8.1 INTRODUCTION

Hyperlipidemia is a broad term which is also called hyperlipoproteinemia, is a common disorder in developed countries and is the major cause of coronary heart diseases. It results from abnormalities in lipid metabolism or plasma lipid transport or a disorder in the synthesis and degradation of plasma lipoproteins.

The term “dyslipidemia” now a days is increasingly being used to describe abnormal changes in lipid profile, replacing the old term hyperlipidemia. Hyperlipidemia means abnormal increase in fat levels of blood. These fats include cholesterol and triglycerides. These are important for our body to function, but when their levels are high they, can cause heart disorders.

Hyperlipidemia is manifested as hypercholesterolemia and hypertriglyceridemia. Hypercholesterolemia is the most common hyperlipidemia. The lipids that are involved in hypercholesterolemia are cholesterol, an essential component of cell membrane and a precursor of steroid hormone synthesis and triglycerides are important energy source, they are transported in blood as lipoproteins. The consequence of hyperlipidemia is to cause atherosclerosis, thus the risk of coronary heart diseases and strokes. The risk of heart diseases in future also depends on many other factors that influence the health of a person’s level of cholesterol, blood vessels and blood circulation.\(^1\)

LDL is strongly associated with a higher risk, and HDL is associated with a lower risk, of coronary heart diseases (CHD). Lowering lipids through dietary or pharmacological therapy has been shown to decrease the incidence of
atherosclerotic events. Since lipid levels have been observed to track into adulthood, adolescents with hyperlipidemia are also at greater CHD risk. The extent of abnormal lipids and other cardiovascular risk factors during childhood and adolescence is related to the severity of atherosclerosis seen in autopsies of young adults.²

Patients with Diabetes mellitus DM are at significantly increased risk of CHD compared with non diabetic patients of similar age. DM patients without known CHD appear to have a risk for first myocardial infarction (MI) similar to the risk for recurrent MI of non-DM patients with CHD and a prior coronary event. Patients with type 2 diabetes commonly have other risk factors (hypertension, high LDL-C, low HDL-C, obesity) that increase risk for cardiac events.³

High lipid levels can speed up a process called atherosclerosis, or hardening of the arteries. From inside, arteries are normally smooth and unobstructed, but as increase in age, a sticky substance called plaque forms in the walls of arteries, which is made of lipids and other materials circulating in blood.
As more plaque builds up, arteries can narrow and stiffen. Eventually, enough plaque may build up to reduce blood flow through arteries.

Hyperlipidemia is typically asymptomatic and is frequently detected during routine screening.

Hyperlipidemia often results from delayed or defective clearance, or overproduction of VLDL by the liver, which is subsequently transformed into LDL. Hypercholesterolemia involves defective hepatic and nonhepatic LDL receptors. Excess intake of saturated fats increases the liver's production of VLDL and triglycerides via a molecular mechanism involving protein activators. Saturated fats are found in animal products, such as meat, whole milk dairy products (milk, cream, cheese), and butter, and tropical oils (palm, palm kernel, and coconut).

High concentrations of total and LDL cholesterol and low levels of high-density lipoprotein (HDL) cholesterol, predicts cardiovascular risk in both men and women. High triglyceride levels have been associated with greater risk in women only. The risk of cardiovascular disease increases by an average of 2%,
for each corresponding 1% rise in total cholesterol. Adolescents with high TC or LDL may have a genetic disorder of lipid metabolism such as familial hypercholesterolemia or familial combined hypercholesterolemia. Those with homozygous forms of these disorders can experience myocardial infarction or other events during childhood or early adolescence. Familial hypercholesterolemia is often diagnosed in adolescence and is characterized by high LDL levels that can be refractory to dietary treatment. These patients can present clinically with xanthomas or xanthelasma– cholesterol deposits under the skin on the hands, elbows, knees, heel or eyelids. 

8.1.1 Types of Hyperlipidemia:

Depending on the complexity of the disease, Hyperlipidemia classified into two types.

1) Primary Hyperlipidemia.

2) Secondary / Acquired Hyperlipidemia.

I) Primary Hyperlipidemia:

Several genetic conditions are known to responsible for primary Hyperlipidemia, such as lipoprotein lipase deficiency, apolipoprotein C-II deficiency etc. The primary hyperlipidemia may be treated by anti-lipidemic drugs. Primary Hyperlipidemia are again classified into 5 types

1. Type-I Hyperlipidemia: Severe elevation of chylomicrons (CMs) with resultant elevation of TGs.

2. Type-II (A) Hyperlipidemia: Elevations of LDL –C only.
3. Type-II (B) Hyperlipidemia: Elevations of both LDL-C and triglycerides (TG’s).

4. Type-III Hyperlipidemia: It develops due to defect in VLDL remnant Clearance.

5. Type-IV Hyperlipidemia: It is characterized by hyper TG’s

6. Type-V Hyperlipidemia: Characterized by elevated levels of CMs and VLDL.

II) Secondary Hyperlipidemia:

In this many factors can influence the level of TGs in circulation like diabetes, obesity etc. Secondary Hyperlipidemia demands treatment of original diseases rather than Hyperlipidemia.

Causes of secondary Hyperlipidemia:

A. Metabolic influences: Diabetes, obesity, hyperuricemia, glycogen storage diseases.

B. Harmonal influences: Insulin, estrogen, thyroxine

C. Nutritional influences:-Alcohol, high carbohydrate intake

D. Disease states:-Renal diseases, renal failure, nephrotic syndrome

E. Drugs: - Diuretics

Beta-blockers

Glucocorticoids

Estrogen replacement therapy
There are several secondary causes of abnormal lipids that may occur in adolescence. Children, infants and geriatritics have been shown to have higher levels of cholesterol, especially those who exhibit poor catch-up growth.\(^4\) The starved state that occurs in anorexia and the use of anabolic steroids are both associated with abnormal lipids. Certain medications for acne, seizure disorders, immunosuppression, and contraception can adversely affect lipids as can a high carbohydrate diet or a ketogenic diet sometimes prescribed for refractory epilepsy. Adolescents with a history of a transplant also tend to have an abnormal lipoprotein panel despite a TC in the normal range.

### 8.1.2 Risk factors:  

#### a. Positive risk factors:

1. Age (males > 45 years, females > 55 years or menopause < age 40)
2. Family history of premature coronary artery disease; definite myocardial infarction (MI) or sudden death before age 55 in father or other male first-degree relative, or before age 65 in mother or other female first-degree relative
3. Current cigarette smoker
4. Hypertension (systolic blood pressure > 140 mmHg or diastolic blood pressure > 90 mmHg confirmed on more than one occasion, or current therapy with antihypertensive medications)
5. Diabetes mellitus (DM)
6. High-density lipoprotein (HDL)-cholesterol < 40 mg/dl
b. Negative risk factor:

1. Elevated HDL cholesterol, > 60 mg/dl

8.1.3 Etiology:

The etiology can be classified into primary and secondary causes.

Primary causes are due to single or multiple gene mutations resulting in a disturbance of LDL and triglyceride production or clearance. They vary in location of genetic defect, inheritance pattern, prevalence, clinical features, and treatment. At least 18 separate entities have been described. The suspicion for a primary lipid disorder should be especially high in patients with premature atherosclerotic disease, a family history of early atherosclerotic disease, a significantly elevated serum cholesterol level (>240 mg/dl), are physical signs of hyperlipidemia. Primary dyslipidemia are most commonly seen in children, young adults and a small percentage of adults were prone to this disorder.

Most adult cases of dyslipidemia are secondary in nature. In Western civilizations, sedentary lifestyle and excessive consumption of saturated fats, trans-fatty acids, and cholesterol are the most important secondary causes. Certain medical conditions are commonly associated with dyslipidemia, including chronic renal insufficiency, renal failure, diabetes mellitus, hypothyroidism, cholestatic liver disease, and alcohol dependency. Certain drugs, including high-dose thiazide diuretics, oral estrogens, glucocorticoids, anabolic steroids, and atypical antipsychotics such as olanzapine and clozapine have also been implicated in causing mild-to-moderate degrees of dyslipidemia. Use of atypical
Antihyperlipidemic Studies

Antipsychotics, such as olanzapine and clozapine, and of beta-blockers without intrinsic sympathomimetic or alpha-blocking activities is associated with reduced HDL-cholesterol levels.

Table-8.01: Etiologies of Hyperlipidemia

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Elevated Particles</th>
<th>Major Lipid Abnormality</th>
<th>Frequency</th>
<th>Etiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Chylomicrons</td>
<td>TG</td>
<td>Very rare</td>
<td>LPL deficiency, apoC-II deficiency, inhibition of LPL (eg, systemic lupus erythematus)</td>
</tr>
<tr>
<td>IIA</td>
<td>LDL</td>
<td>LDL-C</td>
<td>Common</td>
<td>FH, FCH, polygenic hypercholesterolemia, hypothyroidism, renal disease, biliary tract disease, diabetes mellitus</td>
</tr>
<tr>
<td>IB</td>
<td>LDL and VLDL</td>
<td>LDL-C, TG</td>
<td>Common</td>
<td>Similar to type IIA HLP</td>
</tr>
<tr>
<td>III</td>
<td>IDL</td>
<td>TC, TG</td>
<td>Rare</td>
<td>ApoE-2 homozygosity (E-2/E-2) plus obesity, diabetes mellitus, renal disease, hypothyroidism, or liver disease</td>
</tr>
<tr>
<td>IV</td>
<td>VLDL</td>
<td>TG</td>
<td>Common</td>
<td>FCH, FH, metabolic/endocrine disease*, renal disease, liver disease, ethanol use/abuse, pregnancy, drug use.</td>
</tr>
<tr>
<td>V</td>
<td>Chylomicrons and VLDL</td>
<td>TG</td>
<td>Uncommon</td>
<td>Usually results from a combination of any two conditions that cause type IV HLP</td>
</tr>
</tbody>
</table>
8.1.4 Pathophysiology

Hypercholesterolemia develops as a consequence of abnormal lipoprotein metabolism, mainly reduction of LDL receptor expression or activity, and consequently diminishing hepatic LDL clearance from the plasma. It is a major predisposing risk factor for the development of atherosclerosis. This mechanism is classically seen in familial hypercholesterolemia and when excess saturated fat or cholesterol is ingested. In addition, excessive production of VLDL by the liver, as seen in familial combined hyperlipidemia and insulin resistance states such as abdominal obesity and Type -II diabetes, can also induce hypercholesterolemia or mixed dyslipidemia.

A current theory for the initiating event in atherogenesis is that apoprotein B-100 containing lipoproteins are retained in the sub endothelial space, by means of a charge-mediated interaction with extracellular matrix and proteoglycans. This allows reactive oxygen species to modify the surface phospholipids and unesterified cholesterol of the small LDL particles. Circulating LDL can also be taken up into macrophages through unregulated scavenger receptors. As a result of LDL oxidation, isoprostanes are formed. Isoprostanes are chemically stable, free radical-catalyzed products of arachidonic acid, and are structural isomers of conventional prostaglandins. Isoprostane levels are increased in atherosclerotic lesions, but they may also be found as F2 isoprostanes in the urine of asymptomatic patients with hypercholesterolemia.

A strong association exists between elevated plasma concentrations of oxidized LDL and CHD. The mechanisms through which oxidized LDL
promotes atherosclerosis are multiple and include damage to the endothelium, induction of growth factors, and recruitment of macrophages and monocytes.

Vasoconstriction in the setting of high levels of oxidized LDL seem to be related to a reduced release of the vasodilator nitric oxide from the damaged endothelial wall as well as increased platelet aggregation and thromboxane release. Smooth muscle proliferation has been linked to the release of cytokines from activated platelets.

The state of hypercholesterolemia leads invariably to an excess accumulation of oxidized LDL within the macrophages, thereby transforming them into "foam" cells. The rupture of these cells can lead to further damage of the vessel wall due to the release of oxygen free radicals, oxidized LDL, and intracellular enzymes.

This is a metabolically complex disease of lipid-lipoprotein metabolism and the exact etiology is not fully appreciated. The familial type in schnauzers may involve defects lipoprotein lipase and/or Apoprotein C-II, a required cofactor for lipoprotein lipase activity. This defect causes a failure to breakdown chylomicrons and VLDL, and results in excessive levels of circulating triglycerides. It is the elevated concentration of triglycerides that is responsible for the clinical signs.
8.1.5 Lipids\textsuperscript{12}: Lipids are a group of naturally occurring fatty substances, which are present in the blood and tissues of the body. They include cholesterol, cholesterol esters, triglycerides, and phospholipids. Lipids are essential dietary constituents because of their important functions.

8.1.6 Classification of lipids:-

- Fatty acids (palmetic, linoleic, etc)
- Glycerol esters (triglycerides)
- Sterols (cholesterol, hormones, vitamin D)
- Terpenes (vitamin A, E, K)
- Sphingosine derivatives (sphingomyelin)

8.1.7 Lipid functions\textsuperscript{13}:-

- Provide energy required by the body
- Serve as the major structural components of cell membranes
- Aid in the efficient absorption of fat-soluble vitamins
- Serve as insulating material beneath the skin and around certain organs (e.g. kidneys)
- Serve as biosynthetic precursors (e.g., Cholesterol is a precursor for adrenal and gonadal steroid hormones and hepatic bile acids.)
Lipids are insoluble in blood (plasma), they must be transported to the cells by special carriers called lipoproteins. Lipoproteins are spherical particles of high molecular weight. Each lipoprotein particle contains a non-polar core and a hydrophilic surface. The hydrophilic surface makes the lipoprotein soluble in plasma and acts as an interface between the plasma and lipid core. The core consists of hydrophobic lipids, triglycerides and cholesterol esters, surrounded by a hydrophilic surface coat of phospholipids, unesterified cholesterol, and specific proteins termed apolipoproteins or apoproteins. The apolipoproteins provide structural integrity to the lipoproteins and determine the lipoproteins’ metabolic fate by serving as binding sites for receptors and activating enzymes involved in lipid metabolism.

**Table: 8.02 Evaluation of Lipid Levels for People at High Risk for CHD**

<table>
<thead>
<tr>
<th></th>
<th>Total Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>LDL Cholesterol (mg/dl)</th>
<th>HDL Cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desirable levels</td>
<td>&lt;200</td>
<td>&lt;150</td>
<td>&lt;130</td>
<td>&gt;35</td>
</tr>
<tr>
<td>Borderline risk</td>
<td>200-239</td>
<td>150-200</td>
<td>130-159</td>
<td>30-35</td>
</tr>
<tr>
<td>High risk</td>
<td>&gt;240</td>
<td>&gt;200</td>
<td>&gt;160</td>
<td>&lt;30</td>
</tr>
</tbody>
</table>

Modestly elevated Lp (a) levels = 20-30 mg/dl

There is a 10-fold risk of heart attack when Lp (a) levels are > 50 mg/dl in people with high cholesterol levels.
8.1.8 Pathways of Lipid Transport

Cholesterol is absorbed from the intestine and transported to the liver by chylomicron remnants, which are taken up by the low density lipoprotein (LDL)-receptor related protein (LRP). Hepatic cholesterol enters the circulation as very-low-density lipoprotein (VLDL) and is metabolized to remnant lipoproteins after lipoprotein lipase removes triglyceride. The remnant lipoproteins are removed by LDL receptors (LDL-R) or further metabolized to LDL and then removed by these receptors. Cholesterol is transported from peripheral cells to the liver by high-density lipoprotein (HDL). Cholesterol is recycled to LDL and VLDL by cholesterol-ester transport protein (CETP) or is taken up in the liver by hepatic lipase. Cholesterol is excreted in bile. The points in the process that are affected by the five primary lipoprotein disorders and familial hypertriglyceridemia (FHTG), Familial combined hyperlipidemia (FCHL), remnant removal disease (RRD, also known as familial dys-beta-lipoproteinemia), Familial hypercholesterolemia (FH), and hypo-alpha- lipoproteinemia shown. The effects of drug therapy can also be understood from these pathways. Statins decrease the synthesis of cholesterol and the secretion of VLDL and increase the activity of LDL receptors. Bile-acid binding resins increase the secretion of bile acids. Nicotinic acid decreases the secretion of VLDL and the formation of LDL and increases the formation of HDL. Fibrates decrease the secretion of VLDL and increase the activity of lipoprotein lipase, thereby increasing the removal of triglycerides.
Fig:8.03 Pathways of cholesterol movement in the body
### Table-8.03 : Enzymes of importance in lipid transport & metabolism\textsuperscript{15,16.}

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipoprotein lipase</td>
<td>Hydrolysis of TG rich particles some phospholipase activity activated by APO C-II</td>
</tr>
<tr>
<td>Hepatic lipase</td>
<td>Hydrolysis of Tri-, Di- and mono acyl-glycerol’s, Acyl-CoA thioester and phospholipids conversion of HDL2 to HDL1 activated by APO A-I II</td>
</tr>
<tr>
<td>Pancreatic lipase</td>
<td>Hydrolysis of FAs at positions 1 and 3 of emulsified TGs in the intestine.</td>
</tr>
<tr>
<td>Lecithin cholesterol Acyl transferase</td>
<td>Catalysis of lecithin with cholesterol to give lysolecithin and cholesteryl ester activated by APO A-I and APO c-I.</td>
</tr>
<tr>
<td>LCAT</td>
<td></td>
</tr>
<tr>
<td>Pancreatic cholesteroesterase</td>
<td>Esterification of cholesterol in the intestinal lumen</td>
</tr>
<tr>
<td>Acyl co A-cholesterol acyl transferase ACAT</td>
<td>Esterification of cholesterol within the cells</td>
</tr>
<tr>
<td>Cholesterol ester transfer protein CETP</td>
<td>Transfers esterified cholesterol from HDLs to VLDLs and LDLs.</td>
</tr>
<tr>
<td>HMG Co A reductase</td>
<td>Rate limiting enzyme of cholesterol synthesis</td>
</tr>
</tbody>
</table>
8.1.9 Lipoproteins:

Lipoproteins are proteins carrying lipids; cholesterol is one of the lipids. Long chain fatty acids are also carried by these lipoproteins in the form of TG.

Figure: 8.04 General structure of lipoprotein

There are several forms of lipoproteins, very low density lipoproteins, intermediate density lipoproteins (IDL), low density lipoproteins (LDL), and high density lipoproteins (HDL). Lipoproteins are large globular particles which transport cholesterol and triglycerides in the blood stream. They consist of central core of cholesterol or cholesterol esters encased in a hydrophilic coat of polar substance, i.e. phospholipids, free cholesterol and associated proteins (apoprotein).
### Table 8.04: Classification of Lipoproteins

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Composition</th>
<th>Density</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicrons</td>
<td>TG &gt;&gt; C, CE</td>
<td>Low</td>
<td>Large</td>
</tr>
<tr>
<td>VLDL</td>
<td>TG &gt; CE</td>
<td>Higher than chylomicrons</td>
<td>Smaller than chylomicrons</td>
</tr>
<tr>
<td>ILDL</td>
<td>CE &gt; TG</td>
<td>Higher than VLDL</td>
<td>Smaller than VLDL</td>
</tr>
<tr>
<td>LDL</td>
<td>CE &gt;&gt; TG</td>
<td>Higher than ILDL</td>
<td>Smaller than ILDL</td>
</tr>
<tr>
<td>HDL</td>
<td>CE &gt; TG</td>
<td>Highest</td>
<td>Smallest</td>
</tr>
</tbody>
</table>

8.1.10 The six major classes of plasma lipoproteins are:

**a. Chylomicrons:**

These are the largest species of triglyceride rich lipoprotein which are involved in the transportation of dietary fat from intestine to liver.

These are secreted into the lymph. (Fats are broken down into fatty acids in the digestive tract, and then packaged together in groups of three. A triglyceride contains three fatty acids attached to glycerol.).

Fat digesting enzymes break down chylomicrons fairly quickly, so most are gone from the blood after a 12 to 14 hour fast.
b. VLDL: VLDL or "very low-density lipoproteins" involved in transport of endogenous lipids from liver to plasma. Formed in the liver and intestines, VLDL carry about 10 to 15 percent of the cholesterol found in blood.

VLDL delivers cholesterol and triglycerides to cells, which in turn put these lipid products to use.

\[
\text{VLDL} \xrightarrow{\text{Lipase}} \text{IDL} \xrightarrow{\text{Lipase}} \text{LDL}
\]

Through the action of fat-digesting enzymes, VLDL becomes progressively smaller as it circulates through the bloodstream. VLDL also converts to LDL, which is the chief culprit in atherosclerosis.

c. Total cholesterol:

An increasing total cholesterol level is associated with an increased risk of CHD (coronary heart disease).

```
Total cholesterol mg/dl = \frac{\text{Abs TC}}{\text{Abs STD}} \times 200
```

“Desirable” total cholesterol is usually <200>

However, most decisions about treatment are made based upon the level of LDL or HDL cholesterol, rather than the total cholesterol. The total cholesterol can be measured any time of day, without fasting.
d. LDL cholesterol:

The low density lipoprotein (LDL) cholesterol (sometimes called "bad cholesterol") is a more accurate predictor of CHD than total cholesterol.

\[
[\text{LDL-chol}] = [\text{Total chol}] - [\text{HDL-chol}] - (\frac{\text{TG}}{5})
\]

Where all concentrations are given in mg/dl.

Higher LDL cholesterol concentrations have been associated with an increased incidence of CHD in a large number of studies. They have longest plasma half-life, of about 1.5 days.

Ideally, LDL cholesterol levels should be less than 100 mg/dl in patients who have had CHD in the past or CHD risk equivalents. People with levels of 160 mg/dl or higher have a high risk of CHD. Intermediate levels — 130 to 159 mg/dl — predict an intermediate risk of CHD.
Antihyperlipidemic Studies

LDL particles are finally delivered to hepatic and certain extra hepatic tissues for further liposomal degradation to release the cholesterol which can be utilized in cell membrane formation.

The LDL cholesterol can only be determined accurately on a blood test after fasting for 12 to 14 hours.

e. IDL cholesterol:-

These are the lipoproteins obtained when the triglyceride content of VLDL are partially digested in capillaries by the action of extra hepatic lipoprotein lipase and having the diameter of 20-35 nm.

f. Triglycerides:

Elevated levels of triglycerides are also associated with an increased risk of CHD.

- Normal - less than 150 mg/dl (1.69 mmol/l)
- Borderline high - 150 to 199 mg/dl (1.69 to 2.25 mmol/l)
- High - 200 to 499 mg/dl (2.25 to 5.63 mmol/l)
- Very high - greater than 500 mg/dl (5.65 mmol/l)

Triglycerides in mg/dl = Abs T/Abs STD×200

Like LDL cholesterol, triglycerides should only be measured in a blood specimen obtained after fasting for 12 to 14 hours.
**g. HDL cholesterol:**

This is a group of heterogeneous lipoprotein having low lipid content and is also called as good cholesterol. HDL enhances the removal of cholesterol from the arterial wall. Hence, chances of development of atherosclerotic lesions are more when HDL value falls below normal.

Similar to total cholesterol, the HDL-cholesterol can be measured in blood specimen without fasting.

\[
\text{HDL cholesterol mg/dl} = \frac{\text{Abs TH}}{\text{Abs STD}} \times 50
\]

**Conversion Factors:**

- **Cholesterol:** \( \text{mmol/L} \times 38.7 = \text{mg/dl} \) \( \text{mg/dl} \times 0.026 = \text{mmol/L} \)
- **Triglycerides:** \( \text{mmol/L} \times 885.5 = \text{mg/dl} \) \( \text{mg/dl} \times 0.0113 = \text{mmol/L} \)
- **Phospholipids:** \( \text{g/L} \times 0.01 = \text{mg/dl} \) \( \text{mg/dl} \times 10 = \text{g/L} \)

Simple blood tests can determine levels of Lipoproteins. Including Total cholesterol, LDL and HDL cholesterol, and triglycerides.
8.1.11 Some of the risk factors for developing hyperlipoproteinemia are:

- Obesity
- Diabetes
- Hyperthyroidism
- Nephrotic disorder
- Liver disease
- Hypertension
- Family history of high cholesterol or heart disease
- Diet high in fat and cholesterol
- Cigarette smoking

8.1.12 Causes:

Hyperlipidemia is caused by lifestyle habits or treatable medical conditions. Lifestyle habits include obesity, sedentary life without exercise, smoking. Medical diseases that may result in Hyperlipidemia are diabetes, kidney disorders, pregnancy, and an underactive thyroid gland. Common secondary causes of hypercholesterolemia are hypothyroidism, pregnancy, and Kidney failure. Common secondary causes of hypertriglyceridemia are diabetes, excess alcohol intake, obesity, and certain prescription medications\(^\text{18}\).
8.1.13 Symptoms and diagnoses of Hyperlipidemia:\textsuperscript{19}

Generally hyperlipidemia condition does not show apparent symptoms and it is discovered and diagnosed during routine examination or evaluation for atherosclerotic cardiovascular disease. However, deposits of cholesterol may be formed under the skin in individuals with familial forms of the disorder or in persons with very high levels of cholesterol in the blood. In individuals with hypertriglyceridemia, several pimple-like lesions may be developed across their bodies. Pancreatitis, a severe inflammation of the pancreas that may be life-threatening can also be developed due to extremely high levels of triglycerides. For diagnosis of hyperlipidemia, levels of total cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol, and triglycerides are measured in blood sample. It is important to note that the lipid profile should be measured in all adults 20 years and older, and the measurement should be repeated after every 5 years. Food or beverages may increase triglyceride levels temporarily, so people must fast at least 12 hours before giving their blood samples. Blood tests are carried out to identify the specific disorders, when lipid levels in the blood are very high. Specific disorders may include several hereditary disorders, which produce different lipid abnormalities and have different risks.
8.1.14 **Laboratory Testing:** Patients are subjected to fasting for at least 12 hours before collecting the blood sampling. Because, chylomicron clearance can take up to 10 hours. However, a fasted sample is not required for simple cholesterol screening.

Laboratory testing of the lipid profile measures total plasma cholesterol, HDL, and triglycerides levels directly. VLDL cholesterol levels are calculated by dividing the triglyceride value by 5. LDL cholesterol is calculated by subtracting HDL cholesterol and VLDL cholesterol from total cholesterol. When triglycerides are above 400 mg/dl, LDL calculation is inaccurate, and specialized laboratory tests are required.

8.1.15 **Treatment**\textsuperscript{20,21}:

The goals of treatment are to lower total and LDL cholesterol in order to reduce the risk of first or recurrent events such as

- Myocardial infarction,
- Angina,
- Heart failure,
- Ischemic stroke, or other forms of peripheral arterial disease such as carotid stenosis or abdominal aortic aneurysm
Most patients should receive 3 month TLC trial before initiating pharmacologic therapy unless very high risk

If patient unable to reach goals with TLC alone choose lipid-lowering drugs based on lipoprotein disorder

Combination therapy may be necessary to monitor closely: increased risk of drug interactions, adverse effects

8.1.16 General approach:

The National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) recommends that a fasting lipoprotein profile and risk factor assessment be used in the initial classification of adults.

There are four categories of risk that modify the goals and modalities of LDL-lowering therapy.

The highest risk category is having known CHD or CHD risk equivalents; the risk for major coronary events is equal to or greater than that for established CHD (i.e., >20% per 10 years, or 2% per year).

The next category is moderately high risk, consisting of patients with two or more risk factors in which 10-year risk for CHD is 10% to 20%.

The lowest risk category is persons with zero to one risk factor, which is usually associated with a 10-year CHD risk of <10%.

ATP III recognizes the metabolic syndrome as a secondary target of risk reduction after LDL-C has been addressed.
This syndrome is characterized by abnormal obesity, atherogenic dyslipidemia (elevated triglycerides, small LDL particles, low HDL cholesterol), increased blood pressure, insulin resistance (with or without glucose intolerance), and pro thrombotic and pro inflammatory states.

If the metabolic syndrome is present, the patient is considered to have a CHD risk equivalent.

Other targets include non-HDL goals for patients with triglycerides >200mg/dl.

Non-HDL cholesterol is calculated by subtracting HDL from total cholesterol, and the targets are 30 mg/dl greater than for LDL at each risk stratum.

8.1.17 Pharmacologic Therapy:

Treat all secondary problems resulting from acute or chronic disease (e.g. Diabetes, seizures)

After proper diagnosis of hyper lipoproteinemia and before medicinal intervention, lifestyle changes and risk factor reduction is warranted. This includes diet modification, weight loss, exercise, smoking cessation, and control of underlying disorders such as diabetes and hypertension. Such changes can lead to significant reductions in plasma lipoproteins. If dietary and lifestyle changes fail, hypolipoproteinemic (cholesterol-reducing) drugs are advised. The types of drugs used to lower blood lipids and block atherogenesis are:
1. Cholesterol biosynthesis inhibitors (e.g. Lovastatin)
2. Fibrates (e.g. Clofibrate)
3. Bile acid sequesterants (e.g. Cholestyramine)
4. Cholesterol absorption inhibitors
5. LDL oxidation inhibitors (e.g. Probucol)

8.1.18 Experimental Induced Methods of Hyperlipidemia:

I. Cholesterol Induced Hyperlipidemia:

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases. Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death. Hyperlipidemia is characterized by elevated serum total cholesterol, low density lipoprotein, very low density lipoprotein and decreased high density lipoprotein levels. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease. Among these hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease. 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. It is actively involved in the screening of herbal formulations and synthetic drugs for its anti-hyperlipidemic activity.\textsuperscript{23}
II. Atherogenic Diet Induced Hyperlipidemia in Rats:

In rats, hyperlipidemia can be induced by daily oral administration of 1% cholesterol, 0.5% Cholic acid suspended in 25% coconut oil over a period of 26 days. The test compounds were administered simultaneously along with cholesterol diet.

High intake of saturated fat and cholesterol increases serum LDL-C, probably by decreasing the amount of and/or activity of LDL receptors in the liver. Elevated and modified LDL is one of the principal factors in the development of atherosclerosis. Feeding the high fat diets causes fatty liver with accumulation of TG and TC$^{24}$.

III. Fructose Induced Hyperlipidemia in Rats$^{25}$:

Carbohydrate, fructose plays an important role in the pathogenesis of experimental and clinical hypertriglyceridemia and hyperinsulinemia. High fructose fed (HFF) diet induces significant hyperinsulinemia and hypertriglyceridemia in rats. An adverse effect of fructose on insulin sensitivity of rat is well established. This phenomenon is believed to be related hypertriglyceridemic effect of fructose. In rats hyperlipidemia can be produced by administration of fructose (66% fructose), once daily for 30 days.

Fructose feeding stimulates the hepatic production TGs, both by promoting the reesterification of circulating non-esterified FAs and by stimulating fatty acid synthesis. Increased delivery of TGs to the muscle interferes with the utilization of glucose, through the principles of Randle cycle, impairing the insulin action.
IV. Triton WR 1339(TR) Induced Hyperlipidemia in Rats:

The systemic administration of the nonionic surfactant TR (iso octyl polyoxyethylene phenol /Tyloxipal) to rats results in a biphasic elevation of plasma cholesterol and TGs. TR induced hyperlipidemia occurs in 2 phases.

**Phase –I (synthesis phase):**

It is thought to be due to increased hepatic synthesis of cholesterol, which reaches the elevated lipid level at the end of 24th hr through the ability to interfere with the uptake of plasma lipid levels, by the tissues. Drugs interfering with cholesterol biosynthesis were shown to be active in this phase.

**Phase –II (excretory phase):**

In this phase, the elevated lipid levels almost reach normal by the end of 48th hr. While drugs interfering with cholesterol excretion and metabolism were shown to be active in this phase.

The biphasic nature of TR induced hyperlipidemia is helpful in understanding the mode of action of hypolipidemic agents.
8.2 MATERIALS AND METHODS:

Wistar albino rats of either sex (150-200gm)

Petroleum ether extract of *V. mung*

Chloroform extract of *V. mung*

Ethanolic extract of *V. mung*

Petroleum ether extract of *V. radiata*

Chloroform extract of *V. radiata*

Ethanolic extract of *V. radiata*

Petroleum ether extract of *V. unguiculata*

Chloroform extract of *V. unguiculata*

Ethanolic extract of *V. unguiculata*

High Cholesterol diet pellets

5% Aqueous gum acacia

**Animal models:** Wistar albino rats

**Standard drug:** Atorvastatin- ATOCOR™ 80 supplied by Dr.Reddy Labs
8.3 EXPERIMENTAL PROCEDURE

In the present study we aimed to screen various extracts of selected plants on Cholesterol induced hyperlipidemic rat model\textsuperscript{27,28}.

Healthy Wistar albino rats weighing between 150-200gm were acclimatized to the laboratory at temperature \((25\pm1)\)°c, relative humidity \((50\pm15)\) %, 12hrs light-dark cycles, kept in standard polypropylene cages and given standard diet and water \textit{ad-libitum}. The animals were divided into control, toxic, standard and test groups of V.M.P.E 100mg/kg, V.M.C.E 100mg/kg, V.M.E.E 100mg/kg b.w p.o, suspended in 5% gum acacia solution, daily once. Each comprising of 6 animals in all sets of experiments. Animals in the normal control group, received normal saline orally. Except control group rest other groups were fed with rich cholesterol diet pellets supplied by M/s Rayans biotechnologies Pvt.Ltd., Hyderabad. Standard group received Atorvastatin 10mg/kg b.w p.o suspended in 5% gum acacia solution. The treatment was given for 20 days. In between mean body weight of the animals was checked time to time. Feeding the animals with cholesterol supplied diet induces hyperlipidemia, especially hypercholesterolemia and hypertriglycerideridemia. Cholesterol feeding has been often used to elevate serum or tissue cholesterol levels to assess hypercholesterolemia-related metabolic disturbances in experimental animal .On 21\textsuperscript{st} day the blood samples were withdrawn from the arterial damage. All the lipid profile parameters were determined. Total cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL), very low density lipoproteins (VLDL), low density lipoproteins (LDL) were analysed from serum.
Antihyperlipidemic Studies

Same control, Toxic and standard groups were maintained for all the three selected plant extracts, for screening antihyperlipidemic activity against cholesterol induced diet in wistar albino rat model.

Table:8.05  Protocol for study of antihyperlipidemic activity using whole plant extracts of Vigna mung Linn in albino rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (20)days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Normal saline</td>
</tr>
<tr>
<td>Group II</td>
<td>Cholesterol diet</td>
</tr>
<tr>
<td>Group III</td>
<td>Cholesterol diet + Atorvastatin (10mg/kg b.w) suspended in 5% gum acacia solution</td>
</tr>
<tr>
<td>Group IV</td>
<td>Cholesterol diet + V.M.P.E (100 mg/kg b.w) suspended in 5% gum acacia solution</td>
</tr>
<tr>
<td>Group V</td>
<td>Cholesterol diet + V.M.C.E (100mg/kg b.w) suspended in 5% gum acacia solution</td>
</tr>
<tr>
<td>Group VI</td>
<td>Cholesterol diet + V.M.E.E (100mg/kg b.w) suspended in 5% gum acacia solution</td>
</tr>
</tbody>
</table>
Table :8.06 :Protocol for study of antihyperlipidemic activity on whole plant extracts of *Vigna radiate* Linn in albino rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (20)days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Normal saline</td>
</tr>
<tr>
<td>Group II</td>
<td>Cholesterol diet</td>
</tr>
<tr>
<td>Group III</td>
<td>Cholesterol diet + Atorvastatin (10mg/kg b.w) suspended in 5% gum acacia solution</td>
</tr>
<tr>
<td>Group IV</td>
<td>Cholesterol diet + V.R.P.E (100 mg/kg b.w) suspended in 5% gum acacia solution</td>
</tr>
<tr>
<td>Group V</td>
<td>Cholesterol diet + V.R.C.E (100mg/kg b.w) suspended in 5% gum acacia solution</td>
</tr>
<tr>
<td>Group VI</td>
<td>Cholesterol diet + V.R.E.E (100mg/kg b.w) suspended in 5% gum acacia solution</td>
</tr>
</tbody>
</table>

The animals were divided into control, toxic, standard and test extracts of V.R.P.E 100mg/kg, V.R.C.E 100mg/kg, V.R.E.E 100mg/kg groups. Each comprising of 6 animals in all sets of experiments. Animals in the normal control group received normal saline orally. Except control group rest other groups were fed with rich cholesterol diet pellets. Standard group received atorvastatin 10mg/kg orally. The treatment was given for 20 days. In between mean body weight of the animals was checked time to time. On 21st day the blood samples were withdrawn from
the arterial damage. Total cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL), low density lipoproteins (LDL) were analysed from serum.

**Table:8.07 Protocol for study of antihyperlipidemic activity on whole plant extracts of *Vigna unguiculata* Linn in albino rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (20)days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Normal saline</td>
</tr>
<tr>
<td>Group II</td>
<td>Cholesterol diet</td>
</tr>
<tr>
<td>Group III</td>
<td>Cholesterol diet + Atorvastatin (10mg/kg b.w) suspended in 5% gum acacia solution</td>
</tr>
<tr>
<td>Group IV</td>
<td>Cholesterol diet + V.U.P.E (100 mg/kg b.w) suspended in 5% gum acacia solution</td>
</tr>
<tr>
<td>Group V</td>
<td>Cholesterol diet + V.U.C.E (100mg/kg b.w) suspended in 5% gum acacia solution</td>
</tr>
<tr>
<td>Group VI</td>
<td>Cholesterol diet + V.U.E.E (100mg/kg b.w) suspended in 5% gum acacia solution</td>
</tr>
</tbody>
</table>

The animals were divided into control, toxic, standard and test extracts of V.U.P.E 100mg/kg, V.U.C.E 100mg/kg, V.U.E.E 100mg/kg groups. Each comprising of 6 animals in all sets of experiments. Animals in the normal control group received normal saline orally. Except control group rest other groups were fed with rich cholesterol diet pellets. Standard group received atorvastatin.
10mg/kg orally. The treatment was given for 20 days. In between mean body weight of the animals was checked time to time. On 21st day the blood samples were withdrawn from the arterial damage. Total cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL), low density lipoproteins (LDL) were analysed from serum.

8.3.1 Biochemical Estimations:-

At the end of experimental period, rats were anesthetized with ether. Blood samples were collected by cardiac puncture method. Serum total cholesterol, triglycerides, high density lipoproteins- cholesterol using beacon diagnostic Pvt ltd kits. Serum LDL, VLDL was determined by calculation.

8.3.2 Procedures for testing parameters:

1. Estimation of serum of triglycerides:

Diagnostic kit was used for estimation of triglycerides, which followed end point colorimetry enzymatic test using glycerol-3-phosphate oxidase.

Principle:

The enzyme, lipoprotein lipase catalyzes hydrolysis of TGs to glycerol and Free acids. Glycerol then is phosphorylated in an ATP - requiring reaction catalyzed by glycerophosphate. The formed glycerophosphate is oxidized to dihydroxyacetone and H₂O₂ in a glycerophosphate oxidase catalyzed reaction. H₂O₂ then reacts with 4 -AAP and 4 -chlorophenol under the catalytic influence of
peroxidase to form coloured quinoneimine complex, the intensity of which was measured at 505nm

\[
\text{Lipase}
\]

\[
\text{Triglyceride} + 3\text{H}_2\text{O} \rightarrow \text{Glycerol} + 3 \text{fatty acids}
\]

\[
\text{Glycerokinase}
\]

\[
\text{Glycerol} + \text{ATP} \rightarrow \text{Glycerol} -3\text{-phosphate} + \text{ADP}
\]

\[
\text{Glycerophosphate oxidase}
\]

\[
\text{Glycerol} -3\text{-Phosphate} + \text{O}_2 \rightarrow \text{DHAP} + \text{H}_2\text{O}
\]

\[
\text{Peroxidase}
\]

\[
2\text{H}_2\text{O} + 4\text{-AAP} + 4\text{-chlorophenol} \rightarrow \text{Quinoneimine} + \text{HCl} + 4\text{H}_2\text{O}
\]
### Table: 8.08: Reagents used

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Reagent composition</th>
<th>Conc. in the final test mixed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pipes butter 50mmol/l</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>4-Chlorophenol 5mmol/l</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Mg$^{2+}$ 5 mmol/l</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>ATP 1 mmol/l</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Lipase $\geq$ 5000 U/l</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Peroxidase $\geq$ 1000 U/l</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Glycerol Kinase $\geq$ 400 U/l</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Glycerol - 3- phosphate oxidase $\geq$ 4000 U/l</td>
<td></td>
</tr>
</tbody>
</table>

**Standard:** The concentration of standard triglyceride used was 200mg/dl

**Assay & Procedure:** Fresh clear and unhaemolysed serum was used for the estimation.
Table: 8.09: Reaction parameters:

<table>
<thead>
<tr>
<th></th>
<th>Reaction type</th>
<th>End point</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Wave Length</td>
<td>505 nm</td>
</tr>
<tr>
<td>3</td>
<td>Optical length</td>
<td>1 Cm</td>
</tr>
<tr>
<td>4</td>
<td>Temperature</td>
<td>(37^\circ C)</td>
</tr>
<tr>
<td>5</td>
<td>Measurement</td>
<td>Against reagent blank</td>
</tr>
</tbody>
</table>

Table: 8.10 Summary of assay details:

<table>
<thead>
<tr>
<th>Pipetted in to test tube</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>1000 μl</td>
<td>1000 μl</td>
<td>1000 μl</td>
</tr>
<tr>
<td>Standard Triglyceride (200mg/dl)</td>
<td>-</td>
<td>10 μl</td>
<td>-</td>
</tr>
<tr>
<td>Sample (serum)</td>
<td>-</td>
<td>-</td>
<td>10 μl</td>
</tr>
</tbody>
</table>

The reaction mixtures were mixed well and incubated for 10 min at \(37^\circ C\). The absorbance of sample and standard were measured against reagent blank at 505 nm.

**Calculations:**

Serum triglycerides (mg/dl) = \(\frac{\text{Abs of test}}{\text{Abs of STD}} \times \text{Conc of standard}\)
2. Estimation of serum Total Cholesterol:-

The reagents kits intended for the \textit{In-vitro} quantitative determination of cholesterol in serum/plasma.

\textbf{Principle: -}

The cholesterol esters are hydrolysed by enzyme cholesterol esterase to give free cholesterol and fatty acid molecules. This free cholesterol gets oxidised in presence of cholesterol oxidase to liberate cholest4en-3one and H$_2$O$_2$. Liberated H$_2$O$_2$ by this reaction combines with phenol and 4 amino antipyrine in presence of peroxidase to form red colour quinonimine complex, the intensity of which is measured at 505 nm.

\textbf{General system parameters: -}

Wave length: 505 nm (490-530nm)

Incubation: 5 min

Sample volume: 10\textmu l

Reagent volume: 1.0ml

Standard concentration: 200mg/dl
Table: 8.11: Process for estimation of Total cholesterol levels

<table>
<thead>
<tr>
<th></th>
<th>Procedure for 1ml</th>
<th>Procedure for 3ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>S</td>
</tr>
<tr>
<td>Enzyme reagent</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>Standard cholesterol (200mg/dl)</td>
<td>-</td>
<td>10µl</td>
</tr>
<tr>
<td>Sample (serum)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Procedure:**

Bring all the reagents of assay to room temperature. Mix well and incubate for 5 min at room temperature. Mix well and measure the absorbance of standard and test against the reagent blank at 505 nm.

**Calculation:**

\[
\text{Total cholesterol mg/dl} = \frac{\text{Abs TC}}{\text{Abs STD}} \times 200
\]
3. Estimation of serum High-Density Lipoprotein cholesterol (HDL-C):

Diagnostic kit was used for estimation of HDL cholesterol, which followed Cholesterol oxidase / peroxidase (CHOD-POD) method.

**Principle:**

HDL-C is measured in the supernatant after the precipitation of the lipoproteins including chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), intermediate-density lipoproteins (ILDL) directly from serum polyanions like phosphotungstic acid and along with MgCl$_2$ are added to an aliquot of serum an immediate heavy precipitation is formed. From the clear supernatant and HDL cholesterol is measured. Which, is estimated by enzymatic method as described earlier in estimation serum of TC.$^{91}$

**Table 8.12: Reaction parameters:**

<table>
<thead>
<tr>
<th></th>
<th>Reaction type</th>
<th>End point</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Wavelength</td>
<td>505 nm</td>
</tr>
<tr>
<td>3</td>
<td>Optical path</td>
<td>1 cm</td>
</tr>
<tr>
<td>4</td>
<td>Temperature</td>
<td>$37^\circ$ C</td>
</tr>
<tr>
<td>5</td>
<td>Measurement</td>
<td>Against reagent blank</td>
</tr>
</tbody>
</table>
Preparation:-

Take 0.5 ml of serum/plasma in to glass tube. Add 50 µl precipitating reagent. Mix well, leave it at R.T. For 10 min. centrifuge at 3000 r.p.m. for 10 min, take the clear supernatant for HDL cholesterol estimation.

Table:8.13 : Procedure for cholesterol estimation

<table>
<thead>
<tr>
<th>Enzyme reagent</th>
<th>B</th>
<th>S</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>0.01 ml</td>
<td>-</td>
</tr>
<tr>
<td>Supernatant sample</td>
<td>-</td>
<td>-</td>
<td>0.01 ml</td>
</tr>
</tbody>
</table>

Mix well and incubate for 5 min at 37°c. Measure the absorbance of HDL & STD at 510 nm.

Calculations: - HDL cholesterol mg/dl = Abs T/Abs STD×200

4. Estimation of VLDL:

It can be determined by dividing Triglycerides with 5.

5. Estimation of LDL:

The difference between HDL and VLDL gives LDL value.
Table: 8.14: Effect of *Vigna mung* Linn extracts on body weight of hyperlipidemic rat models:

<table>
<thead>
<tr>
<th>Days</th>
<th>Mean body weight(gm) change in body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>0(^{th}) day</td>
<td>140</td>
</tr>
<tr>
<td>5(^{th}) day</td>
<td>143</td>
</tr>
<tr>
<td>10(^{th}) day</td>
<td>148</td>
</tr>
<tr>
<td>15(^{th}) day</td>
<td>151</td>
</tr>
<tr>
<td>20(^{th}) day</td>
<td>155</td>
</tr>
</tbody>
</table>

The body weight of rats increased from the 0\(^{th}\) day to 20\(^{th}\) day. There is a significant increase in the body weight of cholesterol treated rats, when compare to the normal rats. *V.mung* petroleum ether extract of 100mg/kg b.w p.o treated group also showed significant increase in the body weight, when compare to the normal rats. Less significant increase in the body weight of standard, V.M.E.E & V.M.C.E 100mg/kg treated group.
**Anti Hyperlipidemic Studies**

Table 8.15: Effect of *Vigna mung* Linn extracts in Cholesterol induced hyperlipidemic rat

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>64.89±2.280</td>
<td>53.90±1.666</td>
<td>36.15±1.125</td>
<td>11.76±0.3387</td>
<td>16.00±2.656</td>
</tr>
<tr>
<td>CHOLESTEROL</td>
<td>177.20±2.698**</td>
<td>149.13±2.165**</td>
<td>20.71±1.221**</td>
<td>29.23±0.4326**</td>
<td>116.26±3.507**</td>
</tr>
<tr>
<td>STANDARD</td>
<td>100.22±0.9657*</td>
<td>93.95±1.205*</td>
<td>32.51±0.7098**</td>
<td>18.78±0.2407*</td>
<td>48.89±0.7986*</td>
</tr>
<tr>
<td>V.M.P.E (100mg/kg)</td>
<td>175.61±2.314*</td>
<td>155.12±1.321*</td>
<td>16.23±0.148</td>
<td>36.41±0.552*</td>
<td>119.63±0.143*</td>
</tr>
<tr>
<td>V.M.C.E (100mg/kg)</td>
<td>130.23±1.641*</td>
<td>117.24±2.464*</td>
<td>28.11±0.631</td>
<td>25.34±0.414*</td>
<td>88.61±0.241*</td>
</tr>
<tr>
<td>V.M.E.E (100mg/kg)</td>
<td>169.11±2.412*</td>
<td>140.21±2.314*</td>
<td>20.14±0.145</td>
<td>30.24±0.326*</td>
<td>110.23±0.341*</td>
</tr>
</tbody>
</table>

Values are mean±SEM, n=6 **p<0.01, when compared with control Group
* p<0.01, when compared with Toxic Group
Figure: 8.06 Graphical representation of *Vigna mung* Linn extracts on cholesterol diet induced hyperlipidemic model in wistar albino rats
Table: 8.16: Effect of *Vigna radiate* Linn extracts on body weight of hyperlipidemic rat models

<table>
<thead>
<tr>
<th>Days</th>
<th>Mean body weight(gm) change in body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>0(^{th}) day</td>
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<tr>
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</tr>
<tr>
<td>10(^{th}) day</td>
<td>148</td>
</tr>
<tr>
<td>15(^{th}) day</td>
<td>151</td>
</tr>
<tr>
<td>20(^{th}) day</td>
<td>155</td>
</tr>
</tbody>
</table>

The body weight of rats increased from the 0 day to 20\(^{th}\) day. There is a significant increase in the body weight of cholesterol treated rats, when compare to the normal rats. *V. radiata* petroleum ether extract of 100mg/kg b.w p.o treated group also showed significant increase in the body weight, when compare to the normal rats. Less significant increase in the body weight of standard, *V. radiata* ethanolic extract & *V. radiata* chloroform extract 100mg/kg treated group.
## Anti Hyperlipidemic Studies

Table: 8.17: Effect of *Vigna radiate* Linn extracts in Cholesterol induced hyperlipidemic rat

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>64.89±2.280</td>
<td>53.90±1.666</td>
<td>36.15±1.125</td>
<td>11.76±0.3387</td>
<td>16.00±2.656</td>
</tr>
<tr>
<td>CHOLESTEROL</td>
<td>177.20±2.698**</td>
<td>149.13±2.165***</td>
<td>20.71±1.221***</td>
<td>29.23±0.4326***</td>
<td>116.26±3.507***</td>
</tr>
<tr>
<td>STANDARD</td>
<td>100.22±0.9657*</td>
<td>93.95±1.205*</td>
<td>32.51±0.7098ns</td>
<td>18.78±0.2407*</td>
<td>48.89±0.7986*</td>
</tr>
<tr>
<td>V.R.P.E(100mg/kg)</td>
<td>149.54±1.231*</td>
<td>129.23±3.205*</td>
<td>23.22±0.412</td>
<td>29.52±0.6128*</td>
<td>104.21±0.231*</td>
</tr>
<tr>
<td>V.R.C.E(100mg/kg)</td>
<td>125.21±1.895*</td>
<td>106.45±2.906*</td>
<td>32.97±0.3054</td>
<td>2068±0.5916*</td>
<td>75.55±1.561*</td>
</tr>
<tr>
<td>V.R.E.E(100mg/kg)</td>
<td>146.46±3.669*</td>
<td>125.75±3.978*</td>
<td>28.12±1.546</td>
<td>25.66±0.7609*</td>
<td>95.77±3.825*</td>
</tr>
</tbody>
</table>

Values are mean±SEM, n=6, **p<0.01, when compared with control Group

* p<0.01, when compared with Toxic Group
Figure 8.07: Graphical representation of *Vigna radiate* Linn extracts on cholesterol diet induced hyperlipidemic model in wistar albino rats
Table:8.18 : Effect of *Vigna unguiculata* Linn extracts on body weight of cholesterol induced diet hyperlipidemic rat models

<table>
<thead>
<tr>
<th>Days</th>
<th>Normal</th>
<th>Cholesterol</th>
<th>STD</th>
<th>V.U.P.E (100mg/kg)</th>
<th>V.U.C.E (100mg/kg)</th>
<th>V.U.E.E (100mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>140</td>
<td>142</td>
<td>143</td>
<td>140</td>
<td>142</td>
<td>141</td>
</tr>
<tr>
<td>5&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>143</td>
<td>149</td>
<td>145</td>
<td>150</td>
<td>146</td>
<td>145</td>
</tr>
<tr>
<td>10&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>148</td>
<td>167</td>
<td>152</td>
<td>159</td>
<td>154</td>
<td>150</td>
</tr>
<tr>
<td>15&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>151</td>
<td>175</td>
<td>160</td>
<td>171</td>
<td>163</td>
<td>157</td>
</tr>
<tr>
<td>20&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>155</td>
<td>199</td>
<td>170</td>
<td>192</td>
<td>172</td>
<td>165</td>
</tr>
</tbody>
</table>

The body weight of rats increased from the 0<sup>th</sup> day to 20<sup>th</sup> day. There is a significant increase in the body weight of cholesterol treated rats, when compare to the normal rats. *V.ungiculata* petroleum ether extract of 100mg/kg b.w p.o treated group also showed significant increase in the body weight, when compare to the normal rats. Less significant increase in the body weight of standard, *V.ungiculata* ethanolic extract & *V.ungiculata* chloroform extract 100mg/kg b.w p.o. treated group.
### Table: 8.19: Effect of *Vigna ungingulata* Linn extracts in Cholesterol induced hyperlipidemic rat

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>64.89±2.280</td>
<td>53.90±1.666</td>
<td>36.15±1.125</td>
<td>11.76±0.3387</td>
<td>16.00±2.656</td>
</tr>
<tr>
<td>Cholesterol (Toxic)</td>
<td>177.20±2.698**</td>
<td>149.13±2.165**</td>
<td>20.71±1.221**</td>
<td>29.23±0.4326**</td>
<td>116.26±3.507**</td>
</tr>
<tr>
<td>Standard (10mg/kg)</td>
<td>100.22±0.9657*</td>
<td>93.95±1.205*</td>
<td>32.51±0.7098ns</td>
<td>18.78±0.2407*</td>
<td>48.89±0.7986*</td>
</tr>
<tr>
<td>V.U.P.E (100mg/kg)</td>
<td>159.11±2.323*</td>
<td>142.14±2.516*</td>
<td>30.07±0.611*</td>
<td>30.11±0.516*</td>
<td>106.33±1.45*</td>
</tr>
<tr>
<td>V.U.C.E (100mg/kg)</td>
<td>128.31±0.234*</td>
<td>110.32±2.314*</td>
<td>39.01±0.514</td>
<td>24.73±0.234*</td>
<td>79.56±0.321*</td>
</tr>
<tr>
<td>V.U.E.E (100mg/kg)</td>
<td>153.21±2.713*</td>
<td>134.24±4.614*</td>
<td>32.34±1.643</td>
<td>31.23±0.5412*</td>
<td>102.21±4.312*</td>
</tr>
</tbody>
</table>

Values are mean±SEM, n=6  **p<0.01, when compared with control Group

* p<0.01, when compared with Toxic Group
Fig: 8.08 Graphical representation of *Vigna ungiculata* Linn extracts on cholesterol diet induced hyperlipidemic model in wistar albino rats

**Statistical analysis:** All the data expressed as mean ± S.E.M and analyzed statistically using ANOVA followed by Dunnett’s test and compare with respective control group. A value was of $p<0.05$ was considered significant and $p>0.05$ is ns= non significant.
8.4 RESULTS AND DISCUSSION:

Cholesterol induced hyperlipidemia:

Effect of administration of selected plant extracts (100 mg/kg, p.o., once daily) /Atorvastatin (10mg/kg, p.o, once daily) on serum lipid Parameter levels in rats fed with Cholesterol Diet for 20days.

Effect on serum total cholesterol (serum TC) level:-

- Rats fed with Cholesterol for 20 days had serum TC level of (177.20±2.698 mg/dl) when measured on day 21. This was significantly higher (p<0.001) when compared to serum TC levels in normal control rats (64.89±2.280 mg/dl).

- Cholesterol induced hyperlipidemic rats treated with Atorvastatin (10mg/kg, p.o., once daily) had serum level of 100.22±0.9657 mg/dl when measured on day 21. This was significantly lower (p<0.001) when compared to the serum TC levels in Cholesterol treated toxic control groups (177.20±2.698 mg/dl).

- Cholesterol induced hyperlipidemic rats treated with V.M.P.E 100mg/kg, V.M.C.E 100mg/kg , V.M.E.E 100mg/kg b.w  p.o, once daily, had serum TC level of 175.61±2.314, 130.23±1.641 and 169.11±2.412 mg/dl respectively when measured on day 21. These values were significantly lower (P<0.001) when compared to the serum TC level in Cholesterol control rats (177.20±2.698 mg/dl).
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- Cholesterol induced hyperlipidemic rats treated with V.R.P.E 100mg/kg, V.R.C.E 100mg/kg, V.R.E.E 100mg/kg b.w p.o, once daily, had serum TC level of 149.54±1.231, 125.21±1.895 and 146.46±3.669 mg/dl respectively when measured on day 21. These values were significantly lower (p<0.001) when compared to the serum TC level in Cholesterol control rats (177.20±2.698 mg/dl).

- Cholesterol induced hyperlipidemic rats treated with V.U.P.E 100mg/kg, V.U.C.E 100mg/kg, V.U.E.E 100mg/kg b.w p.o, once daily, had serum TC level of 159.11±2.323, 128.31±0.234 and 153.21±2.713 mg/dl respectively when measured on day 21. These values were significantly lower (p<0.001) when compared to the serum TC level in Cholesterol control rats (177.20±2.698 mg/dl).
Effect on serum triglyceride (serum TG) level:-

- Rats fed with Cholesterol for 20 days had serum TG level of \( (149.13\pm2.165 \text{ mg/dl}) \) when measured on day 21. This was significantly higher (\( p<0.001 \)) when compared to serum TG levels in normal control rats (\( 53.90\pm1.66 \text{ mg/dl} \)).

- Cholesterol induced hyperlipidemic rats treated with Atorvastatin (10mg/kg, p.o., once daily) had serum level of \( (93.95 \pm 1.205 \text{ mg/dl}) \) when measured on day 21. This was significantly lower (\( p<0.001 \)) when compared to the serum TG levels in Cholesterol rats (toxic group) (\( 149.13\pm2.165 \text{ mg/dl} \)).

- Cholesterol induced hyperlipidemic rats treated with V.M.P.E 100mg/kg, V.M.C.E 100mg/kg, V.M.E.E 100mg/kg b.w p.o, once daily, had serum TG level of \( 155.12 \pm 1.321, 117.24\pm2.464 \text{ and } 140.21\pm2.314 \text{ mg/dl} \) respectively when measured on day 21. These values were significantly lower (\( p<0.05 \)) and (\( p<0.001 \)) when compared to the serum TG level in Cholesterol group (\( 149.13\pm2.165 \text{ mg/dl} \)).

- Cholesterol induced hyperlipidemic rats treated with V.R.P.E 100mg/kg, V.R.C.E 100mg/kg, V.R.E.E 100mg/kg b.w p.o, once daily, had serum TG level of \( 129.23\pm3.205, 106.45\pm2.906 \text{ and } 125.75\pm3.978 \text{ mg/dl} \) respectively. When measured on day 21. These values were significantly lower (\( p<0.05 \)) and (\( p<0.001 \)) when compared to the serum TG level in Cholesterol group (\( 149.13\pm2.165 \text{ mg/dl} \)).

- Cholesterol induced hyperlipidemic rats treated with V.U.P.E 100mg/kg, V.U.C.E 100mg/kg, V.U.E.E 100mg/kg b.w p.o, once daily, had serum
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TG level of 142.14±2.516, 110.32±2.314 and 134.24±4.614 mg/dl respectively when measured on day 21. These values were significantly lower (p<0.05) and (p<0.001) when compared to the serum TG level in Cholesterol group (149.13±2.165 mg/dl).

Effect on serum HDL cholesterol (serum HDL-C) level:-

- Rats fed with Cholesterol for 20 days had serum HDL-C level of (20.71±1.221 mg/dl) when measured on day 21. This was significantly lower (p<0.001) when compared to serum HDL-C levels in normal control rats (36.15±1.125 mg/dl).

- Cholesterol induced hyperlipidemic rats treated with atorvastatin (10mg/kg, p.o. once daily) had serum HDL-C level of 32.51±0.7098 mg/dl when measured on day 21. This was significantly higher (p<0.001) when compared to the serum HDL-C levels in Cholesterol control rats (20.71±1.221 mg/dl).

- Cholesterol induced hyperlipidemic rats treated with V.M.P.E 100mg/kg, V.M.C.E 100mg/kg , V.M.E.E 100mg/kg  b.w  p.o, once daily, had serum HDL-C level of 16.23 ± 0.148, 28.11 ± 0.631 and 20.14 ±0.145 mg/dl respectively, when measured on day 21. These values were significantly higher (p<0.05) and (p<0.001) when compared to the serum HDL-C level in Cholesterol control rats(20.71±1.221 mg/dl).

- Cholesterol induced hyperlipidemic rats treated with V.R.P.E 100mg/kg, V.R.C.E 100mg/kg , V.R.E.E 100mg/kg b.w  p.o, once daily, had serum
HDL-C level of 23.22±0.412, 32.97±0.3054 and 28.12±1.546 mg/dl respectively when measured on day 21. These values were significantly higher (p<0.05) and (p<0.001) when compared to the serum HDL-C level in Cholesterol control rats (20.71±1.221 mg/dl).

- Cholesterol induced hyperlipidemic rats treated with V.U.P.E 100mg/kg, V.U.C.E 100mg/kg, V.U.E.E 100mg/kg b.w p.o, once daily, had serum HDL-C level of 30.07±0.611, 33.01±0.514 and 32.34±1.643mg/dl respectively when measured on day 21. These values were significantly higher (p<0.05) and (p<0.001) when compared to the serum HDL-C level in Cholesterol control rats(20.71±1.221 mg/dl).
Effect on serum VLDL cholesterol (serum VLDL-C) level:-

- Rats fed with Cholesterol for 20 days had serum VLDL-C level of (29.23±0.4326 mg/dl) when measured on day 21. This was significantly higher (p<0.001) when compared to serum VLDL-C levels in normal control rats (11.76±0.3387 mg/dl).

- Cholesterol induced hyperlipidemic rats treated with Atorvastatin (10mg/kg, p.o) had serum VLDL-C level of 18.78±0.2407 mg/dl when measured on day 21, showing lower significant change (p<0.001) when compared to the serum VLDL-C levels in Cholesterol control rats (29.23±0.4326 mg/dl).

- Cholesterol induced hyperlipidemic rats treated with V.M.P.E 100mg/kg, V.M.C.E 100mg/kg, V.M.E.E 100mg/kg b.w p.o, once daily, had serum VLDL-C level of 36.41±0.552, 25.34±0.414 and 30.24±0.326 mg/dl respectively, when measured on day 21. These values were significantly lower (p<0.05) and (p<0.001) when compared to the serum VLDL-C level in Cholesterol control rats (29.23±0.4326 mg/dl).

- Cholesterol induced hyperlipidemic rats treated with V.R.P.E 100mg/kg, V.R.C.E 100mg/kg, V.R.E.E 100mg/kg b.w p.o, once daily, had serum VLDL-C level of 29.52±0.6128, 20.68±0.5916 and 25.66±0.7609 mg/dl respectively when measured on day 21. These values were significantly lower (p<0.05) and (p<0.001) when compared to the serum VLDL-C level in Cholesterol control rats (29.23±0.4326 mg/dl).

- Cholesterol induced hyperlipidemic rats treated with V.U.P.E 100mg/kg, V.U.C.E 100mg/kg, V.U.E.E 100mg/kg b.w p.o, once daily, had serum
VLDL-C level of 30.11±0.516, 24.73±0.234 and 31.23±0.5412 mg/dl respectively, when measured on day 21. These values were significantly lower (p<0.05) and (p<0.001) when compared to the serum VLDL-C level in Cholesterol control rats (29.23±0.4326 mg/dl).

Effect on serum LDL cholesterol (serum LDL-C) level:-

- Rats fed with Cholesterol for 20 days had serum LDL-C level of (116.26±3.507 mg/dl) when measured on day 21. This was significantly higher (p<0.001) when compared to serum LDL-C levels in normal control rats (16.00±2.656 mg/dl).
- Cholesterol induced hyperlipidemic rats treated with Atrovastatin (10mg/kg, p.o., once daily) had serum LDL-C level of 48.89±0.7986 mg/dl when measured on day 21. This was significantly lower (p<0.001) when compared to the serum LDL-C levels in Cholesterol control rats 116.26±3.507 mg/dl).
- Cholesterol induced hyperlipidemic rats treated with V.M.P.E 100mg/kg, V.M.C.E 100mg/kg, V.M.E.E 100mg/kg b.w p.o, once daily, had serum LDL-C level of 119.63±0.143, 88.61±0.241 and 110.23±0.341 mg/dl respectively, when measured on day 21. These values were significantly lower (p<0.001) when compared to the serum LDL-C level in Cholesterol control rats (116.26±3.507 mg/dl).
- Cholesterol induced hyperlipidemic rats treated with V.R.P.E 100mg/kg, V.R.C.E 100mg/kg, V.R.E.E 100mg/kg b.w p.o, once daily, had serum LDL-C level of 104.21±0.231, 75.55±1.561 and 95.77±3.825 mg/dl respectively, when measured on day 21. These values were significantly
lower (p<0.001) when compared to the serum LDL-C level in Cholesterol control rats (116.26±3.507 mg/dl).

- Cholesterol induced hyperlipidemic rats treated with V.U.P.E 100mg/kg, V.U.C.E 100mg/kg, V.U.E.E 100mg/kg b.w p.o, once daily, had serum LDL-C level of 106.33±1.45, 79.56±0.321 and 102.21±4.312 mg/dl respectively, when measured on day21. These values were significantly lower (p<0.001) when compared to the serum LDL-C level in Cholesterol control rats (116.26±3.507 mg/dl).

**Discussion:**

It has been well established that nutrition plays an important role in the etiology of hyperlipidimia and atherosclerosis. In our study, we choose cholesterol diet which contains the common ingredients in our daily food. Cholesterol feeding has been often used to elevate serum or tissue cholesterol levels to assess the hypercholesterolemia-related metabolic disturbances in animals. Cholesterol feeding alone however does not affect the serum TG level. It is assumed that a high level of saturated fat in addition to cholesterol is required to significantly elevate serum TG level in rat model.

Hyperlipidemia has been documented as one of the causative factor for atherosclerosis, resulting in coronary heart disease. Elevated cholesterol particularly LDL are the major reasons attributed to CVD. Accordingly to WHO by 2020, 60% of the CVD causes will be of Indian origin.
Development of atherosclerotic disease is a complicated process involving accumulation of lipid-containing particles in the walls of coronary arteries other major arteries in the body. Similarly the present study there was a significant weight gain in cholesterol control (toxic), as compared to normal control groups. Treatment with *Vigna* genus plant extracts significantly reduced the weight gain

Lowering high cholesterol levels significantly reduce the risk of heart attacks, strokes, and death. A rise in the LDL may cause deposition of cholesterol in arteries and aorta and it is a direct risk factor for CHD. In the present study there was a elevation in serum and tissue cholesterol, LDL-C, and VLDL-C level in response to cholesterol induced (toxic) compare to normal control group. Treatment with *Vigna* genus selected plant extracts significantly reduced serum and tissue cholesterol, LDL-C, and VLDL-C levels.

The decrease in triglyceride level is an important finding of experiment. Recent days studies shows that triglycerides are independently related with coronary artery disease. Treatment with *Vigna* genus selected plant extracts showed significant decreased in triglyceride.

HDL is synthesized mainly in intestine and liver. HDL is considered to be a beneficial lipoprotein as it has an inhibitory effect in the pathogenesis of atherosclerosis. Low level of HDL is associated with high risk of coronary artery
disease. In the present study HDL-C level in serum were significantly increased by chloroform extract and ethanolic extract.

The activity of Cholesteryl Ester Transfer Protein (CETP), a key enzyme in reverse cholesterol transport and HDL metabolism increase in high fat diet and mediates the transfer of cholesterol esters from HDL-C to triglyceride-rich particles in exchange for triglycerides. This leads to increased plasma concentrations of TG’s & decreased concentrations of HDL-C.

Lipid profile of Cholesterol induced Hyperlipidemia rats in our study revealed higher levels of serum triglycerides, cholesterol, LDL and VLDL accompanied by decrease of serum HDL-C as compared to controls.

Treatment of Cholesterol induced Hyperlipidemia rats with selected plant extracts and reference standard atorvastatin (10mg/kg b.w), an HMG CoA inhibitor showed a significant decrease of serum triglycerides, cholesterol, LDL and VLDL and significant increase of serum HDL-C levels.

The effect of Vigna genus plant extract cholesterol induced hyperlipidemic rat models observes the changes in lipid levels compare to the control groups. According the results observed upon comparing the results of control group all the lipid levels like TC, TG, LDL, VLDL levels are increased and only HDL levels are decrease when compare to normal rats. The test groups shows significant to the control groups. The standard group shows non-significant when compare to the control group. Decrease of lipid levels in the test group of is the

8.5 CONCLUSION

From this work we conclude that all the *Vigna* genus selected plants posses ability to decrease cholesterol levels in the body. *Vigna radiate* Linn chloroform extract possess highly significant action towards reducing the body cholesterol. Hence the folklore usage has been validated. *Vigna* genus selected plants can be treated as Nutraceuticals.
8.6 REFERENCES


