A STUDY OF THE ANTIHYPERLIPIDAEMIC AND ANTIOXIDANT ACTIVITIES OF ETHANOLIC EXTRACTS OF FRUIT PULP OF *CASSIA FISTULA* IN ALBINO RATS

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ABSTRACT

The present study was designed to evaluate the antihyperlipidaemic and antioxidant activities of fruit pulp of *Cassia fistula*. Aqueous extract was obtained by infusion method and acute oral toxicity tests were performed according to recent OECD guidelines. Hyperlipidaemia was induced by feeding the rats with high fat diet consisting of coconut oil and vanaspati ghee, in a ratio of 2:3 v/v at a dose of 10 ml/Kg body weight. The extract was given at a dose of 500mg/kg body weight. For antihyperlipidaemic activity, total cholesterol, Triglycerides, HDL Cholesterol and LDL cholesterol was measured and for antioxidant activity MDA, CAT and SOD were measured using standard methods. The extract showed a significant decrease in total cholesterol, triglycerides, LDL and MDA in blood. On the other hand, HDL, CAT and SOD were increased significantly. The study demonstrates that the fruit pulp extract of *Cassia fistula* decreases blood lipid levels and lipid peroxidation.

KEYWORDS: Antihyperlipidaemic, antioxidant, *Cassia fistula*.

INTRODUCTION

Hyperlipidaemia is a major risk factor for coronary artery disease and is leading cause of death [1]. It is a highly predictive risk factor for atherosclerosis, coronary artery disease, and cerebral vascular disease. Clinical trials showed conclusively that lowering serum cholesterol reduces morbidity and mortality from coronary artery disease in patients with established coronary artery disease and also reduces new coronary artery disease events and mortality in patients without established coronary artery disease[2]. 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 or cerebrovascular disease [3]. A significant number of patients with hypercholesterolaemia do not achieve adequate cholesterol reduction with statins and other lipid lowering drugs[4].
Currently available drugs have been associated with number of side effects. The consumption of synthetic drugs leads to hyperuricaemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function [3].

*Cassia fistula* Linn (family- caesalpinaceae) is commonly known as the golden shower Indian Laburnum[5]. Known as Sonaru in Assamese[6] and Amaltas in Hindi, it is native to India, the Amazon, Sri Lanka and is extensively diffused in various countries[7]. It is a tropical ornamental tree with trunks consisting of hard reddish wood, growing up to 40 feet tall. It has showy racemes, up to 2” long, with bright, yellow, fragrant flowers. The fruit are dark brown cylindrical pods, also 2” long, which also hold the flattish, brown seeds (up-to 100 in one pod) these seeds are in pods each containing a single seed [5].

Active constituents extracted from *Cassia fistula* includes biflavonoids, triflavonoids and proanthocyanidins like catechin, epicatechin, procyanidin B-2, and epiafzelechin. Besides phenolics and their derivatives, a certain amount of alkaloids, triterpenes and diterpenes are also found to be present [8].

*Cassia fistula* has been used extensively in the folklore medicine for the treatment of a variety of diseases [9]. Its extract showed antibacterial, antifungal, anti-tumor and hepatoprotective activities[10].

In Ayurvedic system of medicine, it is useful in treatment of biliousness, ulcers, erysipelas, vomiting, vaginal complains, fever, inflammations and leprosy. In Unani system of medicine, it is useful in piles, nose disease, gonorrhea and to lessen inflammation. The aerial root is styptic, useful in syphilis, biliousness, dysentery, inflammation of liver etc. Medicinally it has been various pharmacological activities like antimicrobial, antifungal, antipyretic, analgesic, larvicidal, anti-inflammatory, anti-oxidant, anti-tumour, hepatoprotective and hypoglycaemic activities[5].

Nowadays there has been a revival of interest in plant derived drugs and there is a good scope for using herbal medicines possessing antihyperlipidaemic and antioxidant property with adequate safety and efficacy as an alternative to synthetic drugs.

The antihyperlipidaemic and antioxidant activities of this plant have not been scientifically evaluated so far. Hence, the present study was undertaken to evaluate the antihyperlipidaemic and antioxidant activities of this plant on albino rats fed with high fat diet.

**MATERIAL AND METHODS**

*Plant material:*
Cassia fistula fruits were collected from Assam Medical College and Hospital campus, Dibrugarh in the month of April to August, 2012 and plant materials were authenticated by Dr. L.R. Saikia, Reader, Department of Life Sciences, Dibrugarh University, Dibrugarh, Assam.

**Preparation of the extract:**

Aqueous extract of the pulp *Cassia fistula* (AECF) was prepared by infusion method. The pulp was separated from the hard outer shell and seeds of the fruits and dried at room temperature separately. It is then mixed with water, shaken and stirred at regular intervals to obtain a homogeneous mixture and then allowed to stand for 15-20 minutes. Finally the mixture is filtered through 4 layers of muslin cloth and the filtrate obtained is collected in Petri dishes and allowed to dry. The dried extract is then collected in sterile bottles and stored. Fresh extract was prepared each day for the required period of treatment [11].

**Animals:**

The study was carried out in healthy albino rats of Wistar strain (*Rattus norvegicus*) of either sex of body weight between 150-200 g. Animals were procured from The Central Animal House Assam Medical College and Hospital, Dibrugarh. They were given standard animal diet consisting of Bengal gram, wheat, maize and carrot in sufficient quantity and water was given *ad libitum* during the entire period of the experiment. The animals were housed in standard conditions with natural light and dark cycle and adequate ventilation. The study was permitted by the Institutional Animal Ethics Committee (IAEC), Assam Medical College, Dibrugarh, Assam and conducted in accordance to the CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals) guidelines.

**Sample collection:**

Under all aseptic and antiseptic measure and under ether inhalation anaesthesia, blood samples were collected from the retro orbital sinus with the help of a capillary tube. The capillary tube was inserted at the medial canthus into the retro-orbital plexus with gentle rotation so that blood flowed into it by capillary action[12].

**Acute Toxicity Tests:**

The acute toxicity of Extract of fruit pulp of *Cassia fistula* was determined on female albino rats of Wistar Strain weighing 150-200g. After administration with different doses of the extract, the mortality with each dose was noted as per OECD (Organization for Economic Cooperation and Development, 2006) guidelines 425[13].

As the extract was found to be safe at 2000mg, one fourth of the dose of the extract of the plant from the test was decided to be considered for the study.
**Induction of hyperlipidaemia:**

A high fat diet, consisting of coconut oil and vanaspati ghee, in a ratio of 2:3 v/v at a dose of 10 ml/Kg body weight, was fed to the animals, per orally, daily, in addition to normal diet [14] for a period of 8 weeks[15].

**Experimental design:**

A total of 20 animals of either sex weighing 150-200g were divided into four groups of five animals each and were treated as follows:

Group I: (Normal Control) Received normal diet and normal saline at a dose of 10 ml/Kg/day.

Group II: (Hyperlipidaemic Control) Received high fat diet at a dose of 10 ml/Kg/day.

Group III: (Hyperlipidaemic Test): Received high fat diet at a dose of 10 ml/Kg/day and EECF at a dose of 500 mg/Kg/day.

Group IV: (Hyperlipidaemic Standard): Received high fat diet at a dose of 10 ml/Kg/day and Simvastatin at a dose of 1.8 mg/Kg/day.

The drugs were administered once daily, orally for a period of 8 weeks by intragastric feeding tube. At the end of 8 weeks, all the animals were kept fasting for 18 hours and blood samples were collected from each rat for assessing the various parameters of lipid profile and antioxidant status.

**Estimation of the biochemical parameters:**

Serum was separated out from the blood after clotting and centrifuged for 5 minutes at 3000 rpm. The serum thus obtained was used for biochemical estimations.

The total serum cholesterol estimation was done by the method described by Allain CC *et al* (1974)[16] using Qualigens-Diagnostics Cholesterol Kit manufactured by Sigma Diagnostics (India) Pvt. Ltd., Baroda.

Triglycerides were measured by enzyme colorimetric method as described Fossati P *et al* (1982)[17] using Qualigens-Diagnostics Triglyceride Reagent GPO manufactured by Sigma Diagnostics (India) Pvt. Ltd., Baroda.

HDL–cholesterol was assayed by the method of Izzo C *et al* (1981)[18] using Qualigens-Diagnostics HDL-cholesterol Kit manufactured by Sigma Diagnostics (India) Pvt. Ltd., Baroda.

LDL–cholesterol was measured by using the formula of Friedewald WT *et al* (1972)[19].
The Atherogenic Index (AI) and Percent Protection was calculated using the following formulae[20]

\[
\text{Atherogenic Index (AI)} = \frac{\text{Total serum Cholesterol}}{\text{HDL Cholesterol}}
\]

\[
\text{Percent protection} = \frac{\text{AI of hyperlipidaemic control} - \text{AI of treated group}}{\text{AI of hyperlipidaemic control}} \times 100
\]

**Estimation of antioxidant status:**
Malondialdehyde (MDA) was measured in plasma while Catalase (CAT) and Superoxide Dismutase (SOD) was measured in the erythrocytes. MDA level was estimated by the method described by Satoh K[21], CAT level was estimated by the method described by Beers and Sizer[22] and SOD level was estimated by the method described by Kakkar et al[23].

**Statistical analysis:**
Statistical Analysis was done using the software Graph pad Prism version 5. All the values were expressed as mean ± SEM. The results were analyzed for statistical significance by using one way ANOVA, followed by the Dunnett’s test. P values which were <0.05 were considered as significant.

**RESULTS**

**Result of Acute Toxicity Test.**
There was no mortality recorded for the extracts of the leaves of *Cassia fistula* among the rats upt0 the maximum dose of 2000 mg/Kg when administered orally. Hence, the LD₅₀ can be said to be above 2000mg/kg.

**Changes in blood lipid profile (Table 1):**
At the end of the experiment the hyperlipidaemic control group showed a significant (p<0.05) elevation of total cholesterol, triglycerides and LDL cholesterol together with a significant (p<0.05) decrease in HDL- cholesterol when compared with the normal control group. In the hyperlipidaemic test group as well as in the hyperlipidaemic standard group there was a significant (p<0.05) reduction in total cholesterol, triglycerides and LDL cholesterol. HDL cholesterol was also increased significantly (p<0.05) in both the groups. This indicates that EECF
is effective in reducing total cholesterol, triglycerides and LDL cholesterol and increasing HDL cholesterol.

Table 1. Changes in Serum Lipid Profile:

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Total Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>Atherogenic Index</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>72.44 ± 1.98</td>
<td>68.28 ± 1.73</td>
<td>23.91 ± 1.77</td>
<td>36.07 ± 1.57</td>
<td>3.03</td>
<td>-</td>
</tr>
<tr>
<td>Hyperlipidaemic</td>
<td>239.38 ± 2.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>217.10 ± 1.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.44 ± 2.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>184.52 ± 1.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.92</td>
<td>-</td>
</tr>
<tr>
<td>Hyperlipidaemic</td>
<td>105.88 ± 52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80.00 ± 83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>46.43 ± 55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.45 ± 22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.28</td>
<td>89.10</td>
</tr>
<tr>
<td>Hyperlipidaemic</td>
<td>75.36 ± 2.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>59.82 ± 1.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.71 ± 1.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.69 ± 1.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.17</td>
<td>89.63</td>
</tr>
</tbody>
</table>

Values are expressed as MEAN ± SEM; (n=5).
One Way ANOVA followed by Dunnett’s multiple comparison tests is done.
<sup>a</sup>p<0.05 when compared to the normal control group.
<sup>b</sup>p<0.05 when compared to the hyperlipidaemic control group.

Changes in lipid peroxidation and blood antioxidant levels (Table 2):

At the end of the experiment there was a significant increase (p<0.05) in serum MDA levels in the hyperlipidaemic control group when compared to the normal control group. While blood CAT and SOD levels are significantly (p<0.05) decreased in the hyperlipidaemic control group when compared to the normal control group.

There was a significant (p<0.05) decrease in serum MDA levels in the hyperlipidaemic test group and Hyperlipidaemic standard group when compared to hyperlipidaemic control group. Blood CAT and SOD levels were increased significantly in hyperlipidaemic test group and hyperlipidaemic standard group when compared to hyperlipidaemic control group.

This indicates that EECF decreases lipid peroxidation and increases the antioxidant enzymes in blood.
Table 2. Changes in lipid peroxidation and blood antioxidant levels:

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>MDA (nmol/mL blood)</th>
<th>CAT (u/mg protein)</th>
<th>SOD (u/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>1.52±0.72</td>
<td>396.2±0.86</td>
<td>7.2±0.21</td>
</tr>
<tr>
<td>Hyperlipidaemic Control</td>
<td>4.34±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>143.8±0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.1±0.61&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hyperlipidaemic Test (EECF)</td>
<td>2.05±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>257.14±33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.83±0.74&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hyperlipidaemic Standard</td>
<td>1.82±0.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>327.8±3.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.9±0.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as MEAN ± SEM; (n=5).
One Way ANOVA followed by Dunnett’s multiple comparison tests is done.
<sup>a</sup>p<0.05 when compared to the normal control group.
<sup>b</sup>p<0.05 when compared to the hyperlipidaemic control group.

DISCUSSION

The present study was undertaken to evaluate the antihyperlipidaemic and antioxidant activities of *Cassia fistula*.

In hyperlipidaemic control group the total cholesterol, triglycerides and LDL cholesterol levels in blood are significantly (p<0.05) increased together with a decrease in HDL cholesterol level. Elevated levels of all lipoproteins except the HDL are associated with increased risk of atherosclerosis[24].

The hyperlipidaemic control group fed with HFD and EECF showed significant (p<0.05) decreases in the total cholesterol, triglyceride and LDL Cholesterol which is almost comparable to the Standard group fed with HFD and simvastatin.

On the other hand HDL cholesterol level is significantly (p<0.05) increased in the hyperlipidaemic test group as well as in the standard group. HDL cholesterol is referred to as the ‘good’ cholesterol because, HDL is involved in transport of cholesterol from peripheral tissues to liver and thereby reducing the amount stored in the tissue and the possibility of developing atherosclerotic plaques[24].

Atherogenic index (AI) calculated as the ratio between total cholesterol and HDL cholesterol is used as a marker to assess the susceptibility of atherogenesis[25].

It is an important indicator of CHD risks at both high and low serum cholesterol level[26]. When compared with the hyperlipidaemic control group there is a significant decrease in atherogenic index in the group fed with EECF (2.28), which is almost comparable to the standard (2.17).

The persistence of hypercholesterolemic state causes enhanced oxidative stress, leading to the development of atherosclerosis, coronary artery disease (CAD) and other complications of
obesity [27]. Hypercholesterolemia increases the levels of the lipid peroxidation product Malondialdehyde. The increase in Malondialdehyde (MDA) levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals [28]. Oxygen free radicals have been implicated in the development of hyperlipidaemic atherosclerosis. SOD and Catalase mimics effective for detoxification of Oxygen free radical and Hydrogen peroxide among these SOD converts the highly toxic superoxide ($O_2^-$) to less toxic hydrogen peroxide and $O_2$ this is the first line of defense to protect the cells from the injurious effects of superoxide. Hydrogen peroxide produced by superoxide dismutase further metabolized by the catalase [29]. The hyperlipidaemic test group fed with HFD showed a significant increase in MDA and decrease in CAT and SOD, indicating increased lipid peroxidation and decreased antioxidant enzyme levels. The hyperlipidaemic test group which was fed with EECF showed a significant decrease in MDA and increase in CAT and SOD.

Several naturally occurring active components found in *Cassia fistula* may be the reason behind its antihyperlipidaemic and antioxidant activity. Polyphenols and flavonoids are reported to be present in *Cassia fistula* fruit pods are the most likely constituents behind these activity. The present study suggests that *Cassia fistula* has antihyperlipidaemic and antioxidant activity. Further pharmacological and biochemical investigations are needed to determine the precise mechanism and site of action and the active constituents involved, so that these herbal drugs can be used as a safer alternatives to synthetic drugs.

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