EFFECT OF *CASSIATORA* SEED EXTRACT ON LIPID PROFILE OF HYPERLIPIDAEMIC RABBITS

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Summary

Ethanolic extract (50%v/v) of *Cassia tora* seed extract has been investigated for the protective effects against experimentally induced hyperlipidaemia in rabbits. Hyperlipidaemia was induced in rabbits by feeding atherogenic diet containing coconut oil, hydrogenated vegetable oil and cholesterol for 90 days. Rabbits were divided into three groups, each group comprising of six rabbits. Group I served as normal control, Group II (hyperlipidaemic control) was fed with atherogenic diet and cholesterol (400 mg/kg bodyweight /day/rabbit), Group III was concurrently fed with *Cassia tora* seed extract (500 mg/kg bodyweight /day/rabbit) along with atherogenic diet and cholesterol.

Significant increase (P<0.05) in serum total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides was observed by feeding atherogenic diet and cholesterol to rabbits for 45 and 90 days. Simultaneous administration of *Cassia tora* seed extract significantly (P<0.05) prevented the rise in serum total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides level as compared to hyperlipidaemic control group.

Total cholesterol in liver (mg/ gm tissue) and heart (mg/ gm tissue) were increased significantly (p<0.05) in rabbits fed with atherogenic diet and cholesterol for 90 days. Concurrent administration of *Cassia tora* seed extract with atherogenic diet and cholesterol caused a significant decrease (P<0.05) in liver and heart in total cholesterol content. A significant increase (P<0.05) in cholesterol content of faeces (mg/ gm dry faeces) was observed in *Cassia tora* seed extract treated group as compared to hyperlipidaemic control group after 90 days.

**Keywords:** *Cassia tora*, cholesterol, anti-hyperlipidaemic, Lipid profile, Rabbits
Introduction

Cardiovascular diseases (CVD) are the most common cause of death worldwide. In Western countries where CVD is declining and is considered as a disease of the aged, 23% of CVD deaths occur below the age of 70; this compares with 52% of CVD deaths occurring among people under 70 years of age in India (1,2).

Coronary heart disease (CHD) is the major cause of CVD mortality. The incidence of CHD is on an increase in the Indian subcontinent (including India, Pakistan, Bangladesh, Sri Lanka and Nepal). There is high rate of CHD mortality, CHD manifests almost 10 year earlier on an average in this region compared with the rest of the world, resulting in a substantial number of CHD deaths occurring in the working age group (3).

There is strong link between the elevated blood cholesterol (particularly LDL cholesterol) and the occurrence of CHD (4-8). In spite of the availability of various anti-hyperlipidaemic agents, there is increase in the incidence of CHD. The current investigations in the field of cholesterol lowering drugs are directed towards finding an efficacious, safe and economic hypolipidaemic agent. The currently available synthetic drugs are effective but they frequently produce side effects, some of which are of serious in nature. They are also very expensive and have poor patient compliance, which limit their usefulness on a long-term basis. Thus, there is still considerable interest in the evaluation of new anti-hyperlipidaemic agents (synthetic and herbal). Hence, the present study was undertaken to explore the hypolipidaemic activity of the plant *Cassia tora*, which is available as common weed in India.

Materials & Methods

**Plant material:**
*Cassia tora* Linn. (Fabaceae), is a herbaceous annual occurring as a weed throughout the country in plains, ascending 1500 m in the Central Himalayas (9). Seed is hard, 1 cm long, 3-4 mm thick, oblong or rhombohedral, both ends appear as if cut off obliquely, greenish-brown to brownish-black, smooth and shiny; odourless; taste, bitter.

Seeds contain β-sitosterol, emodin, rubrofusarin, 1,8-dihydroxy anthraquinone while leaves contain myricyl alcohol, β-sitosterol, β-sitosterol glucoside, stigmasterol, mannitol and a ketonic compound, C16H30O (m.p. 79-80°) (10).

**Preparation of crude plant extract**

The dried seeds of *Cassia tora* were crushed to moderately coarse powder. The drug powder was packed in soxhlet apparatus and was defatted with petroleum ether for 72 hours. The defatted material was completely freed of petroleum ether and the marc was extracted with ethanol 50% v/v till exhausted completely. The ethanolic extract so obtained was freed of solvent under vacuum.

**Animal**

Adult albino rabbits weighing between 1.5 and 2.0 kg of both sexes were used for the study. The animals were kept in cages with proper aeration and lighting for 10-14 days. During this
period they were given normal controlled diet consisting of wheat, rizka and water *ad libitum* until they got acclimatized in 10-14 days.

**Experimental Design**

Rabbits were divided into three groups, each group comprising of six rabbits. They were numbered for identification (Table 1).

**Table 1: Group and treatment of rabbits**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Normal Control</td>
<td>6</td>
<td>Control, normal diet</td>
</tr>
<tr>
<td>II Hyperlipidaemic Control</td>
<td>6</td>
<td>Atherogenic diet + Cholesterol feeding (400 mg/kg body weight/day/rabbit)</td>
</tr>
<tr>
<td>III Cassia tora</td>
<td>6</td>
<td>Atherogenic diet + Cholesterol feeding (400 mg/kg body weight/day/rabbit) + <em>Cassia tora</em> (500 mg/kg body weight/day/rabbit)</td>
</tr>
</tbody>
</table>

**Feeding Methodology:**

Group I served as a normal control. Group II and III received atherogenic diet plus cholesterol powder 400 mg/kg body weight/day/rabbit in 5 ml oil mixture for 90 days. In addition, group III received drug, *Cassia tora* seed extract (50% ethanolic) at the dose 500 mg/kg body weight/day/rabbit.

**Ingredients of Atherogenic diet:**

- Rizka green: 60 gm
- Wheat flour: 50 gm
- Vegetable Ghee: 05 gm
- Coconut oil: 05 gm
- Salt: 04 gm
- Cholesterol: 0.4 gm/kg body weight/rabbit/day

The atherogenic diet consisted of supplementing the normal control diet with 400 mg cholesterol + 3ml hydrogenated vegetable oil + 2ml coconut oil per kg per day to each rabbit. The rabbits were subjected to this treatment for 90 days.

**Blood Collection and Serum Separation:**

Blood samples were taken initially, on 45th day and 90th day from each rabbit. No food was supplied for 12 hours before withdrawal of blood samples. Blood samples were taken from
the marginal ear vein of each rabbit (11). The serum was separated for lipid profile and other biochemical estimations.

**Serum biochemistry:**

Serum samples were analyzed for total cholesterol (12), HDL cholesterol (13) and triglycerides (14,15). LDL cholesterol was estimated indirectly after the estimation of total cholesterol, triglycerides, and HDL cholesterol by Friedwald’s formula (16).

**Tissue biochemistry:**

At the end of 90 days, the rabbits were anaesthetized and tissues like heart and liver were removed for respective tests. Total cholesterol in liver and heart was estimated by the method of Zlatkis (17).

**Faecal cholesterol**

Total faecal matter of normal control, hyperlipidaemic control, and *Cassia tora* groups were collected daily during last week of experiments and was dried at 40°C. After weighing, a known quantity of dry faecal matter, was soaked in methanol and chloroform (1:1) for 24 hours and then filtered. This process was repeated three times so as to extract the entire lipid content of faeces (18). The lipid extract so obtained was used for the estimation of total cholesterol.

**Statistical analysis:**

All values are expressed as mean ± standard error of the mean (S.E.M.). Data was analysed by using a one way analysis of variance (ANOVA) followed by least significant difference (L.S.D.) procedure of significance, where appropriate. A value of p < 0.05 was the criterion of significance.

**Results**

Table 2-5 shows the values of serum total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides levels in normal control, hyperlipidaemic control and *Cassia tora* treated groups respectively. Serum total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides levels increased significantly (p<0.05) on 45th and 90th day in hyperlipidaemic control group. Furthermore, the total cholesterol: HDL-cholesterol ratio was increased significantly (P<0.05) on 90th day (Table 6). The LDL-cholesterol: HDL-cholesterol ratio was also increased significantly (P<0.05) on 45th and 90th day (Table 7). Total cholesterol content in liver (mg/ gm tissue) and heart (mg/ gm tissue) were increased significantly (p<0.05) in hyperlipidaemic control group on 90th day (Table 8). A significant increase (p<0.05) in total cholesterol in faeces was observed in hyperlipidaemic control group as compared to normal control group (Table 8).

Concurrent administration of *Cassia tora* seed extract (500 mg / kg b.wt./day) with atherogenic diet and cholesterol (400 mg / kg b.wt./day) caused a significant decrease (p<0.05) in the levels of serum total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides when compared with hyperlipidaemic control group rabbits on 45th and 90th day (Table 2-5). The ratios of total-cholesterol: HDL-cholesterol declined significantly (p<0.05).
on 90th day (Table 6). The LDL-cholesterol: HDL-cholesterol were declined non-significantly (p=n.s.) as compared to hyperlipidaemic control rabbits (Table 7). Total cholesterol content in liver (mg/ gm tissue) and heart (mg/ gm tissue) were decreased significantly (p<0.05) in Cassia tora treated group (Table 8). A significant (p<0.05) increase in cholesterol content of faeces (mg/ gm dry faeces) was observed in Cassia tora treated group as compared to hyperlipidaemic control group after 90 days (Table 8).

Table 2: Effect of Cassia tora seed extract on total cholesterol level (mg/dL) of rabbits

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Total Cholesterol</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial value</td>
<td>45th day</td>
<td>90th day</td>
</tr>
<tr>
<td>Normal Control</td>
<td>66.03 ± 2.54</td>
<td>71.30 ± 4.73</td>
<td>75.51 ± 3.57</td>
</tr>
<tr>
<td>Hyperlipidaemic Control</td>
<td>73.14 ± 3.52</td>
<td>464.97±25.04* (+552.13%)</td>
<td>854.24±20.38* (+1031.29%)</td>
</tr>
<tr>
<td>Cassia tora</td>
<td>72.24±3.92</td>
<td>179.49±11.22* (-61.39%)</td>
<td>342.41±12.93* (-59.91%)</td>
</tr>
<tr>
<td>(Deviation %)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value represents Mean ± SEM. (n=6)

Statistical comparison:
Hyperlipidaemic Control vs. Normal Control, Cassia tora vs. Hyperlipidaemic Control
Significance levels: p < 0.05 = *, Not significant: p = n.s.
Values in parenthesis indicate percent increase (+) or decrease (-)

Table 3: Effect of Cassia tora seed extract on LDL cholesterol level (mg/dL) of rabbits

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>LDL Cholesterol</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial value</td>
<td>45th day</td>
<td>90th day</td>
</tr>
<tr>
<td>Normal Control</td>
<td>28.32±1.24</td>
<td>31.46±1.64</td>
<td>32.06±2.22</td>
</tr>
<tr>
<td>Hyperlipidaemic Control</td>
<td>32.42±1.08</td>
<td>302.11±17.70* (+860.29%)</td>
<td>614.69±33.05* (+1817.31%)</td>
</tr>
<tr>
<td>Cassia tora</td>
<td>29.12 ± 1.56</td>
<td>112.34 ± 9.25* (-62.81%)</td>
<td>236.27 ± 14.91* (-61.56 %)</td>
</tr>
</tbody>
</table>

Each value represents Mean ± SEM. (n=6)

Statistical comparison:
Hyperlipidaemic Control vs. Normal Control, Cassia tora vs. Hyperlipidaemic Control
Significance levels: p < 0.05 = *, Not significant: p = n.s.
Values in parenthesis indicate percent increase (+) or decrease (-)
Table 4: Effect of *Cassia tora* seed extract on HDL cholesterol level (mg/dL) of rabbits

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Initial level</th>
<th>45th day</th>
<th>90th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>26.29±1.97</td>
<td>26.46±2.49</td>
<td>28.25±1.96</td>
</tr>
<tr>
<td>Hyperlipidaemic Control</td>
<td>28.30±2.02</td>
<td>126.78±10.94* (+379.13%)</td>
<td>185.93±12.95* (+558.15%)</td>
</tr>
<tr>
<td><em>Cassia tora</em></td>
<td>28.38 ± 1.89</td>
<td>49.90 ± 6.52* (-60.64%)</td>
<td>85.99 ± 4.31* (-53.75%)</td>
</tr>
</tbody>
</table>

Each value represents Mean ± SEM. (n=6)

Statistical comparison:
- Hyperlipidaemic Control vs. Normal Control, *Cassia tora* vs. Hyperlipidaemic Control
- Significance levels: p < 0.05 = *, Not significant: p = n.s.
- Values in parenthesis indicate percent increase (+) or decrease (-)

Table 5: Effect of *Cassia tora* seed extract on triglycerides level (mg/dL) of rabbits

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Initial level</th>
<th>45th day</th>
<th>90th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>57.04±2.94</td>
<td>66.33±4.47</td>
<td>75.38±4.12</td>
</tr>
<tr>
<td>Hyperlipidaemic Control</td>
<td>61.87±4.05</td>
<td>180.15±14.86* (+171.59%)</td>
<td>268.07±19.79* (+255.62%)</td>
</tr>
<tr>
<td><em>Cassia tora</em></td>
<td>73.14 ± 4.63</td>
<td>86.21 ± 6.73* (-52.15 %)</td>
<td>100.82 ± 6.37* (-62.39 %)</td>
</tr>
</tbody>
</table>

Each value represents Mean ± SEM. (n=6)

Statistical comparison:
- Hyperlipidaemic Control vs. Normal Control, *Cassia tora* vs. Hyperlipidaemic Control
- Significance levels: p < 0.05 = *, Not significant: p = n.s.
- Values in parenthesis indicate percent increase (+) or decrease (-)
Table 6: Effect of *Cassia tora* seed extract on total cholesterol : HDL ratio in rabbits

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Total Cholesterol : HDL</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>45(^{th}) day</td>
</tr>
<tr>
<td>Normal Control</td>
<td></td>
<td>2.55 ± 0.11</td>
<td>2.86 ± 0.40</td>
</tr>
<tr>
<td>Hyperlipidaemic Control</td>
<td></td>
<td>2.69 ± 0.30</td>
<td>3.91 ± 0.57 (^{n.s.})</td>
</tr>
<tr>
<td><em>Cassia tora</em></td>
<td></td>
<td>2.56 ± 0.04</td>
<td>3.95 ± 0.56 (^{n.s.})</td>
</tr>
</tbody>
</table>

Each value represents Mean ± SEM. (n=6)

Statistical comparison:

Hyperlipidaemic Control vs. Normal Control, *Cassia tora* vs. Hyperlipidaemic Control

Significance levels: p < 0.05 = *, Not significant: p = n.s.

Values in parenthesis indicate percent increase (+) or decrease (-)

Table 7: Effect of *Cassia tora* seed extract on LDL : HDL ratio in rabbits

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>LDL : HDL Ratio</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>45(^{th}) day</td>
</tr>
<tr>
<td>Normal Control</td>
<td></td>
<td>1.09 ± 0.04</td>
<td>1.21 ± 0.05</td>
</tr>
<tr>
<td>Hyperlipidaemic Control</td>
<td></td>
<td>1.17 ± 0.08</td>
<td>2.48 ± 0.25*</td>
</tr>
<tr>
<td><em>Cassia tora</em></td>
<td></td>
<td>1.06 ± 0.11</td>
<td>2.40 ± 0.28 (^{n.s.})</td>
</tr>
</tbody>
</table>

Each value represents Mean ± SEM. (n=6)

Statistical comparison:

Hyperlipidaemic Control vs. Normal Control, *Cassia tora* vs. Hyperlipidaemic Control

Significance levels: p < 0.05 = *, Not significant: p = n.s.

Values in parenthesis indicate percent increase (+) or decrease (-)
Table 8: Effect of *Cassia tora* seed extract on liver, heart and fecal cholesterol content in rabbits.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Total cholesterol content liver (mg/gm tissue)</th>
<th>Total cholesterol content in heart (mg/gm tissue)</th>
<th>Total cholesterol in content faeces (mg/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>8.93 ± 0.31</td>
<td>4.70 ± 0.16</td>
<td>36.73 ± 2.81</td>
</tr>
<tr>
<td>Hyperlipidaemic Control</td>
<td>14.76 ± 0.63*</td>
<td>8.26 ± 0.55*</td>
<td>123.95 ± 11.44*</td>
</tr>
<tr>
<td><em>Cassia tora</em></td>
<td>10.72 ± 1.07*</td>
<td>6.90 ± 0.39*</td>
<td>168.67 ± 10.21*</td>
</tr>
</tbody>
</table>

Each value represents Mean ± SEM. (n=6)

Statistical comparison:

Hyperlipidaemic Control vs. Normal Control, *Cassia tora* vs. Hyperlipidaemic Control

Significance levels: $p < 0.05 = *$, Not significant: $p = n.s.$

Values in parenthesis indicate percent increase (+) or decrease (-)

**Discussion**

The aim of present study was to assess the hypolipidemic activity of *Cassia tora* seed extract. Coronary heart disease (CHD) resulting from progressive atherosclerosis, remains the most common cause of morbidity and mortality all over the world (19). In developing countries, the incidence of cardiovascular disease is increasing alarmingly. India is on the verge of a cardiovascular epidemic (20,21).

Various epidemiological, animal and clinical studies clearly indicate that hypercholesterolemia, elevated low-density lipoprotein (LDL) and low high-density lipoprotein (HDL) are unequivocally linked to increased risk for coronary heart disease morbidity and mortality (4-6, 22-25).

Cholesterol feeding in rabbits caused a significant increase in the circulating total-cholesterol, LDL-cholesterol, VLDL-cholesterol, and also in the ratios of total-cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol. These results are consistent with earlier reports which have clearly established a correlation between dietary lipids and serum lipid profile (26,27). A significant increased in the ratios of total-cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol indicates increased risk of atherosclerosis and coronary heart disease.

LDL is one of the most powerful and independent risk factor for CHD. It has been demonstrated that every 1% decrease in LDL-cholesterol is associated with an approximate 1% decrease in CHD-related mortality (28). Simultaneous administration of *Cassia tora* seed extract caused a significant decrease ($p < 0.05$) in serum total cholesterol and LDL-cholesterol suggesting beneficial effect on cholesterol metabolism and turnover.
Elevated serum triglyceride is considered as independent risk factor for cardiovascular disease (29). Evidence of an inverse relationship between triglycerides and HDL-C suggests that both should be considered in CHD risk estimation and as targets for intervention (30). A significant \((p<0.05)\) decline in the serum triglycerides level was observed in \textit{Cassia tora} extract treated rabbits supports the cardiovascular protective influence. In present study, the decrease in serum triglyceride level may be due to increased catabolism of triglyceride. The decline in triglyceride levels could be attributed to the fiber content of \textit{Cassia tora} seed extract. It is known that the dietary fibers decrease intestinal transit time for cholesterol and carbohydrate absorption leading to decreased hepatic lipogenesis and reduction in hepatic and serum triglyceride concentrations (31).

HDL are protective lipoproteins that decreases the risk of CHD thus, high levels of HDL are desirable. There is strong inverse correlation between HDL-cholesterol and CHD in both sexes. Framingham epidemiologic data suggest that a 1 mg/dL increase in HDL results in a 2\% to 3\% decrease in cardiac risk (32). In present study, a significant decrease \((p<0.05)\) in HDL-cholesterol was observed. However, the possibility that feeding cholesterol powder in such a high concentration (400 mg/kg body weight/day) to rabbits may have interfered in expressing the activity of the drug cannot be ruled out.

Total cholesterol content in liver and heart is a good indirect measure of atherosclerotic severity in cholesterol fed rabbits. Feeding of cholesterol rich diet to rabbits increases collagen biosynthesis and promotes accumulation of cholesterol in liver and heart. These results are supported by many earlier studies (33,34). The decrease in cholesterol level is suggestive of the beneficial role of plant products. Simultaneous administration of \textit{Cassia tora} seed extract caused a significant \((P<0.05)\) decline in the cholesterol contents of liver and heart indicating hypolipidemic effect.

The fractions of the seeds not extractable by alcohol have been shown to contain a considerable amount of soluble dietary fiber. The fiber has exhibited greater efficacy than guar gum and pectin in lowering blood lipids of rats fed high-cholesterol diets, with no detectable adverse effects (35). A significant \((P<0.05)\) increase in cholesterol content of faeces was observed in \textit{Cassia tora} seed extract treated group as compared to hyperlipidaemic control group after 90 days, indicating that the \textit{Cassia tora} seed extract is possibly acting by this mode.

\textbf{Conclusion}

On the basis of present study, it is concluded that \textit{Cassia tora} appears to be an effective plant material to provide hypolipidaemic effect. There was a significant increase in faecal cholesterol in \textit{Cassia tora} seed extract treated group. The lipid lowering effect may be due to increased faecal excretion.

\textbf{Acknowledgement}

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