Evaluation of antihyperlipidemic activity of ethanolic extract of *Cassia auriculata* flowers

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Hyperlipidemia is a major risk factor for development of coronary artery disease. *Cassia auriculata* is traditionally used in India for medicinal purposes. In this study, effect of ethanolic extract of *Cassia auriculata* flowers (Et-CAF) was investigated in Triton WR1339-induced hyperlipidemic rats. Treatment with the Et-CAF (450 mg/kg b.wt) significantly reduced the total cholesterol (TC), triglycerides (TG) and low-density lipoprotein-cholesterol (LDL) levels and significantly increased the high-density lipoprotein (HDL) level associated with reduction of atherogenic index in hyperlipidemic rats. However, there was no change in the serum lipid profile of normal rats treated with Et-CAF alone. The results suggest that Et-CAF has a beneficial effect in treating hyperlipidemia and may serve as a potential drug for prevention of hyperlipidemic atherosclerosis.

Keywords: Atherosclerosis, Hyperlipidemia, Triglycerides, Cholesterol, *Cassia auriculata*, Antihyperlipidemic activity

Hypercholesterolemia and hypertriglyceridemia are major risk factors either alone or together and accelerate the development of coronary artery disease, progression of atherosclerosis, myocardial infarction, heart attack, and cerebrovascular diseases. High levels of cholesterol, particularly low-density lipoprotein (LDL) accumulate in the extra-cellular subendothelial space of arteries and can undergo oxidative modifications, which is highly atherogenic and toxic to vascular cells. In addition to atherosclerosis, this can cause a variety of serious diseases such as hypertension, obesity, diabetes, functional depression of some organs, etc. Lipids undergo peroxidative change in the arterial wall and eventually result in tissue injury. Lipid lowering drugs like fibrates, statins and bile acid sequestrants used in the treatment of hyperlipidemia possess toxic side effect. Therefore, there is an urgent need to have a lipid lowering drug with no side effects. A number of herbal medicines are used for controlling hyperlipidemia and related complications in patients.

*Cassia auriculata* L. (Cesalpinaceae, common name: Tanner’s Cassia), a common plant in Asia has been widely used in traditional medicine for rheumatism, conjunctivitis and diabetes. It is widely used in Ayurvedic medicine as ‘Avarai Panchaga Choornam’ and as a constituent of Kalpa herbal tea. It is also reported to possess antiperoxidative and antihyperlipidemic effect in streptozotocin-induced diabetic rats. A number of constituents such as polysaccharides, flavonoids, anthracene derivatives, dimeric procyanidins and β-sitosterol have been reported in *C. auriculata*. However, reports on hyperlipidemic animal models are lacking.

In the present investigation, we have studied the effect of ethanolic extract on *C. auriculata* flower (Et-CAF) on the serum lipid levels, including total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) in triton WR-1339 (TWR)-induced hyperlipidemic rats.

Materials and Methods

Chemicals

Triton WR-1339 was purchased from Acros organic (Thermo Fisher Scientific, USA), cholesterol, glucose, bovine serum albumin and all other chemicals were obtained from M/s Merck Specialties Private Ltd and M/s Himedia Laboratory, Mumbai, India. All chemicals used were of analytical grade.

Preparation of flowers ethanolic extract

Fresh *Cassia auriculata* flowers collected from Bharathidasan University Campus, Tiruchirappalli, Tamilnadu, India were dried under shade thoroughly and powdered. About 100 g of dry flower powder was extracted with 80% ethanol at 80°C for 3 h and the procedure was repeated thrice. The combined ethanolic extract was concentrated in rotary evaporator at reduced pressure to obtain ~22.33% w/w of extract and it was stored at 4°C until further use. During the study, the residual extract was suspended in distilled water and orally administered to the hyperlipidemic rats.
Induction of hyperlipidemia

Hyperlipidemia was induced in experimental rats by a single intravenous (i.v) injection of TWR-1339 (300 mg/kg b.wt). After 48 h of TWR administration, rats showed elevated levels of serum total cholesterol and triglycerides.

Animals and experimental design

Male albino rats of Wistar strain, 140-170 g of body weight were procured from Central Animal Facility, Indian Institute of Science, Bangalore, India and housed under hygienic and standard environmental conditions (temperature: 24 ± 1°C, light/dark cycle: 12/12 h) in Central Animal facility, Bharathidasan University. The rats were fed with standard pellet diet and water ad libitum. Rats selected from colonies were randomized into 7 groups, comprising of six rats each. After induction of hyperlipidemia, blood samples were collected during three different time intervals; Day 0 (before induction of hyperlipidemia), Day 1 (before Et-CAF administration) and Day 8 in both control and experimental rats. Plant extract was administrated to hyperlipidemic rats for 7 consecutive days.

The treatment schedule was as follows: Group-I: control rats; Group-II: control rats administered with Et-CAF alone (450 mg/kg b.wt/day); Group-III: hyperlipidemic rats; Group-IV: hyperlipidemic rats administered with lovastatin (10 mg/kg b.wt/day); Group-V: hyperlipidemic rats administered with Et-CAF (150 mg/kg b.wt/day); Group-VI: hyperlipidemic rats administered with Et-CAF (300 mg/kg b.wt/day); and Group-VII: hyperlipidemic rats administered with Et-CAF (450 mg/kg b.wt/day).

Biochemical analysis

Total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) in serum were determined using enzymatic kits (BioSystems, Spain) according to manufacturer’s instructions. The atherogenic index (A. I) was calculated as A. I = (TC - HDL)/HDL and the LDL was calculated by Friendewald’s formula. Protein was estimated by the method of Lowry et al. using BSA as standard. Blood glucose was estimated by the method of Sasaki et al.

Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA) and a least significant difference (LSD) post-hoc test to compare individual means (SPSS for Windows 11.5; SPSS Inc., Chicago). The results were expressed as mean ± SD of six values in each group. A difference of p<0.05, p<0.001 was considered to be statistically significant.

Results

Body weight

Body weight was increased significantly (p<0.05) in TWR-induced hyperlipidemic rats when compared with control (Group I). Elevated body weight was reversed near to control values after administration of Et-CAF for the period of 1 week (data not shown).

Blood glucose

The effect of Et-CAF on serum levels of glucose in TWR-induced hyperlipidemic rats is given in Table 1. After induction of hyperlipidemia, Et-CAF was administered to hyperlipidemic rats at three different doses (150, 300 and 450 mg/kg b.wt) for 7 consecutive days. Significant antihyperglycemic effect (p<0.001) was observed from dose 150 mg/kg b.wt onwards and was more prominent in Group VII rats (125.2 ± 5.40) treated with 450 mg/kg b.wt compared with hyperlipidemic rats (156.34 ± 4.54). The standard drug lovastatin also reduced the glucose level in hyperlipidemic rats. There was no significant difference between Groups I (98.27 ± 3.49) and II (95.25 ± 3.84) rats.

In vivo antihyperlipidemic activity of Et-CAF on serum lipid profile

The effects of Et-CAF on serum lipid profiles of TWR-induced hyperlipidemic rats are depicted in Table 1. After induction of hyperlipidemia, the rats exhibited significant (p<0.001) increase in serum cholesterol, TG, LDL and VLDL levels (Groups III, IV, V, VI & VII), compared with control. Administration with Et-CAF (Groups V, VI and VII) showed significant (p<0.001) reduction in serum cholesterol (70.55 ± 4.4), TG (59.66 ± 2.92), LDL (31.71 ± 1.68) and VLDL (11.93 ± 0.92), as compared with hyperlipidemic rats (Group III). HDL was significantly increased in Et-CAF treated rats (Group VII; 26.9 ± 2.46) as compared with Group III (15.87 ± 0.01) hyperlipidemic rats (Table 1). The atherogenic index was significantly (p<0.001) decreased in group VII (1.62 ± 0.12) after supplementation of 450 mg/kg of Et-CAF for 7 consecutive days, as compared to Group III (5.23 ± 0.28) hyperlipidemic rats (Table 1). The observed reduction of serum lipid profile was comparable to lovastatin administered rats (Group IV).
Table 1—Effect of Et-CAF on serum glucose and lipid profile in hyperlipidemic rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Control + Et-CAF (450 mg/kg)</th>
<th>HC (10 mg/kg)</th>
<th>HC + Lovastatin (150 mg/kg)</th>
<th>HC + Et-CAF (300 mg/kg)</th>
<th>HC + Et-CAF (450 mg/kg)</th>
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<tr>
<td><strong>Glucose (mg/dL)</strong></td>
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<tr>
<td>Day 0</td>
<td>93.01 ± 2.45</td>
<td>94.10 ± 3.10</td>
<td>93.87 ± 4.18</td>
<td>93.32 ± 3.21</td>
<td>95.54 ± 3.02</td>
<td>93.35 ± 4.21</td>
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<tr>
<td>Day 1</td>
<td>94.27 ± 3.97</td>
<td>96.44 ± 4.08</td>
<td>91.18 ± 5.75</td>
<td>143.51 ± 4.32</td>
<td>140.32 ± 4.92</td>
<td>141.31 ± 6.31</td>
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<td>Day 8</td>
<td>98.25 ± 3.49</td>
<td>95.25 ± 3.84</td>
<td>156.34 ± 4.54</td>
<td>139.24 ± 3.09</td>
<td>140.52 ± 5.41</td>
<td>136.04 ± 3.85</td>
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<td><strong>Cholesterol (mg/dL)</strong></td>
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<tr>
<td>Day 0</td>
<td>46.89 ± 2.42</td>
<td>46.10 ± 1.3</td>
<td>47.01 ± 2.43</td>
<td>45.21 ± 4.32</td>
<td>46.64 ± 3.98</td>
<td>47.11 ± 4.32</td>
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<td>Day 1</td>
<td>47.22 ± 2.61</td>
<td>46.22 ± 2.81</td>
<td>92.82 ± 5.40</td>
<td>93.73 ± 4.21</td>
<td>93.61 ± 4.37</td>
<td>93.61 ± 5.39</td>
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<tr>
<td>Day 8</td>
<td>48.15 ± 2.32</td>
<td>47.75 ± 2.20</td>
<td>98.96 ± 7.86</td>
<td>61.23 ± 3.32</td>
<td>78.96 ± 3.17</td>
<td>75.15 ± 4.02</td>
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<td><strong>Triglycerides (mg/dL)</strong></td>
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<tr>
<td>Day 0</td>
<td>41.98 ± 1.42</td>
<td>41.01 ± 2.90</td>
<td>40.32 ± 3.89</td>
<td>41.75 ± 3.53</td>
<td>42.39 ± 3.45</td>
<td>41.54 ± 3.23</td>
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<td>Day 1</td>
<td>42.26 ± 1.71</td>
<td>45.12 ± 2.71</td>
<td>87.15 ± 4.01</td>
<td>86.71 ± 6.21</td>
<td>87.95 ± 4.16</td>
<td>88.6 ± 5.63</td>
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<tr>
<td>Day 8</td>
<td>45.01 ± 1.86</td>
<td>47.40 ± 2.10</td>
<td>102.74 ± 4.98</td>
<td>47.36 ± 2.21</td>
<td>87.31 ± 5.76</td>
<td>70.43 ± 4.18</td>
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<td><strong>HDL (mg/dL)</strong></td>
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<td>Day 0</td>
<td>25.43 ± 1.98</td>
<td>25.89 ± 2.43</td>
<td>26.10 ± 2.42</td>
<td>25.02 ± 2.65</td>
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<tr>
<td>Day 1</td>
<td>26.73 ± 2.73</td>
<td>25.93 ± 1.64</td>
<td>14.51 ± 1.68</td>
<td>14.83 ± 1.21</td>
<td>15.02 ± 1.15</td>
<td>13.49 ± 1.13</td>
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<td>Day 8</td>
<td>27.97 ± 2.79</td>
<td>26.07 ± 2.14</td>
<td>15.87 ± 1.01</td>
<td>26.62 ± 1.83</td>
<td>17.56 ± 1.69</td>
<td>20.45 ± 1.41</td>
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<td><strong>LDL (mg/dL)</strong></td>
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<tr>
<td>Day 0</td>
<td>13.06 ± 1.34</td>
<td>12.00 ± 1.93</td>
<td>12.84 ± 2.45</td>
<td>11.84 ± 2.02</td>
<td>13.18 ± 2.65</td>
<td>13.47 ± 1.98</td>
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<tr>
<td>Day 1</td>
<td>12.03 ± 1.18</td>
<td>11.26 ± 1.08</td>
<td>60.88 ± 3.63</td>
<td>61.55 ± 2.71</td>
<td>61.00 ± 3.72</td>
<td>62.40 ± 2.62</td>
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<tr>
<td>Day 8</td>
<td>11.17 ± 1.17</td>
<td>12.20 ± 1.16</td>
<td>62.54 ± 3.11</td>
<td>25.13 ± 1.93</td>
<td>43.93 ± 2.97</td>
<td>40.61 ± 2.56</td>
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<td><strong>VLDL (mg/dL)</strong></td>
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<tr>
<td>Day 0</td>
<td>08.39 ± 0.42</td>
<td>08.20 ± 1.90</td>
<td>08.06 ± 0.89</td>
<td>08.35 ± 0.42</td>
<td>08.47 ± 0.45</td>
<td>08.30 ± 0.23</td>
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<tr>
<td>Day 1</td>
<td>08.45 ± 0.71</td>
<td>09.02 ± 0.71</td>
<td>17.43 ± 0.91</td>
<td>17.34 ± 0.32</td>
<td>17.59 ± 0.16</td>
<td>17.72 ± 1.00</td>
</tr>
<tr>
<td>Day 8</td>
<td>09.00 ± 0.86</td>
<td>09.48 ± 0.70</td>
<td>20.54 ± 0.98</td>
<td>9.47 ± 0.51</td>
<td>17.46 ± 0.79</td>
<td>14.08 ± 0.86</td>
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<tr>
<td><strong>A.I</strong></td>
<td>0.72 ± 0.11</td>
<td>0.83 ± 0.64</td>
<td>5.23 ± 0.28</td>
<td>1.30 ± 0.21</td>
<td>3.49 ± 0.21</td>
<td>2.67 ± 0.32</td>
</tr>
</tbody>
</table>

HC, hyperlipidemic control; ad statistically different (p<0.05; p<0.01) when compared with control rats; ab statistically different (p<0.05; p<0.001) when compared with hyperlipidemic rats; NS not significant, compared with control rats.

Discussion

*Cassia auriculata*, a traditionally well known medicinal plant has shown diverse biological activities and pharmacological functions, including reduction of blood glucose and serum lipids. It has long been used to treat diabetes mellitus, renal injury, and related antiperoxidative efficacy. Its leaf extracts are found to be effective against alcoholic liver injury, and cancer. Various parts of the plant have also been shown to act against leprosy, asthma, gout, rheumatism and diabetes. The plant is also used in the treatment against skin infections and possesses antipyretic, antulcer properties. Although phytochemical analysis of the plant has shown several active constituents with antioxidant activity, the studies on anti-hyperlipidemic activity of the plant are lacking.

In the present study, we evaluated the lipid-lowering activity of *Et-CAF* in TWR-induced hyperlipidemic rats. Hyperlipidemia was induced by single i.v injection of TWR (300 mg/kg b.wt). It is a well known that TWR (a non-ionic detergent) elevates total TC and TG in blood by altering the hepatic lipid metabolism. This TWR model has been used as a screening method for hypolipidemic agents and also for elucidating lipid metabolism.

Hyperlipidemic rats showed hyperlipidemia along with hyperglycemia. The increased risk of coronary artery disease by the lipoprotein abnormalities associated with diabetes mellitus. *Et-CAF* exhibited antihyperglycemic activity in a dose-dependent manner and the maximum effect was observed at 450 mg/kg b.wt. (Table 1). Anti-hyperglycemic effect of this plant has been reported in several studies and might be due to production of insulin by pancreatic β-cells in islets of langerhans or due to enhanced transport of blood glucose to peripheral tissue.

Cholesterol is an essential component of cell membrane and is a starting material for synthesis of bile acid, steroid hormones, and vitamin D. TG is a...
major component of chylomicron and VL DL, both of which are energy substrates in liver, peripheral tissue and muscles. Increased serum cholesterol and TG leads to the development of coronary atherosclerosis, which warrants for the development of novel and more effective anti-hyperlipidemic agents.

Serum TC, TG, LDL, VLDL and A.I level were decreased significantly (p<0.001) in Et-CAF treated hyperlipidemic rats (Group VII) accompanied with increased levels of HDL. Treatment with Et-CAF significantly (p<0.001) decreased the serum TC at a dose of 450 mg/kg b.wt. Most of the anti-hyperlipidemic drugs do not decrease TG level, however, interestingly we observed a significant reduction of TG level after treatment with Et-CAF. Among the lipoproteins, LDL plays a crucial role in the development of atherosclerotic lesions, progress of fatty streaks and ulcerated plaques. In the present study, LDL level decreased and serum HDL-cholesterol level increased after administration of Et-CAF. It is well known that increase in HDL is beneficial in hyperlipidemic conditions. HDL exerts an antiatherogenic effect by counteracting LDL oxidation and facilitating the translocation of cholesterol from peripheral tissue like arterial walls to liver for catabolism. The A.I (ratio of LDL to HDL) is commonly used as an index to evaluate the risk for atherosclerosis. Thus, combined reduction of TC, TG and LDL reduces the incidence of atherosclerosis. Treatment with Et-CAF fraction in normal rats did not exert any changes in the lipid profile. Our results clearly showed that Et-CAF possessed anti-atherogenic activity (Table 1).

Anthracene derivatives and dimeric proanthocyanidins are reported to exert antiatherogenic effect and decrease LDL/HDL ratio and LDL oxidation. In addition β-sitostanol reduces blood cholesterol either by interrupting with the recirculation of bile acids or reduces the absorption of cholesterol. Evidences have shown that flavonoids have diverse beneficial effects, including antioxidant activity, decreasing LDL, and increasing HDL. In addition, quick removal of cholesterol from peripheral tissue to liver for catabolism, followed by excretion helps in the prevention of coronary heart disease. Moreover, polysaccharides present in Et-CAF may exert beneficial effects. Our results suggest that Et-CAF extract exerts hypolipidemic effects by regulating the serum lipid profile. However, further studies are needed to characterize and elucidate the active principles and to understand the mechanism underlying the regulation of serum lipid levels by the flower extract.

Acknowledgement
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References

NOTES

33. Sasaki T, Matsu & Sonae A (1972) Rinsho Kagaku 1, 346-353