

Collection and authentication of plant

The heart wood of Caesalpinia sappan L was collected form tirumalla hills in Andhra pradesh and it was authenticated by Dr. K. Madhava chetty, Assistant professor, Department of botany in Sri Venkateswara University, tirupati

Method of extraction process

The heart wood of caesalpinia sappan L was mechanically powdered and subjected to single soxhelt extraction using ethanol. The extract was concentrated to dryness under reduced pressure and controlled temperature to yield a light brown colored mass.

Preliminary Phytochemical analysis

The Ethanolic extract of caesalpinia sappan L was subjected to preliminary phytochemical screening and it shown the presence of steroids, phenol, tannin, saponins, flavanoids.

Acute toxicity studies

The ethanolic extract of plant caesalpinia sappan L was found to be safe up to 2000 mg/kg body wt. by oral route. After 24hr animals were found well tolerated. There was no mortality and no signs of toxicity. So two dose levels i.e. 200mg/kg, and 400mg/kg body weight were selected for the present study.

Antihyperlipidemic studies

Induction of Hyperlipidemia

High Fat Diet (FD) induced hyperlipidemic model

Preparation of Feed

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 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. Thirty Wistar rats were randomly divided into five groups of six each. The chronic experimental hyperlipidemia was produced by feeding the above prepared food for 21 days. The rats are then given test plant extracts i.e., Caesalpinia sappan L (200 and 400 mg/kg, p.o) and Atorvastatin (10 mg/kg, p.o) once daily in the morning orally for 14 consecutive days. During these days, all the groups also received fat diet in the same dose as given earlier. The hyperlipidemic control i.e.,group II animals received the hyperlipidemic diet and the vehicle. The control group animals received the normal laboratory diet and vehicle.

Group 1: Administered vehicle and served as normal control.

Group 2: Fed with fat diet (FD) and served as hyperlipidemic control.

Group 3: Administered CSP (200mg/kg), p.o., and fed with FD.

Group 4: Administered CSP (400mg/kg), p.o., and fed with FD.

Group 5: Administered Atorvastatin (10mg/kg), p.o.,and fed with FD.

On day 15, animals were anaesthetized with Diethyl ether and blood was collected by retro orbital puncture. The blood was subjected to centrifugation for 15 min at 2500 rpm to obtain serum. The collected serum was analyzed for serum total cholesterol,triglycerides, high density lipoprotein cholesterol, low density lipoprotein cholesterol and very low density lipoprotein cholesterol

Statistical analysis

Results were analyzed by one way ANOVA, followed by tukey test, P value less than 0.05 were taken as significant(Table no:1)

Triton X 100 (TR) induced hyperlipidemic model

Hyperlipidemia was induced in Wistar albino rats by single intraperitoneal injection of freshly prepared solution of Triton-X-100 (100 mg/kg) in physiological saline solution after overnight fasting for 18 h .The animals were divided into five groups of six rats each. The first group was given
standard pellet diet, water and orally administered with 5% CMC. The second group was given a single dose of triton administered at a dose of 100mg/kg, i.p. After 72 hours of triton injection, this group received a daily dose of 5% CMC (p.o) for 7 days. To the third, fourth and fifth group were administered with 200mg/kg, 400mg/kg respectively of alcholcoholic extract of Caesalpinia sappan L, daily for 7 days, after inducing hyperlipidemia. Sixth group was administered with the standard 10 mg/kg, p.o. for 7 days.

**Group 1:** Administered vehicle and served as normal control.

**Group 2:** Administered Triton X 100 (TR) and served as hyperlipidemic control.

**Group 3:** Administered CSP (200mg/kg), p.o.,

**Group 4:** Administered CSP (400mg/kg), p.o.,

**Group 5:** Administered Atorvastatin (10mg/kg), p.o.

On the 8th day, blood was collected by retro orbital sinus puncture, under mild ether anesthesia. 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.

**Statistical analysis:** Results were analyzed by one way ANOVA, followed by tukey test, P value less than 0.05 were taken as significant (Table no:2)

### 3. Results and discussions

The dried and powdered heart wood of *Caesalpinia sappan Linn* was subjected to soxhlet extraction with 95% ethanol. Phytochemical analysis of the plant extract showed different phytocomponents viz. glycosides, phytosterols, triterpinoids, alkaloids and flavonoids. Several phytocomponents like glycosides, triterpinoids, Saponins, alkaloids and flavonoids are known to have anti-hyperlipidemic properties. Treatment with CSP (200 & 400mg/kg, p.o.,) for 7 days successfully prevented the elevation of serum Total Cholesterol, Triglycerides, Low Density Lipoproteins Cholesterol (LDL-C), Very Low Density Lipoproteins Cholesterol (VLDL-C), and decrease of serum High Density Lipoprotein Cholesterol (HDL-C) in Triton model rats respectively. Triton induced hyperlipidemia in rats is an acute model for the primary screening of anti-hyperlipidemic agents.

Administration of CSP(200 & 400mg/kg, p.o) for 14 days in fat diet model, successfully prevented the elevation of serum Total Cholesterol, Triglycerides, Low Density Lipoproteins Cholesterol (LDL-C), Very Low Density Lipoproteins Cholesterol (VLDL-C), and decrease of serum High Density Lipoprotein Cholesterol (HDL-C) in Fat diet model rats respectively. It has been well established that nutrition plays an important role in the etiology of hyperlipidemias and atherosclerosis. Fat diet model is used as a chronic model for induction of hyperlipidemia. In our study we chosen fat diet which contain the common ingredients in our daily food. Diet containing saturated fatty acids increases the activity of HMG CoA reductase, the rate determining enzyme in cholesterol biosynthesis; this may be due to higher availability of acetyl CoA, which stimulated the cholesterogenesis rate. Moreover, this could be associated with a down regulation in LDL receptors by the cholesterol and saturated fatty acids in the diet, which could also explain the elevation of serum LDL-C levels either by changing hepatic LDLR (LDL receptor) activity, the LDL-C production rate or both. LCAT enzyme is involved in the transesterification of cholesterol, the maturation of HDL-C and the flux of cholesterol from cell membranes into HDL[8,9]. The activity of the enzyme tends to decrease in diet-induced hypercholesterolemia; therefore the possible mechanism of CSP may involve increase of HDL-C, which is attributed to the mobilization of cholesterol from peripheral cells to the liver by the action of Lecithin Cholesterol O-acyltransferase (LCAT). The increased HDL-C facilitates the transport of TG or cholesterol from serum to liver by a pathway termed ‘reverse cholesterol transport’ where it is catabolised and excreted out of the body. [10] Antihyperlipidemic activity was observed with Atorvastatin (10mg/kg p.o.), and the CSP (400mg/kg) showed better activity than CSP (200mg/kg).

### Table 1: Effect of CSP on serum lipid parameter levels in fat diet induced Hyperlipidemic rats

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Groups</th>
<th>Serum lipid parameters (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total cholesterol</td>
</tr>
<tr>
<td>I</td>
<td>Normal</td>
<td>83.84±1.22</td>
</tr>
<tr>
<td>II</td>
<td>Hyperlipidemic</td>
<td>187.0±10.85</td>
</tr>
<tr>
<td>III</td>
<td>Test 1 (200mg/kg)</td>
<td>123.0±10.83*</td>
</tr>
<tr>
<td>IV</td>
<td>Test 2 (400mg/kg)</td>
<td>107.7±10.74**</td>
</tr>
<tr>
<td>V</td>
<td>Atorvastatin (10mg/kg)</td>
<td>97.67±10.69**</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=6).
Values are statistically significant at* P<0.05
More significant at **P<0.01 vs hyperlipidemic control using one way ANOVA followed by tukey test.
Table 2: Effect of CSP on serum lipid parameter levels in Triton X 100 induced Hyperlipidemic rats

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Groups</th>
<th>Serum lipid parameters (mg/dl)</th>
<th>Total Cholesterol</th>
<th>Triglycerides</th>
<th>HDL-C</th>
<th>LDL-C</th>
<th>VLDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal</td>
<td></td>
<td>84.67±1.28</td>
<td>64.73±8.07</td>
<td>49.27±4.62</td>
<td>24.46±1.61</td>
<td>12.95±1.61</td>
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<tr>
<td>II</td>
<td>Hyperlipidemic</td>
<td></td>
<td>205±12.81</td>
<td>117.9±7.45</td>
<td>20.22±4.40</td>
<td>147.1±15.1</td>
<td>23.58±1.49</td>
</tr>
<tr>
<td>III</td>
<td>Test 1 (200mg/kg)</td>
<td></td>
<td>101.9±15.37***</td>
<td>69.12±2.76***</td>
<td>43.07±4.61</td>
<td>41.01±6.62*</td>
<td>18.56±0.55*</td>
</tr>
<tr>
<td>IV</td>
<td>Test 2 (400mg/kg)</td>
<td></td>
<td>96.57±14.16**</td>
<td>75.19±2.80*</td>
<td>45.03±4.66**</td>
<td>36.09±15.01*</td>
<td>16.52±0.56*</td>
</tr>
<tr>
<td>V</td>
<td>Atorvastatin (10mg/kg)</td>
<td></td>
<td>92.27±13.21**</td>
<td>64.32±3.03**</td>
<td>48.10±4.69*</td>
<td>32.44±12.90*</td>
<td>13.68±0.60*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=6).
Values are statistically significant at* P<0.05
More significant at **P<0.01 vs hyperlipidemic control using one way ANOVA followed by tukey test.

4. Conclusion
The results obtained from the pharmacological screening have led to the conclusions that, ethanolic extract of heart wood of Caesalpinia sappan Linn has significant antihyperlipidemic activity. Hence it can be exploited as an anti-hyperlipidemic therapeutic agent or adjuvant in existing therapy for the treatment of hyperlipidemia.

5. References