Chapter 6

*In-vivo* evaluation of the Anti-hyperlipidemic and Anti-atherosclerotic activities of the selected plant extracts *Boswellia ovalifoliolata, Commiphora caudata, Saccharum spontaneum* and *Garcinia mangostana*.

6.1 Experimental models used in Anti-hyperlipidemic studies

6.2 Results and Discussion
Chapter 6

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6.1 Experimental models used in Anti-hyperlipidemic studies

Development of natural products as antihyperlipidemic agents require suitable models and identification of proper methods for inducing hyperlipidemia. The *in-vivo* models available are all based on the measurements of certain biochemical parameters followed by histopathological observations of the aorta and liver. Victoria *et al.* reported a transgenic atherosclerosis-polygenic hypertension model in dahil salt-sensitive hypertensive rats that over express the human CETP. These rats fed regular rat chow showed age-dependent severe combined hyperlipidemia, atherosclerotic lesions, myocardial infarctions and decreased survival [192]. Certain strains of mice, such as C57BL/6, develop hyperlipidemia as a result of the accumulation of cholesterol-rich remnant particles in plasma and aortic atherosclerosis when fed a high atherogenic diet (AD). ApoE deficient mice also develop hyperlipidemia as a result of the accumulation of remnant lipoproteins that float mainly as VLDL and LDL and massive atherosclerosis even when they are fed a diet of regular chow [193]. Other transgenic mice models for dyslipidemia and atherosclerosis include mutant ApoE transgenic, LDL-receptor deficient and human ApoB transgenic, Apolipoprotein AI (ApoAI) deficient and ApoAI transgenic [194].

In most of the previous studies, dietary obesity has been produced by giving a high fat diet to Sprague-Dawley (SD) rats. Pu-ehr tea aqueous extracts were shown to
lower atherosclerotic risk factors using male SD rats as a hyperlipidemic model [195]. Effect of single intravenous injection of plasmid deoxyribonucleic acid (DNA) encoding human paraoxonase-1 inhibiting hyperlipidemia was shown in hyperlipidemic model of male SD rats, weighing 200±20 g [196].

The inhibitory effect of the methonolic extract of *Pleurospermum kamtschaticum* and its fractions were tested in hyperlipidemic and hypercholesterolemic SD rats using high cholesterol diet as one of the model [197]. Rajasree et al. [198] proved antiatherogenic and antiperoxidative effects of garlic and soy proteins in alcohol fed male SD rats which showed significant increase in liver weight, serum and tissue cholesterol, serum triacylglycerol and phospholipids.

**Experimental models used for evaluation of Hypolipidemic activity were listed below:**

1. High cholesterol diet induced method (HCD.)
2. High fructose diet induced method (HFrD.)
3. Triton induced hyperlipidemic method (TI.)
4. Streptozotocin induced diabetic method (SI.)
5. Alloxan induced diabetic method (AII.)
6. Tylaxapol induced hyperlipidemic method (TyI.)
7. High fat diet induced hyperlipidemic method (HFD.)
8. Hydrocortisone induced hyperlipidemic method (HYI.)
9. Atherogenic diet induced method (AtDI.)
10. Normocholesterolemic method (NC.)
It has been reported that SD rats kept at room temperature do have a higher growth rate and food conversion compared to Wistar rats due to their endocrinal differences [199]. In a study Gao et al observed that serum TG levels in SD and Wistar rats were found to be 194.4% and 86.2% respectively after 5 weeks of feeding AD composing of 1% cholesterol, 10% lard, 10% yolk powder and 79% normal diet. TC in serum also was found to be increased with 76.8% in SD and 48.3% in Wistar rats, showing that SD rats are better model for hyperlipidemia than Wistar rats [200]. Further it has also been proved that female hormones like estrogen have major effects on hepatic cholesterol metabolism. Therefore male SD rats were preferred for the present study [201].

**Methods used in the present work:**

In the present work, antihyperlipidemic activity of selected plant extracts was tested in atherogenic diet induced rats by measuring the serum lipid profiles and physical parameters like body weight. The hepatotoxicity induced by high fat diet and the effect of the extracts was studied by measuring the levels of hepatic enzymes. An increase in the levels of biochemical parameters is a sensitive index of hepatic damage.

**Atherogenic diet (AD)**

Intake of dietary fat has been shown to be important in the development of obesity in humans, and experimental studies have shown that a high-fat diet may be associated with increased oxidative stress in mammals [26]. Many studies have demonstrated that normal rats become obese when offered a high-fat diet ad libitum and it has been suggested that high levels of fat in the diet will increase energy intake, body fat content, and insulin resistance [202]. Xu *et al.* developed a liquid chromatography mass
spectrometry method to detect diosgenin in the plasma of normal and hyperlipidemic male SD rats which were fed high fat-diet containing normal pulverized food (92.8%), lard (5%), cholesterol (2%) and sodium cholate (0.2%) for 4 weeks [26] to show the therapeutic action of human paroxonase gene (pcDNA/PON1) which is a HDL-associated antioxidant enzyme. SD rats were fed rodent chow supplemented with 1% cholesterol, 0.35% sodium cholate and 5% lard for 25 days to produce hyperlipidemic model [196].

Hypolipidemic and antioxidant properties of tocotrienol rich fraction isolated from rice bran oil was studied in hyperlipidemic rats fed atherogenic diet containing 5% hydrogenated fat, 0.5% cholic acid and 1% cholesterol mixed thoroughly with the powdered chow for three weeks [203]. Antiatherosclerotic activity of ibuprofen was performed on hypercholesterolemic animals fed with 2% cholesterol, 1% sodium cholate and 2% coconut oil for 6 days in SD rats where animals showed significant increase in atherogenic index (AI), TC, TG’s, VLDL and LDL levels while decrease in HDL levels as compared to normal animals [204].

Materials

Plant material:

Alcoholic extracts of the selected plants were tested namely, *Boswellia ovalifoliolata* (gum), *Commiphora caudata* (leaves), *Saccharum spontaneum* (whole plant) and *Garcinia mangostana* (fruit pericarp). The preparation of alcoholic extracts of the selected plants was described in chapter 3.
Chemicals and Reagents used

Atorvastatin - Cipla Ltd.
Cholesterol - Spectrochem Pvt Ltd, Mumbai
Choline chloride - SD Fine Chemicals
Sodium chloride - Merck
Sodium carboxy methyl cellulose - Merck
Diethyl ether - SD Fine Chemicals
Formalin - Qualigens
Trichloroacetic acid - SD Fine Chemicals
Thiobarbituric acid - SD Fine Chemicals

Serum HDL diagnostic kit, serum TC diagnostic kit and Serum TG diagnostic kit used in the study were purchased from Swemed Diagnostics. Other chemicals and reagents were of analytical grade.

Equipments and Instruments

The following instruments were used throughout the study.

UV Double beam Spectrophotometer - Shimadzu
Autoanalyzer - Stat Fax 3300
Tissue Homogeniser - Remi Instruments Ltd
Research centrifuge - Remi Instruments Ltd

Preparation of Drugs

3 gm and 6 gm of the test extracts were weighed and suspended in 1% CMC. Each mL of the suspension contained 100 and 200 mg/kg bw respectively. Atorvastatin was dissolved in 1% CMC [205].
**Experimental animals**

Rats were chosen as it accumulates cholesterol in both the liver and blood more readily than the mouse [83]. Also studies have been reported where SD rats have shown higher levels of TC and TG levels in serum in comparison to Wistar rats when fed with AD diet which show that SD rats are better model than Wistar rats for hyperlipidemic studies [199-200]. Adult male SD rats weighing 180-200 gm were used for the experimental study (National Institute of Nutrition, Hyderabad, India). They were housed, three per polypropylene cage under standard laboratory conditions at room temperature 25°C ± 2°C with 12 h light/dark cycle. They had free access to standard pellet diet and water ad libitum except during experimentation.

**Ethical Clearance:** The animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study protocol was approved by institutional Animal Ethics Committee (IAEC).

**Methods**

**Standardization of AD induced hyperlipidemia:**

AD composed of 2% cholesterol, 1% choline chloride and 2% lard [204] was given to one group and another group was fed with normal pellet diet for 30 days. Blood samples were collected on every seventh day till 30th day. The duration for feeding animals to induce hyperlipidemia for the actual study was decided based on this pilot study, where significant elevated serum TG and TC values were obtained on 21st day compared to normal group. Hence the actual study was later conducted for 21 days.
Standardization groups (n=3)

Group 1: Normal pellet diet

Group 2: AD

Experimental induction of atherosclerosis

In rats, hyperlipidemia was induced by daily administration of 2% Cholesterol, 1% choline chloride and 2% lard over a period of 21 days. The cholesterol diet was given at approximately the same time every day.

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. 2% Cholesterol, 1% Choline Chloride and 2% Lard too 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 feed pellets and then mixing with water without the other excipients. The preparation of feed was done once in three days for all the animals.

Experimental design (n=6 animals/group)

Rats were treated with vehicle, standard or alcoholic extracts of the selected plants for 21 days as mentioned.

Group 1: Control group treated with vehicle (1 % CMC)

Group 2: AD Control

Group 3: AD + Atorvastatin at 10 mg/kg.
Group 4: AD+ EBOG (100 mg/kg bw)
Group 5: AD+ EBOG (200 mg/kg bw)
Group 6: AD+ ECCL (100 mg/kg bw)
Group 7: AD+ ECCL (200 mg/kg bw)
Group 8: AD+ ESSW (100 mg/kg bw)
Group 9: AD+ ESSW (200 mg/kg bw)
Group 10: AD+ EGMP (100 mg/kg bw)
Group 11: AD+ EGMP (200 mg/kg bw)

On day 22nd, animals were anaesthetized with diethyl ether and blood was collected by retro orbital puncture. The blood was allowed to clot for 30 min at room temperature and then was subjected to centrifugation at 2000 rpm for 15 minutes to obtain serum [26]. The resulting upper serum layer was collected in clean, dry and labeled micro-centrifuge tubes. This serum was analysed for serum TGs, TC and HDL.

**Biochemical estimations**

**Estimation of serum triglycerides:**

**Method:** Quinoneimine dye

**Principle:** Glycerol released from TGs after hydrolysis by lipoprotein lipase is transformed by glycerol kinase into glycerol-3-phosphate which is oxidized into dihydroxyacetone phosphate and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide oxidizes the chromogen to form purple Quinoneimine dye [206].

\[
\text{TG} + 3\text{H}_2\text{O} \xrightarrow{\text{Lipase}} \text{Glycerol} + 3\text{FFA}
\]
Reagents:

1. TG Reagent: Ready to use
2. Standard: 200 mg/dl

Reaction Parameters:

Wavelength: 505 nm
Optical length: 1 cm
Temperature: 37°C
Measurement: Against reagent blank

Procedure:

Table 6.1: TG Working Procedure

<table>
<thead>
<tr>
<th>Pipetted into cuvettes</th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>500 μL</td>
<td>500 μL</td>
<td>500 μL</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>5 μL</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>5 μL</td>
</tr>
</tbody>
</table>

The reaction mixtures were mixed well and incubated for 10 min at 37°C. The absorbance of sample and standard were measured against reagent blank at 505 nm within 30 minutes.
**Calculation:**

\[
\text{Serum TG (mg/dl)} = \frac{\text{Absorbance of test} \times \text{Concentration of Standard}}{\text{Absorbance of Standard}}
\]

**Estimation of serum total cholesterol:**

**Method:** Quinoneimine dye [207]

**Principle:** The enzyme, cholesterol esterase catalyzed hydrolysis of cholesterol esters to free cholesterol and fatty acid molecules. Then free cholesterol gets oxidized in the presence of cholesterol to form cholesten-3-one and hydrogen peroxide. Liberated hydrogen peroxide reacts with phenol and 4-Aminoantipyrene (AAP) in presence of peroxidase to form red coloured Quinoneimine complex, the intensity of which was measured at 505 nm.

\[
\begin{align*}
\text{Cholesterol ester + H}_2\text{O} & \xrightarrow{\text{Cholesterol ester hydrolase}} \text{Cholesterol + Fatty acid} \\
\text{Cholesterol + O}_2 & \xrightarrow{\text{Cholesterol oxidase}} \text{Cholesten-3-one + H}_2\text{O}_2 \\
2\text{H}_2\text{O}_2 + \text{Phenol + 4AAP} & \xrightarrow{\text{Peroxidase}} 4\text{H}_2\text{O} + \text{Quinone imine dye}
\end{align*}
\]

**Reagents:**

1. TC Reagent: Ready to use
2. Standard: 200mg/dl

**Reaction Parameters:**

Wavelength: 505 nm
Optical length: 1 cm
Temperature: 37°C
Measurement: Against reagent blank

Procedure:

Table 6.2: TC Working Procedure

<table>
<thead>
<tr>
<th>Pipetted into cuvettes</th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>500 µL</td>
<td>500 µL</td>
<td>500 µL</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>5 µL</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>5 µL</td>
</tr>
</tbody>
</table>

The reaction mixtures were mixed well and incubated for 10 min at 37°C. The absorbance of sample and standard were measured against reagent blank at 505 nm within 30 minutes.

Calculation:

\[
\text{Serum TG (mg/dl)} = \frac{\text{Absorbance of test} \times \text{Concentration of Standard}}{\text{Absorbance of Standard}}
\]

Estimation of serum high-density lipoprotein (HDL) cholesterol of the selected plant extracts

Method: Precipitation with phosphotungstic acid and Magnesium chloride [208]

Principle: HDL Cholesterol is measured in the supernatant after the precipitation of the lipoproteins including chylomicrons, VLDL, LDL, IDL directly from serum polyanions like phosphotungstic acid and along with Magnesium chloride are added to an aliquot of serum an immediate heavy precipitation is formed. The precipitate then is sedimented by centrifugation and HDL Cholesterol is measured in the clear supernatant, which is estimated by enzymatic method as described earlier in estimation of TC.
Reagents:

1. HDL Reagent: Ready to use
2. Cholesterol reagent: Ready to use
3. Standard: 25 mg/dl

Reaction parameters:

Wavelength: 505 nm
Optical length: 1 cm
Temperature: 37°C
Measurement: Against reagent blank

Procedure:

Step 1:

Table 6.3(a): HDL Working Procedure

<table>
<thead>
<tr>
<th>Pipetted into centrifuge tubes</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL Reagent</td>
<td>200 μL</td>
</tr>
<tr>
<td>Sample</td>
<td>200 μL</td>
</tr>
</tbody>
</table>

Mix well, leave to stand for 10 minutes at 37°C and centrifuge for 15 minutes at 4000 rpm. The clear supernatant was separated and immediately used to determine the cholesterol content as follows.

Step 2:

Table 6.3(b): HDL Working Procedure

<table>
<thead>
<tr>
<th>Pipetted into cuvettes</th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Redistilled water</td>
<td>50 μL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol Reagent</td>
<td>500 μL</td>
<td>500 μL</td>
<td>500 μL</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>50 μL</td>
<td>-</td>
</tr>
<tr>
<td>Supernatant from step 3</td>
<td>-</td>
<td>-</td>
<td>50 \</td>
</tr>
</tbody>
</table>
The reaction mixtures were mixed well and incubated for 5 min at 37°C. The absorbance of sample and standard were measured against reagent blank at 505 nm within 60 minutes.

**Calculation:**

\[
\text{Serum HDL Cholesterol (mg/dl)} = \frac{\text{Absorbance of test} \times \text{Concentration of Standard}}{\text{Absorbance of Standard}}
\]

**Atherogenic index [209]:**

**Calculation:**

\[
\text{AI} = \frac{\text{LDL} + \text{VLDL}}{\text{HDL}}
\]

\[
\text{LDL-C (mg/dl)} = \frac{\text{TC} - (\text{HDL-C} + \text{Triglycerides})}{5}
\]

\[
\text{VLDL-C (mg/dl)} = \frac{\text{Triglycerides}}{5}
\]

**Body weight:** The body weight of the animals of all the groups was recorded on day 0 and day 21. The percentage change in body weight was calculated and presented in Table 6.7.

**Liver Parameters**

**Method of collection of liver**

The rats were sacrificed by cervical dislocation. Livers were perfused with normal saline to remove blood and then dried and weighed, and homogenized for estimation of LPO.
**Preparation of liver homogenate**

Liver homogenate (10%) was prepared using 0.9% saline by homogenizing with tissue homogenizer. This homogenate was centrifuged at 7000 rpm for 15 min. The supernatant was collected and used for the estimation of LPO.

**Estimation of lipid peroxidation (LPO)**

**Principle:**

Oxidative stress is associated with peroxidation of cellular lipids, which is determined by measurement of thiobarbituric acid reacting substance (TBARS). The concentration of LPO products may reflect the degree of oxidative stress. The increased level of TBARS results in increase of oxygen free radicals, which attacks the polyunsaturated fatty acids in cell membranes and cause LPO. The malondialdehyde (MDA) content, a measure of LPO was assayed in the form of TBARS.

**Procedure**

One mL of suspension medium was taken from 10% of tissue homogenate. To this 1 mL of 30% TCA was added followed by 1 mL of 0.8 % TBA reagent. The tubes were covered with aluminum foil and kept in shaking water bath for 30 minutes at 80°C. After 30 minutes tubes were taken out and kept in ice cold water bath. These were then centrifuged at 3000 rpm for 15 minutes. The absorbance was read at 535 nm at room temperature against appropriate blank. Blank consists of 1 mL distilled water, 1 mL of 30% TCA and 1 mL of 0.8% TBA.

The content of MDA expressed as n moles formed per milligram of protein in tissue was calculated using the formula:

\[
\text{Conc.} = A \times \frac{V}{E} \times P
\]
Where, $A =$ Absorbance

$V =$ Volume of solution

$E =$ Extinction Coefficient ($1.56 \times 10^{-6} \times m^{-1} \times cm^{-1}$)

$P =$ Protein content of tissue calculated as mg of protein per gram of tissue.

**Determination of Aspartate aminotransferase (AST or SGOT), Alanine aminotransferase (ALT or SGPT) and Alanine phosphatase (ALP) levels**

The blood sample was centrifuged at 5000 rpm for 10 min and the plasma was collected in micro-centrifuge tubes. Plasma samples were stored at −20°C for the determination of ALT, AST and ALP levels. These marker enzymes were measured using commercially available kits (ALT kit, AST kit, ALP kit, Merck, Mumbai, India) according to manufacturer’s instructions (5 μl of plasma and 45 μl of specified reagent from AST or ALT or ALP kit). An automated clinical chemistry analyzer was used for measuring the absorbance at 340 nm.

**Statistical analysis**

The results were expressed as mean ± S.D (n=6). The statistical analysis involving eleven groups was performed by analysis of variance (ANOVA) followed by Dunnett multiple comparison test. p value at < 0.05 was considered as statistically significant.

Data were processed with graph pad prism version 6.00 software.

**Results**

**Standardization of AD induced hyperlipidemia**

SD rats were fed with AD diet consisting of 2% cholesterol, 1% Choline and 2% Lard for 30 days to find out induction of hyperlipidemia. Blood samples were collected and analyzed on every 7th day till the 30th day from both AD fed and normal diet fed
groups. The optimum levels of TC and TG were seen on 21\textsuperscript{st} day in comparison to normal pellet diet group fed animals. On 30\textsuperscript{th} day there was not much significant rise in TC and TG in comparison to 21\textsuperscript{st} day. For 7\textsuperscript{th} and 14\textsuperscript{th} day rats did not show much significant increase in serum lipid profile.

**Biochemical estimation in rats treated with selected plant extracts**

The results of *in-vivo* antihyperlipidemic studies of the extracts like the changes in body weight, serum lipid parameters and action on hepatic enzymes were presented in Tables 6.5-6.9 and graphically represented in Fig. 6.1 – 6.7. A significant decrease (p<0.01) in the TC, TG and LDL-C levels were observed in the treatment groups with an increase in HDL-C. The serum AST, ALT and ALP levels were also decreased in extracts treated groups in a dose dependent manner.

**Table 6.4: Effect of atherogenic diet feeding on lipid profile**

<table>
<thead>
<tr>
<th>Duration</th>
<th>Parameter</th>
<th>Normal group</th>
<th>AD group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>TC (mg/dl)</td>
<td>73.4 ±3.67</td>
<td>80.74 ±4.04</td>
</tr>
<tr>
<td></td>
<td>TG (mg/dl)</td>
<td>71.42± 4.78</td>
<td>68.70 ±2.70</td>
</tr>
<tr>
<td>Day 7</td>
<td>TC (mg/dl)</td>
<td>75.58 ±3.18</td>
<td>109.6 ±4.62</td>
</tr>
<tr>
<td></td>
<td>TG (mg/dl)</td>
<td>67.92± 4.43</td>
<td>92.04 ±2.97</td>
</tr>
<tr>
<td>Day 14</td>
<td>TC (mg/dl)</td>
<td>72.79 ±2.90</td>
<td>117.09 ±7.35</td>
</tr>
<tr>
<td></td>
<td>TG (mg/dl)</td>
<td>70.95 ±1.71</td>
<td>132.05 ±3.76</td>
</tr>
<tr>
<td>Day 21</td>
<td>TC (mg/dl)</td>
<td>70.82 ±4.25</td>
<td>149.54 ±5.47</td>
</tr>
<tr>
<td></td>
<td>TG (mg/dl)</td>
<td>66.07± 5.26</td>
<td>127.77 ±3.54</td>
</tr>
<tr>
<td>Day 30</td>
<td>TC (mg/dl)</td>
<td>68.92 ±5.22</td>
<td>143.00 ±12.00</td>
</tr>
<tr>
<td></td>
<td>TG (mg/dl)</td>
<td>68.89± 5.29</td>
<td>127.14 ±4.84</td>
</tr>
</tbody>
</table>

Values are mean ± S.D (n = 3), TC: total cholesterol, TG: triglycerides
Figure 6.1: Effect of atherogenic diet feeding on total cholesterol (TC) and triglycerides (TG)
Table 6.5: Changes of serum lipid parameters in control and treated rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Control</th>
<th>AD Control</th>
<th>AD + Atv</th>
<th>AD + ECCL</th>
<th>AD + EBOG</th>
<th>AD + ESSW</th>
<th>AD + EGMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dL)</td>
<td>77.76±4.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>165.87±3.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>98.91±4.59&lt;sup&gt;hn&lt;/sup&gt;</td>
<td>135.64±8.04&lt;sup&gt;n&lt;/sup&gt;</td>
<td>118.37±3.23&lt;sup&gt;h&lt;/sup&gt;</td>
<td>130.65±7.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>121.13±3.05&lt;sup&gt;hn&lt;/sup&gt;</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>75.07±4.24&lt;sup&gt;i&lt;/sup&gt;</td>
<td>127.62±7.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>78.63±3.46&lt;sup&gt;i&lt;/sup&gt;</td>
<td>97.26±4.64&lt;sup&gt;n&lt;/sup&gt;</td>
<td>90.24±4.43&lt;sup&gt;h&lt;/sup&gt;</td>
<td>99.25±5.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>89.83±4.57&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>27.08±1.23&lt;sup&gt;i&lt;/sup&gt;</td>
<td>21.92±0.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.62±1.03&lt;sup&gt;e&lt;/sup&gt;</td>
<td>24.02±0.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.48±0.66&lt;sup&gt;n&lt;/sup&gt;</td>
<td>23.43±0.68&lt;sup&gt;hn&lt;/sup&gt;</td>
<td>25.08±0.43&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>35.66±4.14&lt;sup&gt;i&lt;/sup&gt;</td>
<td>118.43±4.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.58±4.59&lt;sup&gt;hn&lt;/sup&gt;</td>
<td>92.18±7.99&lt;sup&gt;n&lt;/sup&gt;</td>
<td>74.84±3.13&lt;sup&gt;n&lt;/sup&gt;</td>
<td>87.37±7.51&lt;sup&gt;hn&lt;/sup&gt;</td>
<td>78.09±3.48&lt;sup&gt;hn&lt;/sup&gt;</td>
</tr>
<tr>
<td>AI</td>
<td>1.32±0.19&lt;sup&gt;n&lt;/sup&gt;</td>
<td>5.41±0.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.25±0.18&lt;sup&gt;hn&lt;/sup&gt;</td>
<td>3.85±0.42&lt;sup&gt;n&lt;/sup&gt;</td>
<td>2.94±0.17&lt;sup&gt;n&lt;/sup&gt;</td>
<td>3.73±0.36&lt;sup&gt;n&lt;/sup&gt;</td>
<td>3.11±0.14&lt;sup&gt;n&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.D (n = 6), p values: a<0.05, b<0.01, c<0.001, as compared with normal control; p values: p<0.05, q<0.01, r<0.001, as compared with AD control. (by one-way ANOVA followed by Dunnett multiple comparison test)

ECCL: Ethanolic extract of *C. caudata* leaf,
EBOG: Ethanolic extract of *B. ovalifolia* gum,
AD: Atherogenic diet, Atv: Atorvastatin,
HDL: high density lipoprotein cholesterol,
TC: total cholesterol,
LDL: low density lipoprotein cholesterol,
ECCL: Ethanolic extract of *S. spontaneum* whole plant,
EBOG: Ethanolic extract of *G. mangostana* pericarp.
TG: triglycerides.
AI: Atherogenic index.
Figure 6.2: Effect of treatment on total cholesterol levels

Figure 6.3: Effect of treatment on triglyceride levels

ECCL: Ethanolic extract of *C. caudata* leaf,
EBOG: Ethanol extract of *B. ovalifoliata* gum,
AD: Atherogenic diet,
ESSW: Ethanolic extract of *S. spontaneum* whole plant,
EGMP: Ethanolic extract of *G. mangostana* pericarp.
Atv: Atorvastatin,
**Figure 6.4:** Effect of treatment on HDL levels

**Figure 6.5:** Effect of treatment on LDL levels

ECCL: Ethanolic extract of *C. caudata* leaf,  
EBOG: Ethanolic extract of *B. ovalifoliat*a gum,  
AD: Atherogenic diet,  
ESSW: Ethanolic extract of *S. spontaneum* whole plant,  
EGMP: Ethanolic extract of *G. mangostana* pericarp,  
Atv: Atorvastatin.
Figure 6.6: Effect of treatment on Atherogenic index

ECCL: Ethanolic extract of *C. caudata* leaf,
EBOG: Ethanolic extract of *B. ovalifoliata* gum,
AD: Atherogenic diet,

ESSW: Ethanolic extract of *S. spontaneum* whole plant,
EGMP: Ethanolic extract of *G. mangostana* pericarp,
Atv: Atorvastatin,
Table 6.6: Effect of the alcoholic extracts of the selected plants on body weight in control and treated rats

<table>
<thead>
<tr>
<th>Day</th>
<th>Normal Control</th>
<th>AD diet Control</th>
<th>Atorvastatin 10 mg/kg</th>
<th>ECCL 10 mg/kg</th>
<th>ECCL 100 mg/kg</th>
<th>ECCL 200 mg/kg</th>
<th>EBOG 100 mg/kg</th>
<th>EBOG 200 mg/kg</th>
<th>ESSW 100 mg/kg</th>
<th>ESSW 200 mg/kg</th>
<th>EGMP 100 mg/kg</th>
<th>EGMP 200 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>190.83 ± 4.07</td>
<td>192.50 ± 5.01</td>
<td>191.17 ± 4.07</td>
<td>188.83 ± 6.37</td>
<td>189.33 ± 4.76</td>
<td>189.67 ± 3.88</td>
<td>192.00 ± 5.10</td>
<td>189.04 ± 2.69</td>
<td>189.33 ± 4.27</td>
<td>187.50 ± 5.24</td>
<td>190.33 ± 4.55</td>
<td></td>
</tr>
<tr>
<td>Day 21</td>
<td>232.67 ± 5.35</td>
<td>334.33 ± 15.65</td>
<td>257.00 ± 10.00</td>
<td>286.50 ± 13.84</td>
<td>283.17 ± 13.26</td>
<td>296.33 ± 14.42</td>
<td>289.83 ± 13.98</td>
<td>305.00 ± 13.01</td>
<td>296.17 ± 14.25</td>
<td>293.33 ± 14.22</td>
<td>276.83 ± 12.43</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± S.D (n = 6)

Table 6.7: Effect of the alcoholic extracts of the selected plant on % body weight gain

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Control</th>
<th>AD diet Control</th>
<th>Atorvastatin 10 mg/kg</th>
<th>ECCL 10 mg/kg</th>
<th>ECCL 100 mg/kg</th>
<th>ECCL 200 mg/kg</th>
<th>EBOG 100 mg/kg</th>
<th>EBOG 200 mg/kg</th>
<th>ESSW 100 mg/kg</th>
<th>ESSW 200 mg/kg</th>
<th>EGMP 100 mg/kg</th>
<th>EGMP 200 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Body weight gain</td>
<td>21.99 ±4.60</td>
<td>73.78 ±9.22</td>
<td>34.55 ± 7.44</td>
<td>51.96 ± 10.95</td>
<td>49.66 ± 8.52</td>
<td>56.38 ± 10.00</td>
<td>51.05 ± 8.35</td>
<td>61.41 ± 8.40</td>
<td>56.52 ± 8.94</td>
<td>56.47 ± 6.84</td>
<td>45.53 ± 7.63</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± S.D (n = 6), p values: a<0.05, b<0.01, c<0.001, as compared with normal control; p values:p<0.05, q<0.01, r<0.001, as compared with AD control (by one-way ANOVA followed by Dunnett multiple comparison test)

ECCL: Ethanolic extract of C.caudata leaf,
EBOG: Ethanolic extract of B. ovalifoliata gum,
ESSW: Ethanolic extract of S.spontaneum whole plant,
AD: Atherogenic diet,
EGMP: Ethanolic extract of G.mangostana pericarp.
Atv: Atorvastatin
Table 6.8: Effect of alcoholic extracts on lipid peroxidation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Control</th>
<th>AD diet Control</th>
<th>Atorvastatin</th>
<th>ECCL 10 mg/kg</th>
<th>ECCL 200 mg/kg</th>
<th>EBOG 10 mg/kg</th>
<th>EBOG 200 mg/kg</th>
<th>ESSW 10 mg/kg</th>
<th>ESSW 200 mg/kg</th>
<th>EGMP 10 mg/kg</th>
<th>EGMP 200 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPO</td>
<td>3.93±0.35(^a)</td>
<td>7.53±0.47(^b)</td>
<td>3.84±0.14(^i)</td>
<td>5.46±0.32(^b)</td>
<td>4.85±0.47(^b)</td>
<td>5.43±0.41(^b)</td>
<td>4.86±0.38(^b)</td>
<td>5.53±0.48(^b)</td>
<td>5.58±0.41(^b)</td>
<td>5.18±0.33(^b)</td>
<td>4.29±0.32(^i)</td>
</tr>
</tbody>
</table>

Table 6.9: Effect of alcoholic extracts on hepatic enzymes in diet-induced hyperlipidemic rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Control</th>
<th>AD diet Control</th>
<th>ECCL 100 mg/kg</th>
<th>ECCL 200 mg/kg</th>
<th>EBOG 100 mg/kg</th>
<th>EBOG 200 mg/kg</th>
<th>ESSW 100 mg/kg</th>
<th>ESSW 200 mg/kg</th>
<th>EGMP 100 mg/kg</th>
<th>EGMP 200 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP (U/L)</td>
<td>211.0±10.07(^a)</td>
<td>507.7±35.42(^b)</td>
<td>321.1±8.33(^b)</td>
<td>277.20±10.32(^b)</td>
<td>302.22±8.54(^b)</td>
<td>270.51±5.31(^b)</td>
<td>432.40±61.40(^b)</td>
<td>390.40±48.83(^b)</td>
<td>287.80±6.69(^b)</td>
<td>257.38±13.93(^e)</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>36.15±3.71(^a)</td>
<td>75.61±8.41(^b)</td>
<td>50.82±6.25(^b)</td>
<td>44.74±6.89(^b)</td>
<td>52.57±4.25(^b)</td>
<td>48.95±6.12(^b)</td>
<td>67.94±7.24(^b)</td>
<td>61.61±6.47(^b)</td>
<td>48.96±4.92(^b)</td>
<td>41.79±3.45(^e)</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>59.13±7.76(^e)</td>
<td>96.47±7.29(^b)</td>
<td>71.08±4.86(^e)</td>
<td>68.05±6.26(^e)</td>
<td>71.95±5.39(^b)</td>
<td>67.97±7.34(^b)</td>
<td>89.78±7.24(^b)</td>
<td>88.15±4.76(^b)</td>
<td>66.21±7.34(^b)</td>
<td>64.72±5.03(^i)</td>
</tr>
</tbody>
</table>

Values are mean ± S.D (n = 6), p values: a<0.05, b<0.01, c<0.001, as compared with normal control; p values:p<0.05, q<0.01, r<0.001, as compared with AD control (by one-way ANOVA followed by Dunnett multiple comparison test)

ECCL: Ethanolic extract of *C. caudata* leaf,
EBOG: Ethanolic extract of *B. ovalifoliata* gum,
ESSW: Ethanolic extract of *S. spontaneum* whole plant,
EGMP: Ethanolic extract of *G. mangostana* pericarp.
AD: Atherogenic diet,
Fig 6.7: Effect of the alcoholic extracts on % body weight gain

ECCL: Ethanolic extract of *C. caudata* leaf,  
EBOG: Ethanolic extract of *B. ovalifoliata* gum,  
AD: Atherogenic diet,  
ESSW: Ethanolic extract of *S. spontaneum* whole plant,  
EGMP: Ethanolic extract of *G. mangostana* pericarp,  
Atv: Atorvastatin.
Figure 6.9: Effect of treatment on ALP in diet-induced hyperlipidemic rats

ECCL: Ethanolic extract of *C. caudata* leaf,

EBOG: Ethanolic extract of *B. ovalifoliata* gum,

AD: Atherogenic diet,

ESSW: Ethanolic extract of *S. spontaneum* whole plant,

EGMP: Ethanolic extract of *G. mangostana* pericarp,

Atv: Atorvastatin,
6.2 Results and Discussion:

Though lipids are essential in the formation of cell membrane and hormone synthesis, excess of cholesterol leads to development of atherosclerosis. LDL-C is considered as the major bad cholesterol as it transports cholesterol not only to peripheral tissues but also to the arterial wall [210]. The increase in concentration of blood cholesterol through diet results in accumulation of LDL-C whose oxidative modification is implicated in atherosclerosis.

In the present study the lipid levels of atherogenic diet (AD) control group were significantly higher than the control group which indicates that AD is a potential source of dietary cholesterol. Animals in control group showed no change in the lipid profile and lie in the normal range. Treatment with ECCL, EBOG, ESSW and EGMP at 100 and 200 mg/kg showed significant decrease in body weight and lipid levels in a dose dependant manner. Animals treated with atorvastatin, a known hypolipidemic drug at a dose of 10 mg/kg showed a marked decrease in the levels of serum TC, TG, LDL and atherogenic index. Besides, the levels of HDL were elevated in the treated groups. HDL is considered as good cholesterol as it takes up the excess cholesterol from the cells and delivers to the liver for metabolism [211]. Therefore an increase in HDL levels indicates lower risk of atherosclerosis. Atherogenic index (AI) which is defined as LDL-C to HDL-C is an important factor for the diagnosis of atherosclerosis. The results clearly indicate that the extracts significantly decreased the AI.

A significant (p<0.05) decrease in the body weight was observed in all the extract treated groups except for ESSW (100 mg/kg). The percentage increase in body weight was found to be highest in AD group (73%) while EGMP (200 mg/kg) showed least
increase in body weight among all the extracts (45%). Atorvastatin treated groups showed on increase of 34% in body weight. The results indicate that the extracts have effect on the body weight.

Oxidative stress caused by atherogenic diet is associated with peroxidation of cellular lipids, which is determined by measurement of thiobarbituric acid reacting substance (TBARS). The concentration of LPO products may reflect the degree of oxidative stress. The increased level of TBARS results in increase of oxygen free radicals, which attacks the polyunsaturated fatty acids in cell membranes and cause LPO. The malondialdehyde (MDA) content, a measure of LPO was assayed in the form of TBARS. Treatment with the ethanolic extracts and atorvastatin significantly (p<0.01) prevented the peroxidation of lipids by reducing the concentration of TBARS (Table 24).

Hyperlipidemia induces liver damage [212] and changes in serum AST, ALT and ALP levels are markers of hepatotoxicity. Animals in group A (Control) showed no change in the AST, ALT, ALP and lies in the normal range. Significant increase of serum AST (96.47 U/L), ALT (75.61 U/L) and ALP (507.76 U/L) levels were detected in AD group compared to normal diet control group. However, significant decrease in the levels of hepatic enzymes was observed in the groups treated with ethanolic extracts which indicate the hepatoprotective potential of the extracts.

Conclusion:

The present study is the first to demonstrate the hypolipidemic activity of Commiphora caudata, Boswellia ovalifoliolata, Saccharum spontaneum and Garcinia mangostana, thereby reducing the risk of cardiovascular complications like atherosclerosis. Among all the extracts tested, Garcinia mangostana was found to
possess highest lipid lowering activity followed by *Commiphora caudata* and *Boswellia ovalifoliolata*. *Saccharum spontaneum* recorded lowest activity among all the extracts tested. The results found were encouraging for further studies on these plants. Though the exact mechanism of hypolipidemic action was not established, the possible beneficial effect of polyphenols can be taken into account as they are potent antioxidants and can prevent the oxidation of Low density lipoprotein cholesterol. Further studies are necessary to establish the exact mechanism of action.

In view of the significant anti-hyperlipidemic activities exerted by the individual extracts of the selected medicinal plants, the author has also prepared a herbal formulation with the selected plant extracts and studied the anti-hyperlipidemic activity. The prepared formulation was tested for anti-hyperlipidemic activity in atherogenic diet induced rat model.