CHAPTER - 1

Introduction

and

Review of Literature
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1.1 INTRODUCTION

1.1.1 Obesity

1.1.1.1 Definition

Obesity can be defined as excess accumulation of body fat arising from a sustained or a periodic positive energy balance which happens when energy intake exceeds energy expenditure. Obesity can be observed as a defined cluster of non-transmissible diseases called “New World Syndrome,” creating a massive socioeconomic and civil health burden in poorer countries. The WHO has described obesity as one of most neglected public health problems, touching every section of the globe (WHO, 2000).

1.1.1.2 Prevalence

In recent years the prevalence of obesity has increased reaching epidemic levels. Worldwide, an estimated number of overweight and obese people increased from 857 million in 1980 to 2.1 billion in 2013. This is one-third of the world’s population. Globally, an increase in the section of adults with a body-mass index (BMI) of 25 kg/m$^2$ or greater, was found between 1980 and 2013 from 28.8% to 36.9% in men, and from 29.8% to 38.0% in women. In the same period the number of kids and teenagers who are overweight and obese has increased significantly in developed countries by 23.8% in boys and 22.6% in girls. It has also increased in kids and teenagers in developing countries, from 8.1% to 12.9% in boys and from 8.4% to 13.4% in girls. In 2010, overweight and obesity were estimated to cause 3-4 million deaths i.e., 3.9% of years of life lost and 3.8% of disability-adjusted life-years worldwide (Ng et al., 2014). According to a recent study, India is just behind US and China in the global hazard list of top 10 countries with highest number of obese people. Also, it was observed that the BMI values were similar in males and females; however, there were more overweight or obese (BMI≥25 kg/m$^2$) females (6.6%) than males (3.5%). In few areas, obesity and its subsequent diseases are posing an massive public health problem (Kalra and Unnikrishnan, 2012).
1.1.1.3 Health consequences

Apart from being a major contributor to chronic disease burden and mortality, obesity particularly abdominal obesity, is also closely related to the metabolic syndrome, a cluster of diseases that encompasses the following conditions described in figure 1.1 (WHO, 2006).

![Figure 1.1: Numerous co-morbid conditions associated with obesity](image)

Though obesity itself is not a disease per se, it is a major risk factor for developing type II diabetes, cardiovascular disease, hypertension, hypercholesterolemia, hypertriglyceridemia, nonalcoholic fatty liver and certain types of cancer at later ages (Calle et al., 2003; Kahn et al., 2006; Pothiwala et al., 2009; Van Gaal et al., 2006). In 2006, the International Diabetes Federation
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published the latest definition of the metabolic syndrome describing a cluster of factors associated with an increased risk for atherosclerotic cardiovascular disease (CVD) and diabetes (Hossain et al., 2007). For a person to be diagnosed with the metabolic syndrome the following criteria have been defined: central obesity measured by waist circumference plus two additional factors such as raised triglycerides (>150 mg/dl), reduced HDL cholesterol (<40-50 mg/dl), raised blood pressure (>130 mm Hg systolic or >85 mmHg diastolic) or raised fasting plasma glucose (>100mg/dl) (Eddy et al., 2008; Grundy et al., 2004). These defined criteria are important in order to diagnose people with metabolic syndrome early and to initiate lifestyle interventions and treatment before the development of diabetes and CVD.

1.1.1.4 Pathophysiology: Hyperlipidemia as the centre of obesity associated complications

Understanding the physiological abnormalities governing the current obesity epidemic is essential for the development of effective interventions to deal with this complex multifactorial disease. This is particularly evident when considering the central role of obesity in the development of CVD and its ability to influence other CVD risk factors (Grundy, 2004) (Figure 1.2). For decades, the association between insulin resistance, type II diabetes, hypertension and dyslipidemia have been recognized.

![Figure 1.2: Obesity as the underlying risk factor of cardiovascular disease](image)

Figure 1.2: Obesity as the underlying risk factor of cardiovascular disease
1.1.1.5 Dysregulated lipolysis: How free fatty acids impair metabolic homeostasis?

Excess adipose tissue increases the risk for a number of diverse conditions such as atherosclerosis, hypertension, insulin resistance and cancer. Much effort has been undertaken to understand the molecular changes in adipose tissue function in the context of obesity that lead to these secondary complications. One important malfunction of adipose tissue during obesity is a detrimental increase in lipolysis along with an excess release of non-esterified fatty acids. Free fatty acids are thought to be one of the major culprits for insulin resistance (Roden et al., 1996; Lam et al., 2003).

Figure 1.3: Dyslipidemia in Obesity (Klop et al., 2013) (The yellow colour represents cholesterol, while the light yellow color represents the TG content within the different lipoproteins. Increase in metabolic processes induced by obesity is marked with green arrows, whereas reductions are marked with red arrows).
The characteristic of hyperlipidemia in obesity is increased levels of fasting and postprandial triglycerides (TG) in combination with the predominance of low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) (Figure 1.3). Elevation in TG might be the major cause of the other lipid abnormalities since it leads to delayed clearing of the TG-rich lipoproteins (Karpe et al., 1994; Meyer et al., 1996; Patsch et al., 1992; Simons et al., 1987) and formation of small dense LDL (Capell et al., 1996; Hokanson et al., 1995).

Lipolysis of lipoproteins is impaired in obesity by reduced mRNA expression levels of lipoprotein lipases (LPL) in adipose tissue (Clemente-Postigo et al., 2011) and reduction in LPL activity in skeletal muscles (Klop et al., 2012). Elevated levels of postprandial free fatty acids leads to detachment of LPL from the endothelial surface (Karpe et al., 1992; Peterson et al., 1990) but it remains attached to very low density lipoproteins (VLDL) and intermediate density lipoprotein (IDL) contributing to added TG depletion. The exchange of TG from these remnants for cholesterol-esters from HDL by cholesterylester-transfer-protein (CETP) with the combined action of hepatic LP, eventually leads to the formation of LDL (Capell et al., 1996; Hokanson et al., 1995). During the state of hypertriglyceridemia, the cholesterol-ester content of LDL decreases, whereas the TG content of LDL increases by the activity of CETP. Nonetheless, the increased TG inside LDL is hydrolyzed by hepatic lipase, which leads to the formation of small, dense LDL particles. The formation of small dense LDL in obesity is generally due to elevated TG concentration and does not depend on total body fat mass (Tchernof et al., 1996). Small dense LDL are comparatively slowly metabolized with increased residence time, which enhances its atherogenicity (Packard, 2003). Chylomicron remnants and LDL can migrate into the subendothelium and get trapped in the sub-endothelial space where they can be taken up by macrophages (Klop et al., 2007; Proctor et al., 2003; Proctor et al., 2002). Also, small dense LDL are more prone to oxidation due to fewer free cholesterol and anti-oxidative content (Subramanian and Chait, 2012). It must be noted that the lipoprotein size is a limiting factor for movement through the endothelium and that LDL particles migrate more easily than chylomicron remnants, but the number of migrated particles does not necessarily translate into more cholesterol deposition since chylomicron remnants contain approximately 40 times more.
cholesterol per particle than LDL (Proctor et al., 2002). Instead, remnants of chylomicrons and VLDL may be transported to the tissues where communication with proteoglycans and lipoprotein receptors leads to particle removal. This procedure takes place in the liver and acts as an anti-atherogenic mechanism, but it can also take place in other tissues where cholesterol cannot be removed proficiently and hence leads to cholesterol buildup (Pacifico et al., 2011; Proctor et al., 2003; Proctor et al., 2004).

Impairment of lipolysis along with increase in chylomicron remnants and VLDL due to obesity, strongly affects HDL metabolism. The increase in TG-rich lipoproteins leads to increased CETP activity, which exchanges cholesterol esters from HDL for TG from VLDL and LDL (Subramanian and Chait, 2012). Moreover, lipolysis of these TG-rich HDL occurs by hepatic lipase resulting in small HDL with a reduced affinity for apo A-I, which leads to dissociation of apo A-I from HDL. This will eventually lead to low levels of HDL-C and a decrease in circulating HDL particles with impairment of reverse cholesterol transport (Deeb et al., 2003).

1.1.1.6 Drugs and treatments

The most effective treatment for people with metabolic syndrome, is change in lifestyle, which includes weight loss, a healthy diet, regular physical activity and giving up smoking. A very effective option to reduce weight is bariatric surgery including gastroplasty, gastric banding, gastric bypass and biliopancreatic diversion (Buchwald et al., 2004; Rubino et al., 2009). Interestingly, gastric surgery has beneficial effects on glucose control and insulin resistance independent of weight loss within days of the surgery; the mechanisms for these effects are currently unclear (Schauer et al., 2003; Scopinaro et al., 2005). However, due to concerns regarding pre-operative mortality and surgical complications, these procedures are reserved for the critically obese patients (Field et al., 2009; Melnikova and Wages, 2006). Other than invasive surgery, medical treatment for different features of the metabolic syndrome is common practice (Grundy et al., 2006).

For the treatment of obesity, most currently available drugs exert their effect on the central nervous system, for example, sibutramine inhibits noradrenergic and serotonergic uptake in the hypothalamus (Arterburn et al., 2004) or disturbs
the cannabinoid receptors (Van Gaal et al., 2005). Another strategy to reduce weight pharmacologically is the use of fat absorption blockers like orlistat, through the inhibition of gastric and pancreatic lipases (Curran and Scott, 2004), which are most effective in combination with a reduced calorie diet.

Conventional pharmacological mono therapies for obesity are even though initially successful in achieving weight loss, are frequently subject to counter-regulation (Adan et al., 2008; Vemuri et al., 2008). It is therefore important to design or discover newer drugs to concurrently target more than one biological mechanism and that might eventually be more effective in producing sustained weight loss and improvement in co-morbidities.

1.1.1.7 Safety issues with weight-loss drugs

Drugs can be very effective in inducing weight loss. The history of dietary supplements is full of success stories in terms of efficacy, but this success is matched by tragedy with regard to safety (Table 1.1). Perhaps the most infamous is the combination drug Fen–Phen. A combination of the amphetamine analogs fenfluramine and phentermine, Fen–Phen’s effectiveness helped it to achieve extensive popularity in the 1990s. Unfortunately, it also caused pulmonary hypertension and valvular heart disease, which forced its withdrawal from the market and birthed a legal and financial disaster (Connolly et al., 1997). Other amphetamine analogs and sympathomimetics have had similarly grave risks, including addiction, myocardial toxicity, and sudden death (Asher, 1972). Phenylpropanolamine caused intracranial bleeding and strokes (Kernan et al., 2000). Ephedrine caused heart attacks, hypertension, palpitations, strokes, and sudden death (Haller and Benowitz, 2000). Sibutramine caused increased cardiovascular events (US-FDA, 2010). Rimonabant, an inverse agonist of the endocannabinoid receptor CB1, caused increased depression and suicide (Topol et al., 2010). These unsafe drugs have had their regulatory approval withdrawn, although their unregulated use may continue to some extent. As of September 2013, only one drug, orlistat, has been approved by both the FDA and the European Medicines Agency (EMA) for chronic weight management. Orlistat is also the only FDA-approved weight-loss drug that is available without a prescription. It was approved in 1999 for prescription sale and in 2007 for over-the-counter sale. Orlistat inhibits gastrointestinal lipases, reducing fat absorption.
Consequently, its most common adverse effect is steatorrhea. Despite its approved status, orlistat has had a number of safety issues, including hepatotoxicity, nephrotoxicity, pancreatitis, and kidney stones. In 2010, the FDA rejected two proposed weight-loss drugs, lorcaserin and phentermine/topiramate (extended-release formulation) due to safety and efficacy issues. Major concerns over lorcaserin included limited efficacy, carcinogenesis, cardiovascular events, cognitive impairment, and psychiatric disorders. Major concerns over phentermine/topiramate included cardiovascular events, fetal toxicity, and suicidal ideation. In 2012, the FDA reversed its position and granted approval to both lorcaserin and phentermine/topiramate. Nonetheless, the FDA has required postmarketing safety studies to address the original cardiovascular concerns. Furthermore, the EMA has rejected both lorcaserin and phentermine/topiramate. The EMA rejected lorcaserin due to its opinion that the drug’s benefits did not outweigh its risks, particularly the potential risk for tumors (EMA, 2013) and phentermine/topiramate due to concerns over the potential cardiovascular and central nervous system effects associated with its long-term use, its teratogenic potential, and its use by patients for whom it is not indicated (EMA, 2013). Despite their limitations, lorcaserin and phentermine/topiramate represent the first new antiobesity drug approvals in 13 years.
Table 1.1: Current status of antiobesity medications (Kim et al., 2014)

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<th>Mechanism of action</th>
<th>Effects and safety concerns</th>
<th>Status</th>
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<td>Phentermine</td>
<td>Suppresses Appetite. Stimulates anorexic signaling in hypothalamus. Sympathomimetic agent similar to norepinephrine with central nervous system stimulatory activity</td>
<td>Appetite repression and weight loss Dizziness, dry mouth, insomnia, irritability, nausea, vomiting, diarrhea, or constipation. Has withdrawal symptoms</td>
<td>Approved by the FDA in 1959</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>Anorexia and weight loss Nervousness, restlessness, excitability, dizziness, headache, fear, anxiety, and tremor. Increases blood pressure and heart rate. Has withdrawal symptoms</td>
<td></td>
<td>Off-label usage; approved for attention-deficit hyperactivity disorder</td>
</tr>
<tr>
<td>Lorcaserin</td>
<td>Serotonin, dopamine, and norepinephrine reuptake inhibitor that potentiates the neurotransmitter activity in the central nervous system</td>
<td>Limited weight-loss efficacy and increases in cancer risk Headache, infection, sinusitis, nausea, depression, anxiety, and suicidal thoughts. Possible concerns of cancer risk</td>
<td>Approved by the FDA in 2012</td>
</tr>
<tr>
<td>Desvenlafaxine</td>
<td>Anorexia, but effect on body weight is unclear. Vision problems, headache, low libido, dry mouth, dizziness, insomnia, taste problems, vomiting, anxiety, sexual dysfunction, depression, high blood pressure, stomach ache, numbness and tingling, fatigue, and involuntary quivering</td>
<td></td>
<td>Off-label usage; approved for depression</td>
</tr>
<tr>
<td>Medicine</td>
<td>Action</td>
<td>Side Effects</td>
<td>Approval Status</td>
</tr>
<tr>
<td>--------------------------</td>
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<td>---------------------------------------------------------------------------------</td>
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<tr>
<td>Sibutramine (Meridia)</td>
<td>Limited weight-loss efficacy</td>
<td>Increased risk for cardiovascular events and stroke</td>
<td>Approved by the FDA approval in 1997 but withdrawn in 2010 due to cardiovascular effects</td>
</tr>
<tr>
<td>Orlistat (Xenical)</td>
<td>Weight loss</td>
<td>Increased number of bowel movements and potential changes in the bowel function and microbiota</td>
<td>Approved by the FDA in 1999</td>
</tr>
<tr>
<td>Exenatide (Byetta, Bydureon)</td>
<td>Decreased blood glucose level and body weight</td>
<td>Gastrointestinal symptoms, acute pancreatitis, dizziness, and headache. It might increase risks of sulfonylurea-induced hypoglycemia and thyroid cancer</td>
<td>Off-label usage; approved for diabetes</td>
</tr>
<tr>
<td>Liraglutide (Victoza)</td>
<td>Maintains normal blood glucose and body weight.</td>
<td>Increases risks of C-cell carcinoma and thyroid C-cell focal hyperplasia</td>
<td>Off-label usage; approved for diabetes</td>
</tr>
<tr>
<td>Pramlintide (Symlin)</td>
<td>Decreases blood glucose level and body weight</td>
<td>Nausea, hypoglycemia, vomiting, headache, abdominal pain, and fatigue</td>
<td>Off-label usage; approved for diabetes</td>
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1.1.2 Hyperlipidemia

1.1.2.1 Definition

Hyperlipidemia or hyperlipoproteinemia is the condition of abnormally elevated levels of any or all lipids and/or lipoproteins in the blood (Pande, 2009). These lipids include cholesterol, cholesterol esters, phospholipids and triglycerides. They are transported in the blood as part of large molecules called lipoproteins (Cheng, 2004). Lipoproteins include Chylomicrons, VLDL, LDL and HDL. Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis-associated conditions such as CHD, ischemic cerebro-vascular (ICV) disease and peripheral vascular disease (Jain et al., 2007). The prevalence of hyperlipidemic patients has dramatically increased worldwide due to a modern lifestyle and an increase of consumption of high-carbohydrate and high-fat diets (Misra and Khurana, 2009).

1.1.2.2 Prevalence

In most of the developed countries hyperlipidemia and atherosclerosis are the leading cause of cardiac illness and death (Misra and Vikram, 2004; Saravanan et al., 2007). According to WHO nearly 60% of Indians will contribute the world cardiac demographic class amounting about 100 million patients. Moreover, a WHO survey reveals that India is predicted to have large number of mortalities due to CAD by the year 2015 (D’Souza et al., 2007; Patel et al., 2009). Hyperlipidemia is an important risk factor in the initiation and progression of atherosclerotic impasse. Therefore, prime consideration in the therapy for hyperlipidemia and atherosclerosis is to attenuate the elevated blood serum/plasma levels of lipids (Dikshit et al., 2009; Ghule et al., 2009; Xu et al., 2009). In these complex disease groups hypolipidemic drugs are of crucial importance for preventing further disease risk and ameliorating the quality of everyday life (Mayes, 2003; RL, 2008).

1.1.2.3 Subtypes of hyperlipidemia

Hyperlipidemia is divided into primary and secondary subtypes

I. **Primary hyperlipidemia** is usually due to genetic causes such as a mutation in a receptor.

II. **Secondary hyperlipidemia** also called as Acquired hyperlipidemia, may mimic primary forms of hyperlipidemia and can have similar
consequences. They may result in increased risk of premature atherosclerosis or other complications. (Cheng et al., 2004).

1.1.2.4 An overview of lipoprotein metabolism
Numerous metabolic processes are involved in the uptake, transport and storage of lipids. Dietary fat is absorbed through the gastrointestinal tract in the form of circulating chylomicrons and VLDL, part of which is metabolized to provide energy and the rest of which enters the liver and adipose tissues for short- and long-term storage, respectively (Figure 1.4). As a gauge of the level of energy reserves, adipose tissues secrete several adipokines, such as leptin, which regulates energy homeostasis by signalling to the brain and peripheral tissues.

Figure 1.4: Adipocytes at the crossroads of energy homeostasis (Shi and Burn, 2004).
Adipose tissues, through the lipolysis and re-esterification process, are also the main sites for fatty-acid cycling, thereby securing the energy supply to oxidative tissues, such as skeletal muscle and the heart. The liver has an important role as a homeostat for transient energy fluctuation; it protects other tissues from postprandial triglyceridaemia by temporarily storing FAs from the circulation as a benign derivative, triacylglycerol (TAG), and secreting them as VLDL when the period of maximum lipid load has passed. The liver is also an important site for energy conversion, exchanging energy sources from one form to another, such as glycogen to glucose, fatty acid to TAG and saturated fatty acid to unsaturated fatty acid. (Shi and Burn, 2004).

After the ingestion of a meal containing fat, TG are lipolyzed in the intestinal lumen into FFA and 2-monoacylglycerols (MAG) and are taken up by the enterocytes via passive diffusion and specific transporters like CD36 (Pan and Hussain, 2011). Cholesterol is taken up by the enterocytes via the specific cholesterol transporter Niemann-Pick C1 Like 1 protein (NPC1L1) (Altman et al., 2004; Davis et al., 2004). Once in the enterocyte, cholesterol is transformed into cholesterol-esters, whereas FFA and MAG are assembled into TG again. Finally, cholesterol-esters and TG are packed together with phospholipids and apolipoprotein (apo) B48 to form chylomicrons (Pan and Hussain, 2011; Klop et al., 2012). After assembly, the chylomicrons are secreted into the lymphatics and finally enter the circulation via the thoracic duct. The liver synthesizes TG-rich lipoproteins called very low density lipoproteins (VLDL), which increase postprandially when food derived TG and FFA reach the liver (Klop et al., 2012). The assembly of VLDL is almost identical to the synthesis of chylomicrons, but apo B100 is the structural protein of VLDL (and its remnants, i.e., IDL and LDL. The human liver lacks the editing complex necessary to change the apo B100 molecule into the smaller apoB48, by post-transcriptional modification of one base leading to a premature stop codon (Innerarity et al., 1987).

Chylomicrons and VLDL deliver FFA to the heart, skeletal muscle and adipose tissue for energy expenditure and storage. Adequate lipolysis of TG-rich lipoproteins is necessary for FFA to be released in the circulation. This process is regulated by several enzymes and proteins acting as co-factors. Lipoprotein lipase (LPL) is the primary enzyme for TG lipolysis in the circulation and is strongly expressed in tissues that require large amounts of FFA like the heart, skeletal
muscle and adipose tissue (Goldberg et al., 2009). The amount of liberated FFA from chylomicrons and VLDL depends on the activity of LPL, which is stimulated by insulin (Karpe et al., 2011; McQuaid et al., 2011). In addition, chylomicrons compete with endogenous VLDL for the action of LPL (Brunzell et al., 1973).

The postprandial rise in insulin is one of the most important regulatory mechanisms for fuel storage. The postprandial increase of insulin results in the effective inhibition of hormone sensitive lipase, which is the key enzyme for hydrolysis of intracellular lipids. Despite the uptake of FFA by adipocytes and myocytes, a proportion of FFA remains in the plasma compartment (“spill over”) where the FFA are bound by albumin and transported to the liver (Evans et al., 2002). When delivery of FFA for energy expenditure is insufficient like in the fasting state, FFA can be mobilized by adipose tissue for oxidation in energy demanding tissues like cardio myocytes. Insulin is also an important regulator of FFA mobilization from adipose tissue (Karpe et al., 2011).

Besides the above described TG and LDL metabolism, the intestine and liver also play an important role in the reverse cholesterol transport by the synthesis of HDL particles. HDL promotes the uptake of cholesterol from peripheral tissues, including the arterial wall, and returns cholesterol to the liver. Enterocytes and hepatocytes synthesize apo A-I which is the structural protein of HDL. Nascent HDL particles acquire free cholesterol from peripheral tissues. In the liver, hepatic lipase hydrolyses HDL-associated TG and also phospholipids inducing the formation of smaller HDL particles which can contribute again to the reverse cholesterol transport. Therefore, lipid metabolism is highly dynamic and depends on numerous factors including the postprandial state, TG-rich lipoprotein concentrations, HDL levels and function, energy expenditure, insulin levels and sensitivity and adipose tissue function (Klop et al., 2012).

1.1.2.5 Pathophysiology of hyperlipidemia (Jackson, 1991)

An understanding of the biology of the lipoproteins and the pathophysiology of hyperlipidemtic states is essential to the rational choice of treatment regimen (Figure 1.5).
**Exogenous pathway: route of uptake of dietary lipids**

Chylomicrons are complexes of TG, cholesteryl esters (CE), and apoproteins. After the removal of TG they become chylomicron remnants. Chylomicrons are degraded by lipoprotein lipase on endothelial cells of adipose tissue and muscle. After removal of TG for storage, the chylomicron remnants are transported to the liver. This results in dietary TG stored in adipose tissue and muscles.

**Endogenous pathway: route for distribution of cholesteryl esters (CE) from liver to target cells**

VLDL is secreted by the liver into plasma and transported to adipose tissue and muscles, where lipoprotein lipase extracts most triglycerides. The remnant IDL is either taken up by the liver or circulated until the remaining triglycerides are removed forming LDL particles, rich in cholesterol. LDL is cleared from plasma through LDL receptor-mediated endocytosis. This results in transfer of TG from liver to target cells via VLDL, as well as, transfer of CE from liver to target cells via LDL.

**Route for cholesterol recovery**

Reverse cholesterol transport is a pathway where cholesterol is transported from atherosclerotic plaques or other lipids back to liver to be excreted into the feces via bile. As cell dies and the cell membranes turnover, free cholesterol is released into the plasma. It is immediately absorbed into HDL particles, esterified with a long chain fatty acid by lecithin cholesterol acyl transferase (LCAT), and transferred to VLDL or IDL by a cholesteryl ester transfer protein in plasma. Eventually, it is taken up by the liver as IDL or LDL, thus resulting in the recovery of cholesterol from cell membranes and reincorporation into LDL pool or return to liver.

**De novo cholesterol biosynthesis**

Liver synthesizes 2/3rd of the total cholesterol made in the body. The rate limiting enzyme is 3-hydroxy-3-methylglutaryl (HMG) CoA reductase and provides feedback regulation by controlling the cholesterol concentrations in cells.
**Cholesterol excretion by enterohepatic circulation**

Bile salts are synthesized from cholesterol in the liver, released into the intestine, and recycled. A small amount of bile acid is excreted. This results in conversion of liver cholesterol to bile salts for excretion.

![Exogenous and endogenous pathway of cholesterol](image)

**Figure 1.5:** Exogenous and endogenous pathway of cholesterol.
1.1.2.6 Drugs used in hyperlipidemia

The five major pharmacological classes of drugs routinely used in the treatment are:

1. Statins
2. Fibric acid derivatives
3. Bile acid sequestrants
4. Cholesterol absorption inhibitors
5. Nicotinic acid derivatives

For the control of dyslipidemia, the most commonly used drugs are statins, which inhibit HMG-CoA reductase thereby blocking cholesterol synthesis (Robinson et al., 2005). Another popular class of drugs are the fibrates, a group of PPARα agonists that primarily enhance fatty acid oxidation (Staels and Fruchart, 2005). Other possibilities are cholesterol-absorption blockers (Pearson et al., 2005) that lower LDL serum levels.

Although many efficacious lipid-lowering drugs exist, none is effective for all lipoprotein disorders, and all such agents are associated with some adverse effects. The use of statins in the treatment of hyperlipidemia causes concern in both patients and physicians about the safety associated with such medications. Muscle toxicity or myopathy, is a common adverse effect of this class of drugs. Myopathy progressing to rhabdomyolysis and renal failure is the most serious side effect associated with all statins either in monotherapy or in combination therapy and appears to be dose related. As statins therapy is for a long term basis, there may be a risk of chronic toxic effects like carcinogenic, teratogenic and mutagenic over a life time of use (Forrester et al., 2005; Kumar et al., 2007; Mahley, 2006; Rinki, 2011; Rang and Ritter, 2007; Vani et al., 1997).

1.1.3 Lipid Metabolizing Enzymes: Drug Targets for Treatment of Obesity and Hyperlipidemia

A recent analysis concerning antiobesity drugs reported a mean weight loss in subjects but marked improvement in dyslipidemia were absent (Zhou et al., 2012). Hence it is imperative that the drugs should have a multi target feature to ameliorate hyperlipidemia along with obesity condition. LPL inhibitors like Orlistat, which reduces the lipolysis of TG within the gastrointestinal system and thus prevents absorption of intestinal fats, shows only a modest reduction in
LDL-C. Sibutramine, which increases the sensation of satiety by modulation the central nervous system has less effect in lowering TG levels whereas rimonabrant does not show improvement in hyperlipidemia (Zhou et al., 2012).

Overall it has to be noted that the development of new drugs targeting features of the metabolic syndrome of obesity has had limited success over the past years (Grundy, 2006). The multifactorial nature of the metabolic syndrome poses the problem of polypharmacy and hence finding suitable drugs that reduces multiple metabolic factors like obesity along with hyperlipidemia, are required.

Hence, efficacy of selected plant extracts were checked for their effect on pancreatic lipase as well as HMG CoA reductase. Pancreatic lipase is the enzyme responsible for mobilization of fats in the system and HMG CoA reductase enzyme is the key enzyme responsible for the production of cholesterol in the system.

1.2 REVIEW OF LITERATURE
1.2.1 Need for Alternative Approach
Till date, there are very few natural medications available in the market to treat hyperlipidemia. Therefore it is a need of the day to search for natural medicaments because of their fewer side effects and less expensive as compared with synthetic drugs. Ancient literature mentions many herbal medicines for treating various diseases like obesity and cardiovascular diseases. It has also seen an increase in the popularity and use of natural remedies in developed countries, including herbs, herbal medicines, over-the-counter health foods, nutraceuticals and herbal medicinal products. The use of herbal medicines is especially prevalent in primary health care and for many chronic diseases. But unfortunately many potential plants in India lack scientific documentation. Chemical principles from natural sources have become much simpler and have contributed significantly to the development of new drugs from medicinal plants (Cox, 1990). The valuable medicinal properties of different plants are due to presence of several constituents i.e. saponins, tannins, alkaloids, phenols, flavonoids, terpenoids etc (Tiwari and Singh, 2004). The numerous beneficial effects attributed to phenolic products have given rise to a new interest in finding botanical species with high phenolic content and relevant biological activity (Anila and Vijayalashami, 2002; Harborne, 1998).
The phenolic compounds provide hypolipidemic effect without restricting caloric intake and change in life style (Arulmozhi et al., 2010). But the amount of polyphenols present in our commonly consumed food is very low. Hence, dietary supplements rich in polyphenols are recommended for achieving beneficial results. Evidences on the cholesterol-lowering properties of medicinal plants have been accumulating (Ochuko et al., 2010) and a number of plants have been found to be useful in treatment of hyperlipidemia such as Allium sativum, Commiphora mukul, Boswellia serrata, Emblica officinalis (Singh et al., 2007), Garcinia cambogia, Terminalia arjuna (Srikumar et al., 2007), Trigonella foenum-graecum.

Phyto-chemicals present in plants offer us the safer natural products that can be developed in the form of therapeutics (Table 1.2) (Puri et al., 2012).

**Table 1.2. Antiobesity molecules reported from plants (Singh et al., 2013)**

<table>
<thead>
<tr>
<th>Source</th>
<th>Family</th>
<th>Antiobesity agents</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Oryza sativa</em></td>
<td>Poaceae</td>
<td>Phenylboronic acid</td>
</tr>
<tr>
<td><em>Salvia officinalis</em></td>
<td>Lamiaceae</td>
<td>Carnosic acid (Diterpene)</td>
</tr>
<tr>
<td><em>Platycodon grandiflorus</em></td>
<td>Campanulaceae</td>
<td>Platycodin D (Saponin)</td>
</tr>
<tr>
<td><em>Glycyrrhiz auralensis</em></td>
<td>Fabaceae</td>
<td>Licochalcone A (Polyphenol)</td>
</tr>
<tr>
<td><em>Scabiosa tschiliensis</em></td>
<td>Caprifoliaceae</td>
<td>Prosapogenin (Saponin)</td>
</tr>
<tr>
<td><em>Acanthopanax sessiliflorus</em></td>
<td>Araliaceae</td>
<td>Sessiloside and chiisanoside (Saponins)</td>
</tr>
<tr>
<td><em>Panax japonicas</em></td>
<td>Araliaceae</td>
<td>Chikusetsusaponins (Saponin)</td>
</tr>
<tr>
<td><em>Dioscorea nipponica</em></td>
<td>Dioscoreaceae</td>
<td>Dioscin (Saponin)</td>
</tr>
<tr>
<td><em>Aesculus turbinata</em></td>
<td>Sapindaceae</td>
<td>Escins (Saponin)</td>
</tr>
<tr>
<td><em>Cyclocarya paliurus</em></td>
<td>Juglandaceae</td>
<td>Cyclocariosides (Saponin)</td>
</tr>
<tr>
<td><em>Gardenia jasminoides</em></td>
<td>Rubiaceae</td>
<td>Crocin (Terpene)</td>
</tr>
</tbody>
</table>
1.2.2 Natural Inhibitors of Pancreatic Lipase and HMG CoA Reductase

Medicinal plants have been used as dietary supplements for body weight management and control in many countries. In this sense, presence of PL inhibitors has been demonstrated in different plant species (Table 1.3), although more research is needed for identifying and characterizing effective lipase inhibitors (Gholamhoseinian et al., 2010). Lipase inhibitors of plant origin include certain proteins, such as those from soybean (Gargouri et al., 1984) and from wheat bran and germ (Lairon et al., 1985). Other proteins that strongly inhibit hydrolysis of triglycerides are the basic protein protamine (Tsujita et al., 1996) and ε-polylysine (Tsujita et al., 2003), which could act, as several amphiphilic proteins like ovoalbumin and β-lactoglobulin (Ivanova et al., 1990), by the desorption of lipase from its substrate due to a change in interfacial quality (Gargouri et al., 1984). Other lipase inhibitors from plant origin are basic polysaccharides, especially chitosan oligosaccharides, water-soluble chitosan (46 kDa) and polydextrose when a basic group is introduced (Han et al., 1999; Tsujita et al., 2007), phytic acid and other myo-inositol phosphate esters (Knuckles et al., 1988), phenylboronic acid, a potent inhibitor of lipase from Oryza sativa (Raghavendra et al., 2002) and carnosic acid, a diterpene isolated from the methanolic extract of the leaves of sage (Salvia officinalis) and rosemary (Ninomiya et al., 2004). Korean and Chinese researchers have been very active in the search of new lipase inhibitors of herbal origin. Among the most promising compounds there are platycodin D, isolated from the fresh roots of Platycodon grandiflorum (Zhao et al., 2004; Zhao and Kim, 2005), dioscin, from Dioscorea nipponica (Kwon et al., 2003), licochalcone A, from the roots of Glycyrrhiza uralensis (Won et al., 2007), phenolic constituents from the leaves of Nelumbo nucifera (Ono et al., 2006), the aqueous ethanol extracts of Juniperus communis or common juniper (bark) and Illicium religiosum (wood) (Kim and Kang et al., 2005), the ethanol extract from stem bark and leaves from mango tree (Mangifera indica), which is able to prevent weight gain induced by feeding a high-fat diet to Wistar rats (Moreno et al., 2006), a pomegranate leaf extract rich in ellagic acid and tannins (Lei et al., 2007), Rhei rhizoma (rhubarb) and the combinatorial drug Chunghyuldan (Yang et al., 2003), Prunella vulgaris, Rheum palmatum and other herbs (Zheng et al., 2010).
Table 1.3: Some classes of natural compounds that have been reported to inhibit pancreatic lipase activity \textit{in vitro} (Garza \textit{et al.}, 2011)

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Scientific name</th>
<th>Common name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>\textit{Alpinia officinarum}</td>
<td>Lesser galangal</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>\textit{Taraxacum officinale}</td>
<td>Dandelion</td>
</tr>
<tr>
<td>Flavonoids, Triterpenes</td>
<td>\textit{Actinidia arguta}</td>
<td>Kiwi</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>\textit{Arachis hypogaea}</td>
<td>Peanut</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>\textit{Mangifera indica}</td>
<td>Mango</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>\textit{Medicago sativa}</td>
<td>Alfalfa</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>\textit{Nelumbo nucifera}</td>
<td>Sacred lotus</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>\textit{Salacia reticulata}</td>
<td>Kotala himbutu</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>\textit{Salix matsudana}</td>
<td>Corkscrew willow</td>
</tr>
<tr>
<td>Polyphenols, Proanthocyanidins, Catechins</td>
<td>\textit{Camellia sinensis}</td>
<td>Green, black, oolong tea</td>
</tr>
<tr>
<td>Polyphenols, Saponins</td>
<td>\textit{Ilex paraguariensis}</td>
<td>Yerba mate</td>
</tr>
<tr>
<td>Proanthocyanidins</td>
<td>\textit{Cassia mimosoides}</td>
<td>Nomame herba</td>
</tr>
<tr>
<td>Proanthocyanidins</td>
<td>\textit{Cinnamomum sieboldii}</td>
<td>Cinnamon</td>
</tr>
<tr>
<td>Proanthocyanidins</td>
<td>\textit{Theobroma cacao}</td>
<td>Cocoa</td>
</tr>
<tr>
<td>Proanthocyanidins, Saponins</td>
<td>\textit{Vitis vinifera}</td>
<td>Grape vine</td>
</tr>
<tr>
<td>Saponins</td>
<td>\textit{Aesculus turbinata}</td>
<td>Japanese horse chestnut</td>
</tr>
<tr>
<td>Saponins</td>
<td>\textit{Arctostaphyllos uva-ursi}</td>
<td>Bearberry</td>
</tr>
<tr>
<td>Saponins</td>
<td>\textit{Ardisia japonica}</td>
<td>Marlberry</td>
</tr>
<tr>
<td>Saponins</td>
<td>\textit{Avena sativa}</td>
<td>Oat</td>
</tr>
<tr>
<td>Saponins</td>
<td>\textit{ Coffea arabica}</td>
<td>Coffee</td>
</tr>
<tr>
<td>Saponins</td>
<td>\textit{Cyclocarya paliurus}</td>
<td>Wheel wingnut</td>
</tr>
<tr>
<td>Saponins</td>
<td>\textit{Kochia scoparia}</td>
<td>Burningbush</td>
</tr>
<tr>
<td>Saponins</td>
<td>\textit{Malus domestica}</td>
<td>Apple</td>
</tr>
<tr>
<td>Saponins</td>
<td>\textit{Momordica charantia}</td>
<td>Balsampear</td>
</tr>
<tr>
<td>Saponins</td>
<td>\textit{Olea europeae}</td>
<td>Olive</td>
</tr>
<tr>
<td>Saponins</td>
<td>\textit{Panax ginseng}</td>
<td>Ginseng</td>
</tr>
<tr>
<td>Saponins</td>
<td>\textit{Panax japonicus}</td>
<td>Japanese ginseng</td>
</tr>
<tr>
<td>Saponins</td>
<td>\textit{Panax quinquefolium}</td>
<td>American ginseng</td>
</tr>
<tr>
<td>Saponins</td>
<td>\textit{Platycodi radix}</td>
<td>Doraji</td>
</tr>
<tr>
<td>Saponins</td>
<td>\textit{Platycodon grandiflorum}</td>
<td>Balloon flower</td>
</tr>
<tr>
<td>Saponins</td>
<td>\textit{Sapindus rarak}</td>
<td>Soapberry</td>
</tr>
<tr>
<td>Saponins</td>
<td>\textit{Scabiosa tschiliensis}</td>
<td>Pincushions</td>
</tr>
</tbody>
</table>
HMG CoA reductase reaction is the rate limiting step in the biosynthesis of cholesterol and isoprenoides and is a unique molecular target in anti cancer therapy (Wong et al., 2002; Bochar et al., 1997). Drugs that lower cholesterol level have been used for decades, but the high prevalence of their adverse effects such as myopathy, liver damages and potential drug-drug interactions has been reported too (Deng, 2009). Therefore, finding and development of other therapeutic agents for controlling cholesterol level especially safer agents are warranted. Plants are the best resources of new drug agent and their use for medicinal purposes has a long history (Deng, 2009).

The existence of cholesterol lowering agents and HMG-CoA reductase inhibitors have been demonstrated in different plant species including Garlic (Chetty et al., 2003), Morus alba, Melissa officinalis, Artemisia capillaries (Lee et al., 2008), Vitis vinifera (Koo et al., 2008), Ananas comosus (Xie et al., 2007), Kiwifruit (Jung et al., 2005) and Gynostemma pentaphyllum (Megalli et al., 2005).

1.2.3 Rodent Model for the Study of Obesity-Related Disorders

In lab animals, the origin of obesity is generally related to genetic mutations, but these models are far from that found in humans. Hence, usually high fat or hyperlipidemic diets have been used as tools for obesity induction in animals, because of their similarity to the induction and metabolic responses caused by obesity in humans. Models of diet induced obesity in rodents can be efficiently used when the objective is to study the physiopathology of metabolic complications associated with obesity (Rosini et al., 2012).

There are several widely used rodent models of obesity, are given as follows.

1.2.3.1 Mice models of obesity

*Monogenic Mouse Obesity*

The ob/ob (Zhang et al., 1994) and db/db (Bahary et al., 1990) mice and Zucker (fa/fa) obese rats (Zucker and Zucker, 1961) are classic examples of spontaneous single gene loss-of-function mutations that generates massive obesity. The defect in the ob/ob mouse is a single base pair deletion of the gene product, leptin (Zhang et al. 1994). The db/db and fa/fa rats have mutations in gene coding for the leptin receptor. Leptin is a gene expressed in abundance in the adipose tissue.
The leptin protein plays an important role in appetite control. Hence, ob/ob mice exhibits uncontrollable food intake, obesity, type 2 diabetes, and insulin resistance with hyperinsulinemia. The db (stands for “diabetes”) mutation results in faulty leptin signaling (Chen et al., 1996; Lee et al., 1996). Defective leptin signaling leads to constant hyperphagia and obesity, resulting in insulin resistance, and increased insulin levels.

**Polygenic Mouse Obesity**

Human obesity is mostly mediated by multiple genes. Hence, polygenic models possibly be much more relevant to human obesity.

**New Zealand Obese (NZO) Mouse**

The NZO mice is a polygenic model of obesity that exhibits Type-2 diabetes only in males. NZO mice increases their body weight quickly during the first two months of their life due to hyperphagia. Among polygenic mouse models of obesity, NZO mice exhibit the most severe physical characters, with fat depots more than 40% of total body weight at six months of age (Herberg and Coleman, 1977). Furthermore, NZO mice exhibits decreased exercise activity when compared to control or even ob/ob mice (Jurgens et al., 2006). Thus it suggests that like human obesity, obesity in NZO mice is due to a combined effect of hyperphagia, reduced energy expenditure, and inadequate physical activity.

**Tsumura Suzuki Obese Diabetes (TSOD) Mouse**

The TSOD strain develops obesity with diabetes (Suzuki et al., 1999). Male TSOD mice exhibits polygenic obesity with hyperglycemia and hyperinsulinemia (Suzuki et al., 1999; Hirayama et al., 1999).

**M16 Mouse**

M16 mice exhibits hyperphagia, hyperinsulinemia, and hyperleptinemia compared to controls (Allan et al., 2004).
1.2.3.2 Rat models of obesity

**Zucker Fatty Rat (ZFR)**
ZFR rats are characterized by hyperphagia and early-onset obesity, which appears at 5 weeks of age as an accumulation of subcutaneous fat. Although ZFR also exhibits marked insulin resistance (Zucker and Antoniades, 1972), their blood sugar levels remain normal (Bray, 1977).

**Wistar Fatty Rat**
The WFR strain was developed by transfer of the fa gene from ZFR to Wistar rats, which exhibits poor glucose tolerance (Ikeda et al., 1981). WFR shows obesity from 3 weeks after birth and develops obesity-related complications such as Type-2 diabetes, hyperinsulinemia, and hyperlipidemia. Males show prominent abnormalities however, WFR females display only mild insulin resistance and glucose intolerance (Ikeda et al., 1981). This model is extensively used for research in Type-2 diabetes because aged WFR displays diabetic complications such as nephropathy and neuropathy (Berti-Mattera *et al.*, 1989; Imai *et al.*, 2003; Imai *et al.*, 2003; Matsui *et al.*, 1996).

**Otsuka Long Evans Tokushima Fatty (OLETF) Rat**
OLETF rats are hyperphagic in the beginning of birth, with increase in body weight ultimately progresses to obesity (Kawano *et al.*, 1994). At around 25 weeks after birth, all male OLETF rats display diabetes while only 30% of female OLETF rats develop diabetes.

1.2.3.3 Diet-induced obesity

**High-Fat Diet**
Adopting a hypercaloric or hyperlipidemic diet has been widely used as a template to induce obesity in lab animals. This particular model is extremely useful in research on obesity in laboratory animals due to its close resemblance to the genesis and metabolic responses caused by obesity in humans, i.e., obesity is the consequence of a positive energy balance generated by environmental factors, such as, for instance the excessive intake of high-calorie foods and a sedentary lifestyle (Tschop and Heiman, 2001).
1.2.3.4 Models for study of hyperlipidemia

High fat diet model

The continuous ingestion of high amounts of fat have been used to provoke hyperlipidemia in laboratory animals, in order to understand better the relationship between disorders in cholesterol metabolism and to test possible treatments for the reduction of circulating cholesterol levels. A large number of animal models, such pigeons, chickens, swine, cats, dogs, non-human primates, mice, rabbits and rats, have been tested for hyperlipidemia (Moghadasian et al., 2001; Moghadasian, 2002).

For inducing hypercholesterolemia in rats triglycerides-rich diets containing cholesterol, with or without cholic acid have been used (Lichtman et al., 1999); the level of cholesterol varies substantially as well. The fat sources vary from lard to soybean, canola or sunflower oils. Commercial rations supplemented with cholesterol have also been used (Beynem et al., 1986; Doucet et al., 1987).

Genetically hypercholesterolemic rats

In 1969, Muller et al., interbred 2 males and 4 females selected from different colonies of albino rats, with spontaneous hypercholesterolemia. Hence this strain was established and named as RICO i.e. rats with increased cholesterol. Hypercholesterolemia in RICO rats is due to elevated concentrations of LDL-C and HDL-C. Metabolic studies revealed that in the RICO strain the overall rate of hepatic cholesterol synthesis is accelerated, as a result of higher than normal activity of 3-hydroxy-3-methylglutaryl-CoA reductase. The activity of cholesterol-7 alpha-hydroxylase is decreased in RICO rats, indicating a lower than normal rate of cholesterol catabolism (Muller et al., 1979).

Transgenic animals

A widely used transgenic model for hyperlipidemia is the Apo E knockout mice. These mice have spontaneously elevated plasma cholesterol levels and develop atherosclerosis even on regular chow diet within 3-4 months (Kolovou et al., 2008).
Triton induced hyperlipidemia
The intraperitoneal administration of the surfactant Triton WR 1339 to rats or mice results in a biphasic elevation of plasma cholesterol and triglycerides. Serum cholesterol level increases sharply 2-3 times after 24 hours of administration (Phase I). The hypercholesterolemia decreases nearly to control level within 48 hours (Phase II).

1.2.4 Importance of Medicinal Plants
Medicinal plants are still used by about 80% of the world population, mainly in developing countries, for primary health care because of better tolerability, compatibility and fewer side effects (WHO, 2005). The phytochemical constituents present in the medicinal plant products are a part of the physiological functions of living organisms and hence they have better compatibility with human body. Medicinal plants play a major role in world health. Plants have been a source of drugs for the treatment of humans and animals since thousands of years (Srimar et al., 1997) and their herbal preparations are attracting more attention all over the world (Liang YZ et al., 2004). Hence, with respect to the healing power of plants, use of natural remedies is an absolute requirement of our time (Kamboj, 2000; Ramchoun et al., 2009).

Among the modern drugs in use today about 40% are of natural origin (Freiesleben and Jager, 2014). Due to advancement in the newer technologies, the production of plant-derived crude drugs for use as herbal remedies or raw materials in pharmaceutical industry has increased many folds. Medicinal plant products are also of great importance in the process of drug discovery because of great diversity, permitting the identification of lead molecules for the development of new therapeutic agents. There is an increase in worldwide interest in the use of plant based pharmaceuticals as a complementary or alternative medicine, either to prevent or to ameliorate many diseases (Bisset, 1994; Duke and Martinez, 1994; Mukherjee, 2002).

The Indian region, with a vast heritage of diverse ethnic cultures and rich biodiversity is said to be a great emporium of ethnobotanical wealth (Rana et al., 1999). Most of the flowering plants are used in alternative system of medicines like Ayurveda, Siddha, Unani etc. (Srinivasamurthy et al., 2003). Unfortunately, a key obstacle, which has hindered the acceptance of these alternative systems of
medicine in developed countries, is the lack of documentation. Another major impediment in the herbal drugs industry in India is the lack of quality control, which reduces the reliability of herbal products (Raina, 1999). With the increasing market for the use of herbal medicines and its global expansion, safety has become a concern for health authorities in many countries (Kunle et al., 2012).

1.2.5 Review of the Literature of Plants Selected for the Study

With a view of the literature reviewed, the project aims to evaluate five plants for their potential to be developed as antiobesity and antihyperlipidemic agents. The literature pertaining to the plants included in the present study has been reviewed with respect to their morphology, geographical distribution, phytochemistry, pharmacological and toxicological properties. The list of plants with their common names and the parts used in the study are given below:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Scientific Name</th>
<th>Common Name</th>
<th>Parts Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Amorphophallus campanulatus</em></td>
<td>Elephant foot yam</td>
<td>Corm</td>
</tr>
<tr>
<td>2</td>
<td><em>Foeniculum vulgare</em></td>
<td>Fennel</td>
<td>Seeds</td>
</tr>
<tr>
<td>3</td>
<td><em>Hisbiscus rosa sinensis</em></td>
<td>Shoe flower</td>
<td>Leaves</td>
</tr>
<tr>
<td>4</td>
<td><em>Luffa cylindrica</em></td>
<td>Sponge gourd</td>
<td>Fruit</td>
</tr>
<tr>
<td>5</td>
<td><em>Trichosanthes anguina</em></td>
<td>Snake gourd</td>
<td>Fruit</td>
</tr>
</tbody>
</table>
1.2.5.1 *Amorphophallus campanulatus* (Dennst) Nicolson.

**Common name**: Elephant foot yam  
**Sankrit**: Suranah, Kanda, Kandsuran  
**Hindi**: Suran, Jamikand  
**Kingdom**: Plantae  
**Division**: Angiosperms  
**Class**: Monocots  
**Order**: Alismatales  
**Family**: Araceae  
**Genus**: Amorphophallus  
**Species**: paeonifolius  
**Synonym**: Amorphophallus campanulatus

*Figure 1.10*: Corm of *Amorphophallus campanulatus*
**Geographical Distribution**

*A. campanulatus*, also known as Elephant foot yam is a highly potential tropical tuber crop. It is an important tuber crop of tropical and sub-tropical countries because of its yield potential and culinary properties (Ravindran and George, 2008). It is widely grown and consumed in south eastern countries like India, Philippines, Malaysia, Indonesia. In India, it has gained the status of a cash crop due to its high production potential, market acceptability and lucrative economic returns (Misra et al., 2002). It has a good source of protein as well as starch. In India, it is cultivated in Andhra Pradesh, West Bengal, Gujarat, Kerala, Tamil Nadu, Maharashtra, Uttar Pradesh, and Jharkhand. It has a great export potential since its commercial cultivation is not in other countries (Misra and Shivlingaswamy, 1999; 2001).

**Morphology**

Amorphophallus is a perennial, terrestrial underground hemispherical depressed dark brown corm of approximately 20-25 cm in diameter which bears flowers and fruits in the month of April- May (Cooke, 2001; Yoganarsimshan, 1996). It bears leaves that are solitary which are 30-90 cm broad; Inflorescence consist of a foliar organ, the spathe, which usually envelops a stalk like organ, the spadix. The flowers are tiny, monoecious and strongly reduced and are found at the base of the spadix. Raphides of the *A. campanulatus* Blume isolated from tuber are pointed at one end and square at other end, cross section is ‘X’-shaped at pointed end and they are asymmetrical. Figure 1.5 shows the representative image of corm of *A. campanulatus*.

**Traditional uses**

The tubers are a delicacy in food and rich in nutrients is much popular as a vegetable in various delicious cuisines. The tuberous roots of the plant posses blood purifier properties and have been used traditionally for the treatment of piles, abdominal disorders, tumours, enlargement of spleen, asthma and rheumatism (Kirtikar and Basu, 1989; Misra and Sriram, 2001). They are traditionally used in arthralgia, elephantiasis, tumors, inflammations, hemorrhoids, hemorrhages, vomiting, cough, bronchitis, asthma, anorexia, dyspepsia, flatulence, colic, constipation, helminthiasis, hepatopathy,
spleenopathy, amenorrhea, dysmenorrhoea, seminal weakness, fatigue, anemia and general debility (Arya, 1994; Gupta and Tandon, 2000; Kapoor, 2005). The tuberous roots of the plant have also been reported to possess tonic, stomachic and appetizer properties (Misra and Sriram, 2001).

**Phytochemistry**

*Amorphophallus* is a good source of energy, sugar, starch, proteins as well as minerals. Average nutritional profile contains Starch (11-28%), sugar (0.7-1.7%), protein (0.8-2.60%), fat (0.07-0.40%) mean energy value (236-566.70KJ/100g). The most abundant macro mineral is potassium (327.83 mg/100 g) Phosphorus (166.91 mg/100 g), calcium (161.08 mg/100 g) and iron (3.43 mg/100 g). Macro mineral and soluble oxalate ranges between different varieties (Misra and Sriram, 2001). The mean soluble oxalate content (13.53 mg/100 g) was safe from the viewpoint of accumulation of urinary oxalate leading to kidney stones (Chattopadhyay et al., 2009). The petroleum ether extract contain alkaloids, steroids, fats & fixed oil. The *A. campanulatus* extract contains alkaloids, steroids, flavonoids, tannins and saponins (Dey et al., 2010; Firdouse and Alam, 2011).

**Pharmacology**

Methanolic extract of *Amorphophallus* has gastro protective ability against pylorus ligation induced gastrototoxicity in rats. Treatment with methanol extracts showed reduction in gastric volume, free acidity, total acidity, pH and ulcer score (Natraj et al., 2011). The methanol extract shows significant analgesic activity against frequency of acetic acid-induced writhing in mice (Shilpi et al., 2005). Analgesic activity was also confirmed by tail flick method and acetic acid induced writhing response method (Dey et al., 2010). Petroleum ether extracts of *A. campanulatus* showed dose-dependent activity against onset of convulsion in isoniazid induced mice model (Dey et al., 2012). The methanolic extracts of the tubers showed antihelminthic activity against *Pheretima posthuma* and *Tubifex tubifex* (Dey and Ghosh, 2009). The ethanolic extract of the *A. paeoniiifolius* leaves exhibits reduction in the severity and frequency of diarrhoea produced by castor oil (Purwal et al., 2011). *A. campanulatus* tubers extracts has prominent anti-inflammatory activity on carrageenan induced paw edema model in rats.
(Shankhajit et al., 2010). Petroleum ether, chloroform and benzene extracts of *A. campanulatus* are found to have antibacterial activity against both gram-positive and gram-negative bacteria compared to the other extracts (Khan et al., 2008; Krishnaa et al., 2011). Jayaraman et al., (2010) has described that the ethanol extract of *A. campanulatus* has a potent antioxidant activity in vitro and can be utilized as an effective and safe source of antioxidants. The ethanolic extract of *A. campanulatus* has shown significant antitumor and antioxidant effect in animals. It stimulates both cellular and humoral immunity (Jagadheesh et al., 2010). *A. campanulatus* showed hepatoprotective activity against paracetamol and carbon tetrachloride induced liver damage in rats (Sharstry et al., 2010). *A. campanulatus* tuber confers toxicity against target insects and it can be a promising candidate in preventing crop loss caused due to hemipteran insect attack (Mondal et al., 2012).
1.2.5.2 *Foeniculum Vulgare* Mill.

**Common name**: Fennel  
**Sankrit**: Madhurika, Misyreya  
**Hindi**: Saunf  
**Kingdom**: *Plantae*  
**Class**: *Dicotyledonae*  
**Order**: *Apiales*  
**Family**: *Apiaceae*  
**Genus**: *Foeniculum*  
**Species**: *Foeniculum vulgare*

![Figure 1.8: Seeds of Foeniculum vulgare](image)

**Geographical Distribution**

*F. vulgare* (fennel) is a distinctive aromatic plant belonging to the Mediterranean area. Fennel has two different i.e., bitter or common fennel. Bitter fennel is native to the Mediterranean region and it is cultivated extensively worldwide, including Hungary, Bulgaria, Germany, France, Italy and India. Sweet fennel is thought to have originated from the island of Malta and they were
introduced to the other parts by monks or crusaders, thousands of years ago
(Baytop, 1999; Guilled, 1996; Muckensturm et al., 1997; Puelo, 1980; Yaylayan,

Morphology
Fennel is a robust, perennial, glabrous, aromatic herb that can grow up to 2 m
high and stalks can grow up to 1 m. It grows up to 2 meters high, with feathery
leaves and golden yellow flowers. Plants have finely divided leaves composed of
many linear or awl-shaped segments with greyish compound umbels. The taste of
seeds resembles to anise. Stems are erect, longitudinally striate, and are profusely
branched. The internodes grow hollow when they become older. Fruit is an
ovoid-cylindrical shaped structure with a slightly curved schizocarp, light green
to yellow-brown in color. It splits at maturity into 2 mericarps each with 5
prominent ridges and oil-vittae between the ridges (Bernath et al., 1996). Figure
1.3 shows the representative image of seeds of *F. vulgare*.

Traditional use
Fennel has been used as food and medicine with long history in central Europe
and Mediterranean region as well as in China. It is also a flavor food with health
value (Willett, 1994). Due to unique and preferred flavour and aroma, dried seeds
of sweet fennel are commonly used in cooking as kitchen vegetable. Fennel seeds
are used for savory formulations, sauces, liqueurs, confectionery, cookies,
cheeses, sauces, pickles and beverages, etc. Medicinal diet, also called ‘yaoshan’
in Chinese, was very popular in china, such as ‘sausages fried fennel leaves’. The
swollen bases of fennel were freshly consumed in salad, cooked fish for grilling,
preserve food, and bread bouillons. In addition, fennel provided an excellent
source of potassium, calcium, magnesium, iron, phosphorous and zinc
(Trichopoulou et al, 2000). In western countries, essential oil of fennel fruits
(referred as fennel oil) was used for flavouring purpose, cosmetic and
pharmaceutical products (Bilia et al., 2000). However, this herb or preparations
containing fennel are not suggested for small children or pregnant women and
should not be used for a prolonged period of time, though it was highly
appreciated as a carminative for its mild flavor and good tolerance in infant care
(Raffo et al., 2011).
**Phytochemistry**

The fennel fruits have about 3% relative content of essential oils. The major component of oil is trans-anethole (70.1%) and the compounds with intense odor in fennel fruits are trans-anisole, estragole, fenchone and 1-octen-3-ol. (Diaz-Maroto et al., 2005; Mimica-Dukie´et al., 2003). Napoli et al., (2010) identified 78 compounds from fennel fruits representing more than 98% of the oils. The major compound, estragole ranges from 34 to 89%. The essential oil of bitter fennel fruits have a relatively high concentrations of α-pentene and fenchone, and low concentrations of trans-anethole and estragole, unlike sweet fennel oils (Akgül and Bayrak, 1988). The fruits of fennel contain about 20% fatty acids. Petroselinic acid is a characteristic fatty acid of fennel oil. The level of petroselinic acid could be as high as 70 to 80% (Reiter et al., 1998; Charvet et al., 1991). The chemical analysis of the acetone extract of fennel showed that linoleic acid (54.9%), palmitic acid (5.4%) and oleic acid (5.4%) were major components in acetone extract (Singh et al., 2006). Fennel is rich phenolic compounds such as 3-caffeoylquinic acid, 4-caffeoylquinic acid, quercetin-3-O-galactoside, kaempferol-3-Orutinoside and kaempferol-3-O-glucoside (Parejo et al., 2004). Total flavonoid contents of the hydroalcoholic extracts were about 12.3 ± 0.18 mg/g. Flavonoids such as quercetin, rutin and isoquercitrin were reported to be present in fennel seeds (Cherng et al., 2008)

**Pharmacology**

The volatile oil of the seeds have antibacterial and antifungal properties. These oils can be functional as safe additives for the safety of perishable vegetables because they inhibit various food borne pathogens (Cantore et al., 2004; Elgayyar et al., 2001; Gutierrez et al., 2008; Mohsenzadeh, 2007). Essential oils of fennel have been observed to have strong antioxidant properties (Ozcan et al., 2009). The essential oil of fennel have significant larvicidal activity against the 2 mosquito species i.e., *Anopheles dirus* and *Aedes aegypti* after 24-h exposure (Pitasawat et al., 2007) The essential oil extracts from leaves, flowers and roots of fennel was found to have insecticidal activities against larvae of mosquitoes (Kim et al., 2004). *F. vulgare* seed methanolic extract was found to exhibit inhibitory effects against acute and subacute inflammatory diseases and type IV allergic reactions and shows a central analgesic effect. The essence of
Fennel can be used as a safe and effective herbal drug for primary dysmenorrhea (Nejad, 2006). *F. vulgare* inhibits the growth of prostate tumor xenografts (Ng and Figg, 2003). Anethole suppresses inflammation and carcinogenesis by inhibiting TNF-induced cellular responses by (Chainy *et al*., 2000). Some other studies have also claimed the anticancer properties of fennel and suggest further investigations (Aggarwal *et al*., 2008; Kaileh *et al*., 2007). Also, fennel extracts and oils have been reported to have beneficial effect in gastric disturbances (Birbane *et al*., 2007), respiratory disorders (Mdel *et al*., 2008), glaucoma (Agarwal *et al*., 2008), hypertension (Bardai *et al*., 2001), diabetes (Saraswat *et al*., 2008), CNS disorders (Joshi and Parle, 2006), obesity (Hur *et al*., 2006), skin infections (Singh and Kale, 2008) and also can be used as anticoagulant (Tognolini *et al*., 2007), hepatoprotective (Subehan *et al*., 2007) and diuretic (Wright *et al*., 2007). Fennel oil was also reported to exhibit estrogenic activity, promote menstruation, alleviate the symptoms of female climacteric, and increase libido (Albert-Puleo, 1980).
1.2.5.3 *Hibiscus rosa sinensis* Linn.

**Common name**: Shoe flower or China rose  
**Sanskrit**: Japapushpa  
**Hindi**: Jaswanti  
**Kingdom**: Plantae  
**Class**: Dicotyledonae  
**Subclass**: Polypetalae  
**Order**: Malvales  
**Family**: Malvaceae  
**Genus**: Hibiscus  
**Species**: rosa-sinensis

![Figure 1.6: Flowering shoot of *Hibiscus rosa sinensis*](image)
**Geographical Distribution**

It is colloquially known as the shoe flower or red hibiscus, and is native to China and East Asia but now widely cultivated and grown in India and Philippines. It is widely grown as an ornamental plant throughout the tropics and subtropics. Sometimes it is also planted along the fence or as a hedge plant.

**Morphology**

*Hibiscus* has an inconsistent stature and can be upright or spreading. The leaves are arranged alternately. The leaves can be dark green or spotted with lighter patches and are ovate in shape with toothed margins. The flowers can be of various colours, but the original variety is red and they do not have fragrance. The stalks of the stamens and the style are fused into a long column. The petals can be single or double, smooth or scalloped. Many anthers can be seen parting ways, up the column and five round stigma lobes are visible at the tip of the column. Figure 1.1 shows the representative image of shoot of *H. rosa sinensis* plant.

**Traditional uses**

The medicinal and emollient properties of the plant *H. rosa sinensis* is well known from very early days (Devipriya, 2005). In *Ayurveda*, the flowers have been recommended for use to color hair, eyebrows, food and liquors. In the system of Siddha Medicine, The young leaves and flowers are used for inducing abortion, and as a cure for headache. The plant is useful in menorrhagia, strangury, cystitis and other conditions of the genitourinary tract (Kirtikar and Basu, 2005). The leaves and flowers are useful in healing of ulcers, whereas the flowers have beneficial effect in heart diseases (Nadkarni, 1954, Kurup, 1979, Ali et al., 1997). The leaves and flowers are beaten into paste and poultice onto cancerous swellings and mumps (Quisumbing, 1951). An infusion of petals is widely used in *Ayurvedic* medicine in India as a refrigerant drink in fever (Chatterjee and Prakashi, 1992). Its decoction is given in to cure inflammation of the bronchitis (Chopra et al., 1969). The seeds can be pulverized and mixed with water for curing gonorrhea (Nadkarni, 1989). The flowers ground into a paste are boiled in coconut oil and applied externally to heal wounds, bruises, and
dermatitis. The leaves and flowers are also used as an antiseptic for boils and ulcers (Wong et al., 2010). The plant flowers are also used in folklore medicine as demulcent, refrigerant, aphrodisiac, brain tonic and cardiotonic (Kasture et al. 2000). In India, flowers are used by the natives to blacken shoes. Sankaranarayanan et al., (2010) have reported the ethanobotanical uses of petals of *H. rosa sinensis* along with coconut oil and applied externally for alopecia. Decoction of roots is used for fever in children (Dixit AK, 2011). The plant is laxative, aphrodisiac, emmenagogue, refrigerant. The crushed tender leaves mixed with fresh butter are placed over boils to heal them. Also paste of flowers is boiled with coconut oil and is applied externally to heal wounds, bruises, and dermatitis (Nesamani, 1998). The red flowered variety of the plant species is preferred (Adhirajan et al., 2003). In Unani medicine, *Hibiscus* sharbat has been prescribed as a refrigerant and vitaliser in palpitation, cough, fever and burning sensation of the body. It is considered a cardiotonic and braintonic and is also good for the treatment of schizophrenia and bleeding. In Ayurveda, the flowers are ground in paste and concentrated with raw rice and sugar and consumed for purifying blood. It is also prescribed for the treatment of *raktha pitha* which is nothing but changed and altered metabolic activities of blood (Devipriya, 2005). The fresh root extract of the plant is useful for venereal diseases (Chopra et al., 1974; The Wealth of India, 1959). The powdered root is taken as such (The wealth of India, 1959; Chopra et al., (1974) or the crushed roots may be mixed with oil and administered to control menstrual bleeding. Alternatively, equal quantities of the powdered roots of *Hibiscus* and lotus are mixed with the bark of *Eriodendron anfractuosum* and administered for menorrhagia (Nadkarni, 1989). The decoction of root is used in the treatment of piles. Medicated oil may be prepared from the expressed juice of the fresh root bark of *Hibiscus* or a decoction of the dried root bark as an ingredient can be used for the treatment of alopecia (Devipriya, 2005).

**Phytochemistry**

A large number of phytoconstituents have been identified in various parts of the plant *H. rosa sinensis*. The plant contains calcium oxalate, flavonoids, campesterol, stigmasterol, ergosterol, lipids, phosphorus, thiamine, riboflavin, arachidic, gentsic, myristic, palmitic and oxalic acids, fructose, sucrose and
glucose (Devipriya, 2005). Apart from these, many other phytochemical investigations have revealed the presence of sterols; carbohydrates and glycosides; tannins and phenolic compounds; proteins; triterpenoids and flavonoids in the hydroalcoholic extract of aerial parts of *H. rosa sinensis* (Mandade *et al.*, 2011). Phytoconstituents *viz.* hibiscetin, cyanidide, cyanin glycoside, taraxeryl acetate, β-sitosterol, campesterol, stigmasterol, ergosterol, citric acid, tartaric acid, oxalic acid have been reported by Prajapati *et al.*, (2003). Pattanaik, (1949) have reported that the leaves and petals contain catalase. Srivastava *et al.*, (1979) have identified and reported the presence of fatty acids, fatty alcohols, hydrocarbons in the leaves. Ghaffar and El-Elaimy, (2012) have reported presence of carbohydrates and/or glycosides, steroids, and/or triterpenes, flavonoids, tannins and the same authors reported that alkaloids, saponins and coumarins were absent in the hydroalcoholic extract of leaves of *H. rosa sinensis*. Mohan *et al.*, (2011) have reported presence of flavonoids, tannins and glycosides in the anthocyanidin fraction of flowers of *H. rosa sinensis*. Siddiqui *et al.*, (2005) have isolated and identified five new compounds from the chloroform extract and ten new compounds from the hydro alcoholic extract of the flowers of *H. rosa sinensis*. The flowers contain apigenidin, citric acid, cyanidin diglucoside, cyanin, fructose, glucose, sucrose, gentisic acid, tartaric acid, pelargonidin, quercetin (Sikarwar M *et al.*, 2011). Sheng-Xiang *et al.*, (1998) have isolated Ethyl β-L-arabinopyranoside from the roots of *H. rosa sinensis* L. as a natural monoglycoside. The aqueous root extract of *H. rosa sinensis* showed presence of carbohydrates, proteins, glycosides, phytosterols (Soni *et al.*, 2011). The ethyl acetate soluble fraction of methanol extract of roots showed the presence of flavonoids, glycosides, saponins, alkaloids, and sterols (Nade *et al.*, 2011). Bhaskar *et al.*, (2011) have identified 2, 3-hexanediol, n-Hexadecanoic acid, 1, 2-Benzenedicarboxylic acid and squalene as major components present in the flower extract of *H. rosa-sinensis* by GC-MS analysis.

**Pharmacology**

Sharma *et al.*, (2004) have reported the chemopreventive activity of *H. rosa sinensis* extract and role of gentisic acid in inhibition of tumor promotion response and oxidative stress in mice. Gilani *et al.*, (2005) have reported use of the plant in gastrointestinal disorders with spasmogenic and spasmolytic
constituents in aqueous ethanolic extract of aerial parts of *H. rosa sinensis*. Antioxidant activity of the hydroalcoholic extract of aerial parts of *H. rosa sinensis* was reported by Mandade *et al.*, (2011). An anti-implantation and uterotropistic activity for the root extract of *H. rosa sinensis* has been reported by Vasudeva and Sharma, (2007). Nade *et al.*, (2009) have reported the protective role of methanolic extract of *H. rosa sinensis* roots in reserpin-induced orofacial dyskinesia and oxidative stress. Hypolipidemic activity of the roots of *H. rosa sinensis* has been demonstrated by Kumar *et al.*, (2009). *In vivo* anti-ulcer activity has been reported by Kumari *et al.*, (2010). *H. rosa sinensis* exhibited a protective role against age and scopolamine-induced amnesia, indicating its utility in management of cognitive disorders (Nade *et al.*, 2011). Antipyretic and analgesic potential for the plant has been suggested by Soni and Gupta, (2011). Sachdeva *et al.*, (2001) have reported that aqueous extract of leaves improved glucose tolerance in rats. The insulin secreting activity of leaf extract in diabetes induced rats has been reported by Vimala *et al.*, (2008). Whereas Moqbel *et al.*, (2011) have reported the insulino-tropic as well as protective effect in non-obese diabetic mice using fractions obtained from leaf ethanolic extract. Wong *et al.*, (2010) had reported that leaves possess relatively weak radical scavenging activity but good metal chelating ability. Imafidon, (2010) has reported the dose dependent alterations in liver metabolism with oral administration of aqueous leaves extract. The hypoglycemic and hypolipidemid activities for the ethanolic extract of leaves of the plant have been reported by Mamum *et al.*, (2013). Prakash *et al.*, (1986) have reported that the benzene extract of the flowers led to the termination of pregnancy in about 92% of animals. Alam *et al.*, (1990) has demonstrated the anti-diabetic activity of *H. rosa sinensis* in diabetic rural population. Sharma, (1996) have showed that the flowers are used to reduce the quantity of urine in diabetics. The plant flowers possess anti-spermatogenic and androgenic activities (Reddy *et al.*, 1997). Flowers have been found to be effective in the treatment of arterial hypertension (Dwivedi *et al.*, 1977) and have significant antifertility effect whereas other parts of the plant had no antifertility effect (Kholkute *et al.*, 1977). Sachdeva *et al.*, (2003) have reported the serum glucose and insulin lowering effects of *H. rosa sinensis* flower extracts in diabetic rats. The hydroalcoholic extract of the flowers showed most significant hypotensive activity among different extracts but the isolated active compounds
from the crude extract showed comparatively lowered hypotensive effect. Flowers augments endogenous antioxidant compounds of rat heart and also prevents myocardial injuries. The red pigment anthocyanin prepared from the petals of *H. rosa sinensis* L. can function as an antioxidant. Red flowers of *H. rosa sinensis* showed highest total anthocyanin content and also displayed high ferrous ion chelating ability and lipid peroxidation inhibition activity. Mohan *et al.*, (2011) have demonstrated that the anthocyanidin fraction of flowers of *H. rosa sinensis* possessed antihypertensive and antioxidant properties. Potential antidepressant activity of the anthocyanidins present in the methanolic extract of the flowers has been reported by Shewale *et al.*, (2012). Anti-obesity and anti-atherogenic potential as well as the effect on carbohydrate metabolizing enzymes have been reported for the flowers of *H. rosa sinensis* by Gomathi *et al.*, (2008). Ethanolic extract of the flowers possess anti-genotoxicity effect (Khatib *et al.*, 2009). The aqueous extract of flowers has reversible suppressive effect on cholesterol level, glucose level and spermatogenesis (Mishra *et al.*, 2009).
1.2.5.4 *Luffa cylindrica* (Linn.) M.J. Roem.

**Common name**: Sponge gourd, loofa  
**Sankrit**: Rajakoshataki  
**Hindi**: Ghitarui  
**Kingdom**: Plantae  
**Division**: Mangoliophyta  
**Class**: Mangoliosida  
**Order**: Cucurbitales  
**Family**: Cucurbitaceae  
**Genus**: Luffa  
**Species**: *Luffa cylindrica*

![Fruits of *Luffa cylindrica*](image)

**Figure 1.9**: Fruits of *Luffa cylindrica*
Geographical location

*L. cylindrica* is an annual climbing plant of the sub-tropical region, which requires warm summer temperatures and long frost free growing season when grown in temperate zones. It produces fruits containing fibrous vascular system. It is a vegetable of summer season. It is difficult to assign with accuracy the indigenous areas of luffa species. They have a long history of cultivation in the tropical countries of Asia and Africa. Indo-Burma is reported to be the center of diversity for sponge gourd. The main commercial production countries are China, Korea, India, Japan and Central America (Oboh and Aluyor, 2009).

Morphology

The *L. cylindrical* fruits are smooth and cylindrical shaped. A mature *Luffa* sponge produces at least 30 seeds. *L. cylindrica* leaves are alternate and palmate, with petiole. The leaf is 13 and 30 cm in length and width respectively, with hairless and has serrated edges. The flowers of *L. cylindrica* are yellow in color and blooms in August to September months. *L. cylindrica* flowers are dioecious. The inflorescence of the male flower is a raceme and one female flower exists. Its fruit (gourd) is green in color and has a large cylindrical shape and grows climbing on other physical objects (Oboh and Aluyor, 2009). Figure 1.4 shows the representative image of fruit of *L. cylindrica*.

Phytochemistry

The fruit and leaf of *L. cylindrical* contains triterpenoid saponins like lucysides A to M and lucyn A, lucysides G, N, O, P, Q, R, respectively (Liang et al., 1997; Liang et al., 1994; Takemoto et. al., 1984). They also contain ginsenosides (Liang et al., 1995); flavonoids: apigenin (Khan et al., 1992), etc. The seed contains polypeptides like luffins P1, S (Li et al., 2003), luffacylin (Prakash et al, 2002), etc. Various polyphenols including p-coumaric acid, 1-O-caffeoyl-b-D-glucose, 1-O-[4-hydroxybenzoyl]-glucose, diosmetin-7-O-b-Dglucuronidemethyl ester, apigenin-7-O-b-Dglucuronidemethyl ester and luteolin-7-O-b-D glucuronidemethyl ester have been discovered in *Luffa* pulp (Du et al., 2006). Also, sponge gourd was shown to contain flavonoids, anthocyanins, and ascorbic acid (Reddy et al., 2010).
Traditional use

Apart from being an edible vegetable, *Luffa* also have a wide application in packing medium, shoes mats, sound proof linings, bath sponges, utensil cleaning sponges, adsorbent for removal of heavy metal (such as Nickel, Lead, Chromium, Copper, etc.) in waste water, and immobilization matrix for plant, algae, bacteria and yeast (Demir *et al.*, 2008).

Pharmacology

*Luffin* (a ribosome inactivating protein isolated from *Luffa* seed), has been shown to be effectively acting against parasites, insects, fungi and HIV (Ng *et al.*, 2011). *Luffa* has anti-inflammatory activity on macrophage cells (Bor *et al.*, 2006). Sigualuo, derived from the dried fruit of *Luffa* inhibits carrageenin-induced plantar edema in rats (Kang *et al.*, 1992). Sigualuo possess significant analgesic and sedative activity by inhibiting acetic acid-induced writhing and raising the pain threshold in hot plate and electric shock tests (Kang *et al.*, 1992; Kang *et al.*, 1993). Sigualuo prevented the decrease of heart rate, inhibited the raise in serum lactate dehydrogenase level and myocardial malondialdehyde levels (Guan *et al.*, 2006). It also acts as a effective antihypertriglyceridemic agent by effectively decreasing serum cholesterol and triglyceride levels. It also increases high density lipoprotein-cholesterol, and reduces the body weight (Li *et al.*, 2004). Fruits, leaves and stems potentiated the cytophagic action and acid phosphatase activity of peritoneal macrophages in mice thereby showing its immunostimulating activity (Mao *et al.*, 2004). Sigualuo possess anti-asthma, anti-tussive and expectorant effect on ammonium aerosol-induced cough in mice. It is also reported to prevent hepatic injury, cardiac stimulation, and anti-human immunodeficiency virus activity (Ng *et al.*, 1992). Oral administration of proteins isolated from the seeds exhibited anti-reproductive property in mice (Zhang *et al.*, 1990).
1.2.5.5 *Trichosanthes anguina* Linn.

- **Common name**: Snake gourd
- **Sankrit**: Chichindaka.
- **Hindi**: Padwal, Chichinda
- **Kingdom**: *Plantae*
- **Class**: *Magnoliopsida*
- **Order**: *Curcubitales*
- **Family**: *Curcubitaceae*
- **Genus**: *Trichosanthes*
- **Species**: *Trichosanthes cucumerina*
- **Variety**: *Anguina*

*Figure 1.7*: Fruits of *Trichosanthes anguina*
Geographical Distribution

*Trichosanthes* is indigenous to southern and eastern Asia, Australia and western Pacific islands. It was probably domesticated in ancient times in India, from where non bitter and large fruited types may have migrated to other tropical areas. It is locally grown as a vegetable in many countries of Asia and in home gardens in Africa. It is also exported from India to Malaya and Australia. Snake gourd is the most frequently cultivated variety of *Trichosanthes* species, amongst the various other hundred species in Asia. There are 2 varieties within *Trichosanthes cucumerina* i.e., wild cucumerina and the cultivated variety anguina. (Sandhiya et al., 2010).

Morphology

*T. anguina* is a monoecious annual herb is a creeper with long, slender stems and tendrils. Leaves are variable in shape, lobed, base deeply cordate. Male flowers are arranged in axillary racemes while female flowers solitary, axillary. The leaves are alternate, simple with no stipules. Flowers are unisexual, regular, and white in colour with green and hairy calyx. Fruits are very long, twisted, pale green or white. The seeds are half-ellipsoid, somewhat compressed, undulate, hard, rugose, greyish-brown in color (Gildemacher et al., 1993; Sardseangjun, 1993; Yusuf et al., 2007). Figure 1.2 shows the representative image of fruit of *T. anguina*.

Traditional uses

*T. anguina* is used in the treatment of head ache, alopecia, fever, abdominal tumors, diarrhea, haematuria and skin allergy. *T. anguina* is used as an abortifacient, vermifuge, stomachic, refrigerant, purgative, laxative, hydragogue, hemagglutinant, emetic, and anthelmintic (Nadkarni, 1976). Roots have a strong purgative action and are used for expelling worms. They are also used for diabetes, skin swellings like boils in China. Leaf juice is applied on the body in remittent fevers. The expressed juice of the leaves is emetic. In case of liver dysfunction and skin diseases leaves and stems are used. The fruit is considered to be anti-helmintic. The dried capsules are given in infusion or in decoction with sugar to assist digestion. The seeds have a cooling effect. Seeds are used for their
anti-helmintic and anti-diarrhoeal properties. They have anti-bacterial, anti-spasmodic and insecticidal properties (Madhava et al., 2008; Nadkarni et al., 1976).

**Phytochemistry**

Fruits contain hentriacontane, ceryl alcohol and its D-glucoside, alpha-amyrin, taraxerone, sucrose, potassium nitrate. It is highly constituted with proteins, fat, fibre, carbohydrates, vitamin A, C and E. The total phenolics and flavonoids content is 46.8% and 78.0% respectively (Adebooye, 2008). The crude protein content is 30.18% (Yusuf et al., 2007). The predominant mineral elements present in it are potassium (121.6 mg 100-1g) and phosphorus (135 mg 100-1g). Other elements found in fairly high amounts are Sodium, Magnesium and Zinc (Ojiako and Igwe, 2008). The chemical constituents present in *T. anguina* are cucurbitacin B, cucurbitacin E, isocucurbitacin B, 23,24-dihydroisocucurbitacin B, 23,24-dihydrocucurbitacin E, β-sitosterol and stigmasterol (Datta, 1987). Small amounts of oxalate, phytates and tannins are also present. Analysis showed that the seed of *T. anguina* have high oil content up to 42.5±5%. A galactose-specific lectin and ribosome-inactivating protein named trichoanguin (Azeez and Morakinyo, 2004) are present in aerial parts (Chow et al., 1999; Anuradha, 1999).

**Pharmacology**

Kolte et al., (1997) have reported significant anti-inflammatory activity of the aqueous extracts of *T. anguina* tubers against carrageenin induced mice paw oedema. *T. anguina* root extract and fruit juice have shown cytotoxicity against human breast, colon and lung cancer cell lines (Kongtun et al., 1999). Ethanolic extract of *T. anguina* lowers blood glucose levels in diabetic rats (Kar et al., 2003). Rahuman et al., (2008) showed moderate larvicidal effects with leaf extract of *T. cucumerina*. Arawwawala et al., (2009) reported to improve glucose tolerance in Type II diabetes mellitus induced rats using extract of aerial parts of *T. cucumerina*. Studies shows that the plant possess antidiabetic activity with improvement in oral glucose tolerance and glucose uptake in peripheral tissues (Kirana and Srinivasan, 2008). Sathesh et al., (2009) reported that whole plant of
T. anguina showed significant hepatoprotective activity against carbon tetrachloride induced hepatotoxicity.

Through the present research study, a sincere attempt has been made in evaluating the traditional folklore claims and medicinal uses of the selected plants for their antihyperlipidemic and antiobesity efficacy. Through the literature review done so far for the present study, it was observed that none of these selected plant parts have been evaluated for their antihyperlipidemic and antiobesity activities in a high fat diet model of rats and no such scientifically validated study reports are available. Also while selecting the plants that are to be evaluated for the present study, one of the important and basic selection criteria was to choose and select those plants that are commonly and readily available. Hence for the present study, the antihyperlipidemic and antiobesity effect of five selected plants, viz. Amorphophallus campanulatus, Foeniculum vulgare, Hibiscus rosa sinensis, Luffa cylindrica and Trichosanthes anguina were studied using Triton induced hyperlipidemic model and high fat diet induced obese model.

1.3 RATIONALE
Statins have been found effective in lowering serum low density lipoprotein (LDL) levels; however they have also been found to cause many side effects. As they are basically enzyme inhibitors, so it is likely that they may be inhibiting other critical enzymes in the body. Muscle toxicity or myopathy is a common adverse effect of this class of drugs. As statins therapy is on long term basis, there may be a risk of chronic toxic effects such as carcinogenic, teratogenic and mutagenic effects over a life time of use.

Orlistat, which has inhibitory activity against pancreatic lipase (PL), is one of the best-selling clinically approved drugs for obesity treatment worldwide. However, it has certain gastrointestinal side effects such as oily stools, oily spotting, and flatulence, among others (Derosa et al., 2004). The success of orlistat has prompted research for the identification of new PL inhibitors that lack some of these side effects.

Currently, the potential of natural products for the treatment of obesity is still largely unexplored and might be an excellent alternative strategy for the
development of safe and effective antiobesity drugs (Birari et al., 2007). However, detailed studies are needed to explore the anti-hyperlipidemic activity of herbs. This may, at least in part, help future studies to screen newer anti-hyperlipidemic molecules.

In the present study the potential of five selected medicinal plants viz., *Amorphophallus campanulatus, Foeniculum vulgare, Hisbiscus rosa sinensis, Luffa cylindrical* and *Trichosanthes anguina* for lowering of serum total cholesterol levels in Triton WR 1339 induced hyperlipidemic mice model was assessed. Rodents fed with HFD have been reported as an excellent model for obesity where dietary environment is a major contributor (Bullo et al., 2007).

1.4 AIM AND OBJECTIVES

**AIM**

The aim of the study is to investigate the anti-hyperlipidemic activity of plant extracts on HFD induced obese rat models.

**OBJECTIVES**

1. To screen the antihyperlipidemic activity of extracts of five selected plant extracts in Triton WR 1339 induced hyperlipidemic animal model and to short list the two plants that are most effective.
2. To detect the phytochemical constituents present in the extracts of the shortlisted plants.
3. To determine anti-obesity and anti-hyperlipidemic activities of the plant extracts in HFD induced obese rat model.
4. To elucidate the mode of action of the plant extracts by studying there in vitro and ex vivo effects on lipid metabolic enzymes.

1.5 RESEARCH ENVISAGED

For the present experimental research project, it was proposed that initially five selected plants would be identified and authenticated and methanolic extracts would be prepared. The plant methanolic extracts would be then processed and evaluated for a pilot screening for serum total cholesterol lowering efficacy in Triton WR 1339 induced hyperlipidemic mice model. On the basis of the initial
pilot screening, two plants showing maximum efficacy will be selected for further studies. The phytochemical constituents in the plants will be confirmed by performing TLC and HPTLC. Eventually these two plant extracts would be studied in detail for their antiobesity and antihyperlipidemic activity in a high fat diet induced obese rat model. If the plant extracts shows any inhibitory activity against obesity or hyperlipidemia, then the possible mechanism of action will be found out by performing enzymes assays on two major enzymes in lipid and cholesterol metabolism i.e., pancreatic lipase and HMG CoA reductase, respectively.
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Chapter 1


