CHAPTER 2

MATERIALS AND METHODS
MATERIALS AND METHODS

2.1. Plant Materials:
The tender aerial parts and leaves of Clerodendron colebrookianum Walp (CC) were collected from various localities of Kamrup District, Assam (India). The materials were air dried and made into coarse powder for preparation of the extract.

The identity of the above mentioned plant was confirmed using reference herbarium specimen available in the Department of Botany, Gauhati University, Guwahati, India and with the help of relevant literature (Bordoloi and Borthakur, 1997). The voucher specimen of the collected plant was preserved for reference.

2.2. Chemicals and Regents:
Diagnostic kits (Godkar et al., 1996; Prasanna, 2000; Zou et al., 2005) for measuring total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL) were purchased from Glaxo India Limited and Dr.Reddy’s Diagnostic Laboratories. Rest of the chemicals used, were of analytical grade from Merck and Himedia Ltd, India.

2.3. Extraction of Glycoside:
Air-dried and finely powdered leaves of CC were exhaustively extracted with ethanol in Soxhlet apparatus for about 18 hours. The ethanol extract was concentrated under reduced pressure and then glycoside was precipitated by adding ether in the extract (Chakrabarty and Choudhury, 1973). The precipitated glycoside were filtered off, dried, weighed and dissolved in de-ionized water. The extracted glycoside was confirmed by qualitative test with the help of the procedure adopted by Sharma and Srivastava (1991). The glycoside was administered orally into experimenting animals following effective dose formulation.

2.4. Preparation of Methanol Extract:
Air dried and finely powdered leaves of CC were exhaustively extracted with methanol in soxhlet apparatus for about 18 hours. The concentrated resinous extract was dried, weighed, dissolved in de-ionized water and filtered and tested for glycoside.
This filtrate was administered orally (Gavage) to the experimenting animals following effective dose formulation.

2.5. Extraction of Hepatic Lipid:
For extraction of hepatic lipids 0.5 g liver was placed in a dry and cleaned mortar and grounded thoroughly with approximately 4 g of anhydrous sodium sulphate. The powdered preparation was transferred from the mortar to a clean, dry, 50 ml screw cap vial, the cap of which was lined with tinfoil and sealed in cork. 30 ml of chloroform was added and the screw-cap sealed. The vial was allowed to stand for 72 hours with intermittent shaking by hand (Weil and Stetten, 1947). The chloroform extract was evaporated to dryness under an atmosphere of nitrogen, and the lipid was re-extracted with hot-petroleum ether. The extract contains all the tissue lipids.

2.6. Estimation of Elements:
2.6.1: Preparation of plant materials for elements:
For analysis of element in plant material standard wet digestion method was followed. The leaves of CC were dried in air completely till the weight is constant. The air-dried leaves were crushed to very fine particle with the help of pestle and mortar. To 1 g powdered leaves kept in a small beaker, added with 10 ml of concentrated HNO₃ and allowed to stand overnight. After that it was heated carefully on a hot plate until the production of red NO₂ fumes was ceased. After cooling small amount (2-4 ml) of 70% HClO₄ was added. The sample was heated again and allowed to evaporate to a small volume. The sample was diluted to 50 ml with de-ionized water.

2.6. II: Preparation of Animal Tissue for Element:
About 5 g accurately weighed formalinized tissue placed in a conical flask, to it 25ml de-ionized water and a few glass beads were added and finally 10ml of 1:2 mixtures of concentrated HNO₃ and HClO₄ were added to the tissue and boiled till the solution was clear. The volume of the digested solution was measured and diluted to 100 ml with de-ionized water. The final dilution with de-ionized water (100ml) was adjusted to ensure that the concentration fall within suitable absorbance range. For serum magnesium, the serum was directly diluted with de-ionized water (Kahnke, 1966).
2.7: Animals:
C3H mice (Body weight 20-25g) were taken as experimental model in the present investigation. The animals were randomly selected and reared in the Animal House of the Gauhati University at room temperature 25±2° C. The animals were divided in to the following groups consisting of 10 mice in each group under 5 sets of experimental protocol. The permission to work with the C3H mice were obtained from the Institutional Ethical Committee of the Gauhati University vide letter no: IAEC/04-05 dated 10-06-06

2.8: Experimental Protocol:
2.8.1: 1st set:
The mice were fed with basal diet (Table-2.1); (Farris, 1950) and high fat diet (Table-2.2) in the proportion of 5-7 g/mice and provided with water *ad libitum*. The following groups of mice were considered during the investigation.

**Group –I:** Control animal, received only basal diet (the day on which basal diet was provided considered as 0 day, and the animals were expanded to 28 day).

**Group –II:** Received basal diet and residue of methanol extract of CC (@ 40mg/kg body weight/day) for a period of 28 days.

**Group-III:** Received only high fat diet and expanded to 28 days.

**Group-IV:** Received high fat diet and glycosides of CC (@20mg/kg body weight/day) for a period of 28 days.

**Group-V:** Received high fat diet and Gemfibrozil (@20mg/kg body weight /day) for a period of 28 days.

2.8.2: 2nd set:
The mice were fed with basal diet (Table-2.1) and high fat diet (Table-2.2) in the proportion of 5-7 g/day/mice and provided with water *ad libitum*. The following groups of mice were considered during the investigation.

**Group-I:** Control animals received only basal diet for 45 days.

**Group-II:** Received high fat diet for 45 days.
Group-III: Received high fat diet for 45 days followed by oral administration of glycoside of CC (@20mg/kg body weight/day) for a period of 7 days. The whole experiment was completed in 52 days.

Group-IV: Received high fat diet for 45 days followed by oral administration of glycoside of CC (@20mg/kg body weight/day) for a period of 14 days and the experiment was completed in 59 days.

Group-V: Received high fat diet for 45 days followed by oral administration of glycoside of CC (@20mg/kg body weight/day) for a period of 21 days (i.e. up to 66 days of total period.)

Group-VI: Received high fat diet for 45 days followed by oral administration of glycoside of CC (@20mg/kg body weight/day) for a period of 28 days and the experiment was completed in 73 days.

Group-VII: Received high fat diet for 45 days followed by oral administration of gemfibrozil (@20mg/kg body weight/day) for a period of 28 days and the experiment was completed in 73 days.

2.8.3: 3rd set:
The mice were fed with basal diet (Table-2.1) and high fat diet (Table-2.2) in the proportion of 5-7 g/day/mice and provided with water *ad libitum*. The following groups of mice were considered during the investigation.

Group-I: Control animals received only basal diet.

Group-II: Received high fat diet for 45 days.

Group-III: Received high fat diet for 45 days followed by oral administration of residue of methanol extract (@40mg/kg body weight/day) for 7 days and the whole experiment was completed in 52 days.

Group-IV: Received high fat diet for 45 days followed by oral administration of residue of methanol extract (@40mg/kg body weight/day) for 14 days and the whole experiment was completed in 59 days.

Group-V: Received high fat diet for 45 days followed by oral administration of residue of methanol extract (@40mg/kg body weight/day) for 21 days and the experiment was completed in 66 days.
Group-VI: Received high fat diet for 45 days followed by oral administration of residue of methanol extract (@40 mg/kg body weight/day) for 28 days and the experiment was completed in 73 days.

Group-VII: Received high fat diet for 45 days followed by oral administration of gemfibrozil (@20 mg/kg body weight/day) for 28 days and the experiment was completed in 73 days.

2.8.4: 4th set:
The mice were fed with basal diet in the proportion of 5-7 g/day/mice and provided with de-ionized water. The following groups of mice were considered during the investigation.

Group-I: Received only basal diet.

Group-II: Received basal diet and oral dose of glycoside of CC (@20 mg/kg body weight/day) for a period of 7 days.

Group-III: Received basal diet and oral dose of glycoside of CC (@20 mg/kg body weight/day) for a period of 14 days.

Group-IV: Received basal diet and oral dose of glycoside of CC (@20 mg/kg body weight/day) for a period of 21 days.

Group-V: Received basal diet and oral dose of glycoside of CC (@20 mg/kg body weight/day) for a period of 28 days.

Group-VI: Received basal diet and oral dose of glycoside of CC (@20 mg/kg body weight/day) for a period of 45 days.

2.8.5: 5th set:
The mice were fed with basal diet and Mg-deficient diet (Table-2.3-2.5) in the proportion of 5-7 g/day/mice and provided with de-ionized water. The following groups of mice were considered during the investigation.

Group-I: Received only basal diet.

Group-II: Received Mg-deficient diet for 15 days.
**Group-III:** Received Mg-deficient diet for 15 days followed by oral administration of glycoside of CC (@20mg/kg body weight/day) for 28 days. The whole experiment was completed in 43 days.

**Group-IV:** Received Mg-deficient diet for 15 days followed by oral administration of MgCl₂ @20 mg/kg/body weight/day) for a period of 28 days. The whole experiment was completed in 43 days.

After the stipulated period each animal was sacrificed for blood (from jugular vein), liver and heart from the control and the test groups to assess the lipid and element profile.

a) Lipid profile: Total cholesterol (TC); Triglyceride (TG); High density lipoprotein (HDL); Low density lipoprotein (LDL).

b) Element profile: Magnesium (Mg); Iron (Fe); Copper (Cu), Zinc (Zn) and Manganese (Mn).

### 2.9: Composition of Basal and High Fat Diet:

Each animal was given 5-7 g of diet with the following composition (Farris, 1950).

**Table-2.1: Composition of basal diet**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Whole wheat</td>
</tr>
<tr>
<td>2.</td>
<td>Yellow corn</td>
</tr>
<tr>
<td>3.</td>
<td>Barley</td>
</tr>
<tr>
<td>4.</td>
<td>Anik Spray</td>
</tr>
<tr>
<td>5.</td>
<td>Bone meal</td>
</tr>
<tr>
<td>6.</td>
<td>CaCl₂</td>
</tr>
<tr>
<td>7.</td>
<td>Salt</td>
</tr>
<tr>
<td>8.</td>
<td>Oil</td>
</tr>
<tr>
<td>9.</td>
<td>Vitamin B₁₂</td>
</tr>
</tbody>
</table>

**Table-2.2: Composition of high fat diet**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Whole wheat</td>
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<td>2.</td>
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<tr>
<td>3.</td>
<td>Barley</td>
</tr>
<tr>
<td>4.</td>
<td>Anik Spray</td>
</tr>
<tr>
<td>5.</td>
<td>Bone meal</td>
</tr>
<tr>
<td>6.</td>
<td>CaCl₂</td>
</tr>
<tr>
<td>7.</td>
<td>Salt</td>
</tr>
<tr>
<td>8.</td>
<td>Oil</td>
</tr>
<tr>
<td>9.</td>
<td>Vitamin B₁₂</td>
</tr>
</tbody>
</table>
2.10: Composition of Mg-deficient Diet:

Mg-deficient diet was prepared by mixing semi-synthetic diet with salt mixture. Semi-synthetic food mixture was used as Mg-deficient diet by omitting Mg from the salt mixture (Sos & Szelenyi, 1974).

Table-2.3: Semi synthetic diet.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>General (in%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20</td>
</tr>
<tr>
<td>Fat</td>
<td>9</td>
</tr>
<tr>
<td>Starch</td>
<td>63</td>
</tr>
<tr>
<td>Dried yeast</td>
<td>3</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>1</td>
</tr>
<tr>
<td>Salt-mixture</td>
<td>4</td>
</tr>
</tbody>
</table>

Table-2.4: Salt mixture.

<table>
<thead>
<tr>
<th>Salts mixture</th>
<th>g/kg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>K$_2$HPO$_4$</td>
<td>342</td>
</tr>
<tr>
<td>NaCl</td>
<td>168</td>
</tr>
<tr>
<td>Ca-lactate</td>
<td>160</td>
</tr>
<tr>
<td>CaCO$_3$</td>
<td>152</td>
</tr>
<tr>
<td>Microsalts</td>
<td>8</td>
</tr>
</tbody>
</table>

Table-2.5: Micro-salts.

<table>
<thead>
<tr>
<th>Micro-salts</th>
<th>Wt. in g</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeCl$_3$·7H$_2$O</td>
<td>4.30</td>
</tr>
<tr>
<td>ZnCl$_2$·H$_2$O</td>
<td>1.44</td>
</tr>
<tr>
<td>CuSO$_4$·5H$_2$O</td>
<td>0.24</td>
</tr>
<tr>
<td>CoCl$_2$·6H$_2$O</td>
<td>0.04</td>
</tr>
</tbody>
</table>
2.11: Methodology:
The following biochemical parameters were estimated using Photocolorimeter.

2.12: Lipid Profile:

2.12.1: Estimation of cholesterol: (Enzymatic CHOD-PAP method)

**Principle:**
Cholesterol esterase (CHE) hydrolyzes cholesterol ester. Free cholesterol is oxidized by the cholesterol oxidase (CHO) to cholest-4-en-3-one and hydrogen peroxide. Hydrogen peroxide formed, reacts with 4-amino antipyrine and phenol in presence of Peroxidase (POD) to produce pink coloured quinoneimine dye. The intensity of the colour produced is proportional to the cholesterol concentration.

\[
\text{CHE} \quad \text{Cholesterol ester} + \text{H}_2\text{O} \xrightarrow{} \text{Cholesterol} + \text{Fatty acid}.
\]

\[
\text{CHO} \quad \text{Cholesterol} + \text{O}_2 \xrightarrow{} \text{Cholest-4-en-3-one} + \text{H}_2\text{O}_2
\]

\[
\text{POD} \quad \text{H}_2\text{O}_2 + 4\text{-aminoantipyrine} + \text{Phenol} \xrightarrow{} \text{Quinoneimine dye} + \text{H}_2\text{O}
\]

**Sample material:** Serum and hepatic tissue.
The total cholesterol in the sample material is stable for about 7 days at room temperature and at 4°C (Henry, 1974)

**Reagents:**
1. Enzyme reagent 5× 10 ml.
2. Buffer solution 50 ml.
3. Standard (200 mg %) 3 ml.

All reagents are stable at 2-8°C till the expiry date.

**Reagent preparation:**
One vial of enzyme reagent (1) was reconstituted with equal volume of buffer solution. The reagent is stable for at least 4 weeks at 2-8°C and was kept away from light.
Procedure:
The reagents were pipetted into clean dry test tubes labeled blank (B), standard (S) and test (T).

<table>
<thead>
<tr>
<th></th>
<th>(B)</th>
<th>(S)</th>
<th>(T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme reagent</td>
<td>1ml</td>
<td>1ml</td>
<td>1 ml.</td>
</tr>
<tr>
<td>Distilled water</td>
<td>10µl</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>10µl</td>
<td>-</td>
</tr>
<tr>
<td>Test solution</td>
<td>-</td>
<td>-</td>
<td>10µl</td>
</tr>
</tbody>
</table>

These were mixed well and incubated at 37° C for 5 minutes and the absorbance of test (T) and standard (S) was measured against blank (B) on a photocolorimeter with green filter.

Calculation:
The following equation gives the cholesterol concentration.

\[
\text{Cholesterol in mg %} = \frac{\text{Absorbance of Test (T)}}{\text{Absorbance of standard (S)}} \times 200
\]

2.12. II: Estimation of Triglycerides: (GPO- method)

Principle:
Triglycerides in the sample are hydrolyzed by microbial lipases to glycerol and free fatty acids (FFA). Glycerol is phosphorylated by adenosine 5-triphosphate (ATP) to Glycerol -3-Phosphate (G-3-P) in a reaction catalyzed by Glycerol -kinase (GK). G-3-P is oxidized to dihydroxyacetone phosphate (DAP) in a reaction catalyzed by the enzyme glycerol phosphate oxidase (GPO). In this reaction, hydrogen peroxide (H₂O₂) is produced in equimolar concentration to the level of triglyceride present in the sample. H₂O₂ reacts with 4-aminoantipyrine (4-AAP) and 3, 5, -Dichloro-2-hydroxy
benzene sulfonic acid (DHBS) in a reaction catalyzed by peroxidase (HPOD). The result of this oxidative coupling is a quinoneimine red coloured dye.

The absorbance of this dye in solution is proportional to the concentration of triglycerides in the sample. The series of reaction involved in the assay is given below

\[
\text{Lipases} \quad \text{Triglyceride} \quad \xrightarrow{\text{Glycerol + FFA}} \quad \text{Glycerol + ATP} \quad \xrightarrow{\text{G-3-P + ADP Mg}^{2+}} \quad \text{GPO} \quad \text{G-3-P + O}_2 \quad \xrightarrow{\text{DAP +H}_2\text{O}_2} \quad \text{HPOD} \quad 2\text{H}_2\text{O}_2 + 4\text{AAP} + \text{DHBS} \quad \xrightarrow{} \quad \text{Red quinoneimine dye +H}_2\text{O}
\]

Sample material: Serum and hepatic tissue.

Reagents:
1. Enzyme reagent 100ml (5 x 20 ml)
2. Buffer 100 ml.
3. Triglyceride standard (200 mg %) 3 ml.

Working reagent preparation:
The reagents were allowed to equilibrate at room temperature before activation. One vial of enzyme reagent was dissolved by adding buffer quantity specified on label, cap and mixed the activated the reagent thoroughly and gently.

Procedure:
The required reagents and the samples were brought to 37\(^0\) C before performing the assay as follows

<table>
<thead>
<tr>
<th></th>
<th>(B)</th>
<th>(S)</th>
<th>(T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme reagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>10(\mu)l</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>10(\mu)l</td>
<td>-</td>
</tr>
<tr>
<td>Test solution</td>
<td>-</td>
<td>-</td>
<td>10(\mu)l</td>
</tr>
</tbody>
</table>
The samples were pipetted into clean dry test tube and labeled as blank (B), standard (S) and test (T) and incubated at 37°C for 5 minutes or at room temperature for 15 minutes. The absorbance of test (T) and standard (S) against Blank (B) was measured on a photolorimeter with green filter.

**Calculation:**

\[
\text{Triglyceride concentration (mg/dl)} = \frac{\text{Absorbance of test (T)}}{\text{Absorbance of standard (S)}} \times 200
\]


**Principle:**

The VLDL and LDL fraction of serum sample are precipitated using PTA and then HDL in the supernatant is separated by centrifugation and measured for its cholesterol content. The enzyme cholesterol ester hydrolase (CHE) hydrolyzes the ester cholesterol. Then cholesterol is oxidized by cholesterol oxidase (CHO) to cholest-4-en-3-one and hydrogen peroxide. Hydrogen peroxide in presence of the enzyme Peroxidase (POD) reacts with 4-amino antipyrine and phenol to produce a red coloured complex, whose absorbance is proportional to HDL cholesterol concentration.

Serum + Precipitating reagent \[\rightarrow\] Precipitate (VLDL & LDL)

Precipitating reagent + Supernatant (HDL).

Cholesterol ester + H\textsubscript{2}O \[\rightarrow\] Cholesterol + Fatty acid

Cholesterol + O\textsubscript{2} \[\rightarrow\] Cholest-4-en-3-one + H\textsubscript{2}O\textsubscript{2}

H\textsubscript{2}O\textsubscript{2} + 4 aminoantipyrine + Phenol \[\rightarrow\] Quinoneimine dye + H\textsubscript{2}O

**Reagents:**

1. Precipitating reagent \(1 \times 5\) ml.
2. Enzyme reagent \(4 \times 10\) ml.
3. Buffer \(1 \times 40\) ml.
4. HDL cholesterol standard (50 mg %) \(1 \times 3\) ml.
Reagent preparation:
One vial of enzyme reagent (2) reconstituted with 10 ml. of buffer solution (3). The prepared reagent is stable for at least 4 weeks at 2-8\(^0\) C and protected from light.

Procedure:

Step-I: Precipitation of VLDL & LDL.
In a clean and dry centrifuge tube serum 0.1 ml and precipitating reagent is taken. Both serum and precipitating reagent are mixed well and allowed to stand at room temperature for 5 minutes and centrifuged at 2000-3000 rpm for 10 minutes to get a clear supernatant.

Step –II: Assay of HDL-Cholesterol
Pipetted to three clean and dry test tube labeled as blank (B), standard (s) and test (T).

<table>
<thead>
<tr>
<th></th>
<th>(B)</th>
<th>(S)</th>
<th>(T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme reagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>50(\mu)l</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cholesterol standard (3)</td>
<td>-</td>
<td>50(\mu)l</td>
<td>-</td>
</tr>
<tr>
<td>Sample (serum)</td>
<td>-</td>
<td>-</td>
<td>50(\mu)l</td>
</tr>
</tbody>
</table>

The blank, standard and test were mixed thoroughly and incubated at 37\(^\circ\)C for 5 minutes and the absorbance was taken on a photocolorimeter with green filter.

Calculation:

\[ \text{HDL - cholesterol in mg %} = \frac{\text{Absorbance of test (T)}}{\text{Absorbance of standard (S)}} \times 100 \]
2.12. IV: Estimation of LDL –cholesterol:

LDL –cholesterol was estimated indirectly by Friedwald’s formula (Friedwald’s, 1972).

\[ \text{LDL in mg %} = \text{Total cholesterol} - \left(\text{HDL-cholesterol} - \text{triglyceride} / 5\right) \]

2.13: Selection of Working Dose:

The CC glycoside and residue of methanol extract (ME) were administered at different doses (Table-2.6) orally for four days (96 hour) to separate groups of 10 mice each and the animals were observed for mortality on the 5th day. Extracts showing mortality during the course of treatment or on the 5th day was tested again at lower dose and the dose showing no mortality in mice was selected for effective dose (Sharma, 1991).

Table-2.6: Dose selection for CC glycoside and ME of CC.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Dose (mg/kg b w/day)</th>
<th>No of dead animals out of 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC glycoside</td>
<td>500</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>ME</td>
<td>500</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>

2.14: Calculation of Percentage Reduction (PR) and Overall Percentage Reduction (OPR) of Biochemical Parameter.

The value of estimated serum and hepatic biochemical parameters viz. TC, TG, LDL and HDL were represented by Percentage reduction (PR) and Overall Percentage reduction (OPR) with respect to control and high fat diet fed groups of animal. The objective of representing the data of biochemical parameters in PR and OPR is to
compare the relative potency of methanolic extract and glycoside of CC with respect to control group or high fat diet fed animal groups considering them as 100% reduction.

1. The Percentage Reduction (PR) of each individual lipid profile were calculated from the following equation:

\[
\text{Percentage Reduction (PR)} = \frac{A - B}{A} \times 100
\]

A: mean value of individual lipid profile in control group.
B: mean value of individual lipid profile in plant extract received group.

2. The Overall Percentage Reduction (OPR) of each individual lipid in respect of high fat diet was calculated from the following equation:

\[
\text{Overall Percentage Reduction (OPR)} = \frac{B - C}{B - A} \times 100
\]

A: Aggregate of mean value of all lipid profile in control group.
B: Aggregate of mean value of all lipid profile in high fat diet fed animal group.
C: Aggregate of mean value of all lipid profile in plant extract received group.

2.15: Statistical Analysis:

The application of statistical analysis only made distinct the action of the drug. The results of all biochemical parameters are expressed as mean ± S.D. (Standard deviation). The total variations present in a set of data have been estimated by using one-way analysis of variance (ANOVA). The F-ratio obtained from ANOVA have been compared with the tabulated F-value, if the calculated F value exceeds the tabulated F value, it indicates significant variation amongst the groups under analysis. In order to check the pairs of means, which were different, the data for every biochemical parameter in each set of experiment was analyzed by least significant difference, observed between various groups of mean of particular biochemical parameters.
References


** - Original literature not seen.