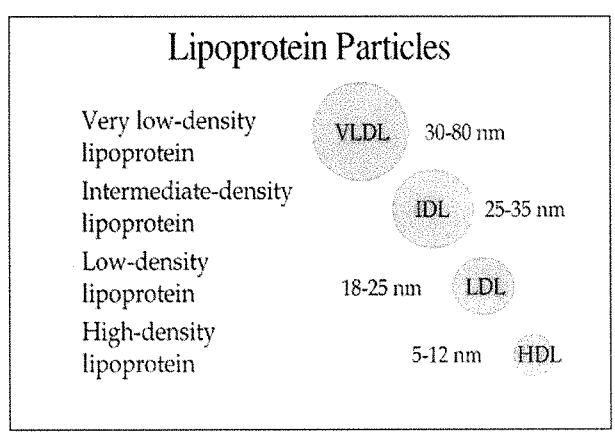
Introduction

INTRODUCTION

Hyperlipidemia: The epicenter of lifestyle disorders

Cholesterol is an important molecule for eukaryotic organisms, since it modulates membrane fluidity and is essential for steroid hormones synthesis. In mammals, a complex system regulates biosynthesis and transport of cholesterol between cells in the body. Cholesterol is transported through serum by lipoproteins that are classified according to their densities as high density lipoprotein (HDL), low density lipoprotein (LDL), intermediate density lipoprotein (IDL), very low density lipoprotein (VLDL) and chylomicrons. LDL and HDL appear to be particularly important. LDL, which is a converted form of VLDL and is rich in cholesterol and cholesterol ester, is regarded as **'bad cholesterol'**, whereas the HDL which contains relatively little cholesterol is popularly known as**'good cholesterol'** (Daneil *et al.,* 2009). Abnormal lipoprotein or lipid metabolism may induce hyperlipidemia, or hypercholesterolemia and hypertriglyceridemia (Figure.1).

Figure.1 Types of lipoproteins



Hyperlipidemia refers to elevated levels of lipids and cholesterol in the blood and, also identified as dyslipidemia to describe the manifestations of various disorders of lipoprotein metabolism. These disorders generally manifest as elevated levels of plasma total cholesterol, LDL and triglycerides and a decrease in the plasma HDL cholesterol. In hyperlipidemia, the blood cholesterol and triglyceride levels are significantly elevated than the baseline level (200mg/dl and 180mg/dl for men and women respectively) in healthy individuals. LDL levels are elevated more than 130mg/dl and HDL levels lowered below 40 mg/dl in men and 50 mg/dl in women (Kreisberg and Reusch, 2005). Hyperlipidemia can be further sub-divided into primary and secondary hyperlipidemia. Primary hyperlipidemia may result due to genetic defect in lipid metabolism such as familial hypercholesterolemia. Familial combined hyperlipidemia (FCHL) is the most common familial lipid disorder that is also responsible for more than 10% of premature coronary artery diseases (CAD) (Ylitalo *et al.,* 2002). On the other hand, secondary hyperlipidemia is a consequence of diabetes, obesity, hypothyroidism, renal ailments, biliary obstruction and blood pressure medication.

A significant change in global lifestyle coupled with a high calorie food intake can be pinned as the two major factors contributing towards onset and progression of hyperlipidemia and related disorders in urban individuals. Moreover, factors such as ageing, gender bias, dietary habits or addictions to nicotine or alcohol also affect lipid metabolism. In the present scenario, diet of an urban individual contains relatively high proportion of fat (30-40% energy) especially saturated fatty acids and cholesterol. Hyperlipidemia and hypercholesterolemia are the root causes for occurrence of various

lifestyle disorders such as obesity, cardiovascular disorders, including atherosclerosis, and type I and type II diabetes. These physiological ailments either individually or in plurality constitute the most common cause of death in developed as well as developing countries (Reiner *et al.*, 2007).

Diabetes: India, a world leader

Diabetes mellitus is a physiological condition that derails body's carbohydrate and lipid metabolisms. The ability of insulin to mediate tissue glucose uptake is a critical step in maintaining glucose homeostasis and in clearing the postprandial glucose load (Bell, 2001). In Non-insulin Dependent Diabetes Mellitus (NIDDM), the insulin stimulated glucose uptake and utilization in liver, skeletal muscle and adipose tissue is impaired (Zimmet, 1982). As per the estimates of World Health Organization (WHO), about 2–10 per 100 individuals will be diabetic during their lifetime and 90% of these will be patients of NIDDM (Nathan, 1993). Today, diabetes threatens to reach pandemic levels by 2030; with a predicted increase from 171 millions in 2000 to 366 million by 2030 worldwide (WHO, 2003a). India faces the greatest challenges amongst Asian countries since International Diabetes Federation (IDF) has declared India as "Diabetic Capital of the world" with more than one fifth (33 million) of the population likely to be diabetic (Kochhar *et al.*, 2007). This is mainly due to the tendency of many Asian races for fat deposition in the abdominal area, known as central adiposity. Increase in visceral fat raises the risk of diabetes and hyperlipidemia by favoring insulin resistance. A positive association between body weight gain and risk of type II diabetes stands established by many cross-sectional and prospective studies (Gregg *et al.*, 2005). Hence, the association

of obesity with type II diabetes is complex and compounded by many heterogeneous factors (Ramachandran, 2004) that further complicates the management of the disease

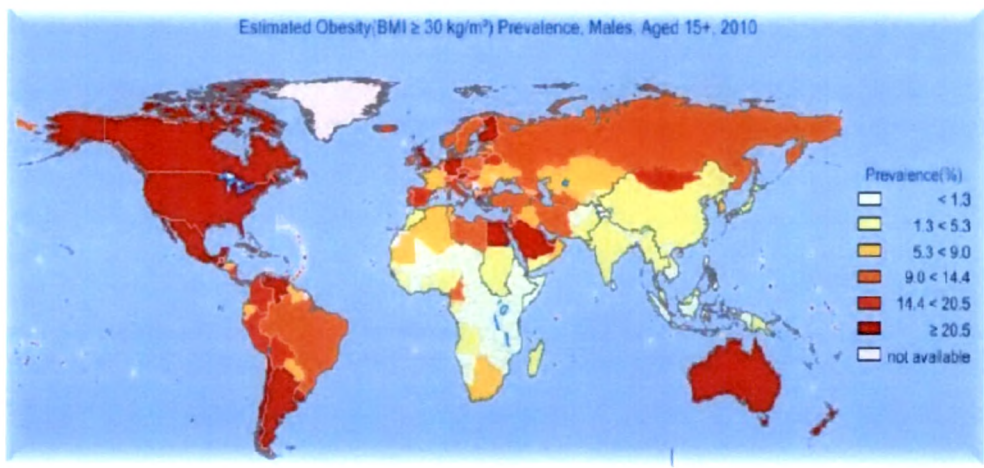
Obesity: A new world syndrome

Obesity describable as the "New World Syndrome" is a chronic metabolic disorder characterized by elevated lipid concentrations in the blood, hypertrophy of adipocytes and increased accumulation of subcutaneous fat (Spiegelman and Flier 1996; Flegal *et al.*, 1998). Obesity is a complex condition, with serious social and psychological dimensions, affecting virtually all ages and socio-economic groups. Obesity once viewed as the result of lack of will power, or a lifestyle "choice" i.e. to over eat and under exercise, now projects more appropriately in the modern world as a chronic disease, which requires effective strategies for management. Obesity has reached epidemic proportions globally, with more than 1 billion adults being overweight - at least 300 million of them clinically obese - and is a major contributor to the global burden of chronic disease and debility (WHO, 2003b). Two key factors appear to influence obesity - genetic and socioeconomic status. Though genes are important in determining a person's susceptibility to be overweight, societal changes and worldwide nutritional transition are the main engines driving the obesity epidemic (Nammi *et al.*, 2004). Economic growth, modernization, urbanization and globalization of food markets are fueling this epidemic. (Spiegelman and Flier, 1996; Nammi *et al.*, 2004).

Statistical data reveals that the problem of obesity has increased from 12–20% in men and from 16–25% in women over the last ten years (Flegal *et al.*, 1998). Recent studies suggest that nearly 15–20% of the middle-aged European populations are obese

(Bjorntorp *et al.,* 1997). About one third of adults aged 20 to 74 years are categorized as obese in United States alone (NCSH, 2006). These statistics also attribute to about 300000 premature deaths each year (US DHSS, 2001; Nammi *et al.,* 2004).

Figure.2 Worldwide prevalence of obesity



From: Ono T, Guthoid R, Strong K. WHO global comparable estimates, 2005.

In the last two decades, North America, United Kingdom, Eastern Europe, Middle East, Pacific Islands, Australasia and China have witnessed a three-fold increase in the number of obese individuals. Current obesity levels range from below 5% in China, Japan and certain African nations, to over 75% in urban Samoa. In India, obesity is emerging as an important health problem, paradoxically co-existing with significant under nutrition prevailing in different sections of the population. The prevalence of obesity has also risen in children and adolescents with childhood obesity growing to epidemic proportions in developed countries. An estimated 17.6 million children under the age of five are reportedly overweight worldwide. In USA, the number of overweight children has doubled while, the number of overweight adolescents have registered a

three-fold increase since 1980 (Figure.2). An alarmingly increased prevalence of type 2 diabetes among children of India and other countries finds attributed to the increased propensity for obesity amongst this population (WHO, 2000). Obesity accounts for 2-6% of total health care costs in several developed countries. The true costs are undoubtedly much greater as not all obesity-related conditions are included in the calculations (WHO, 2003b).

Obesity results due to an imbalance between energy intake and expenditure and, secretion of a number of adipokines from the hypertrophied adipose tissue marks this physiological state (Goossens, 2008). These adipokines in turn affect whole body homeostasis by their favorable or unfavorable effects. The expression of adipokines such as leptin heavily depends on the integrity and physiological status of the adipose tissue. One of the crucial regulatory centres in adipose tissue is the transcription factor - peroxisome proliferator activated receptor (PPARγ), which is a fatty acid and eicosanoid dependent nuclear receptor that plays key roles in adipogenesis. Its over expression affects other regulatory genes (Pagliassotti *et al.,* 1997) that eventually culminates in hypertrophy of adipocytes and development of obesity.

Nonalcoholic steatohepatitis: A concept 'lesser known'

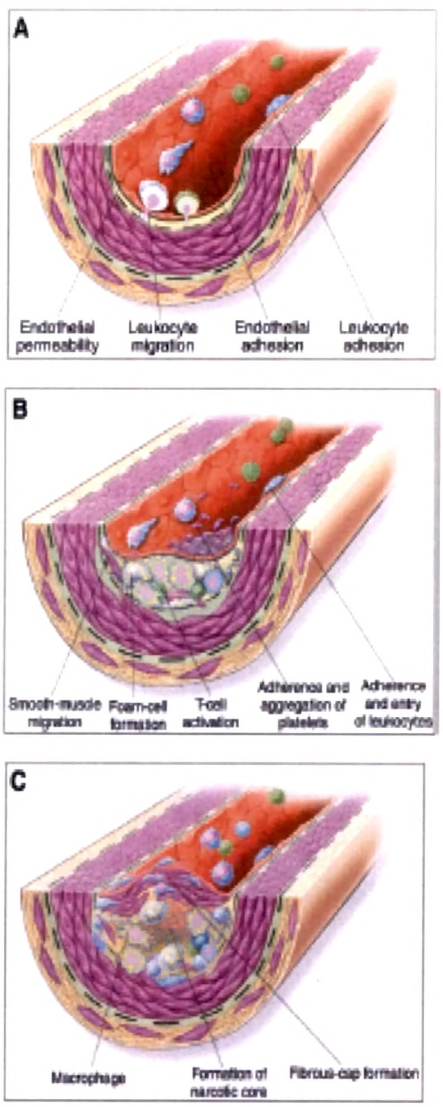
Ludwig *et al.* (1980) described a pathophysiological condition of fatty liver in non-alcoholic individuals for the first time and introduced the term non-alcoholic steatohepatitis (NASH). NASH is considered to be the 3rd most common liver disease after hepatitis C and alcoholic fatty liver in the United States (Patel and Lee, 2001). Global prevalence of NASH stands at 10-24 % amongst the general populace but with a

higher incidence of 25 to 75% in obese diabetic individuals (Angulo and Lindor, 2002). High fat diet leads to elevation in plasma and hepatic lipids that tilts the metabolic balance towards anabolism. This imbalance results in net fat accumulation in hepatocytes. This marks the first step referred to as the "*first hit*" of onset of NASH. Subsequently, fat accumulation and mitochondrial fatty acid oxidation produce reactive oxygen species (ROS) inducing intracellular oxidative stress, inflammation and gross injuries to hepatocytes leading to fibrosis. These set of events together are referred as the "*second hit*" for induction of NASH (Duvnjak *et al.,* 2007). Asymptotic and unexplained increase in the activity levels of plasma aspartate transaminase (AST) and alanine transaminase (ALT) are often correlated with the onset of NASH (Angulo, 2002). Non-invasive radiological imaging techniques such as ultrasonography, computed tomography scan and magnetic resonance imaging are not capable of distinguishing between fatty liver and NASH. These facts make the entire process of diagnostics and therapy of NASH difficult because biopsy is the only comprehensive option for its confirmation (Dabhi *et al.,* 2008). Obese individuals are at maximum risk of developing NASH (Duvnjak *et al.,* 2007) and a strong positive correlation exists between insulin resistance (IR) and development of NASH (Chitturi *et al.,* 2002) and the same has been validated for animals as well (Chidambaram and Venkatraman, 2010).

Atherosclerosis

The primary cause of death in the Western world involves cardiovascular diseases (CVD) such as ischemic (coronary) heart disease, angina pectoris, and myocardial and cerebral infarctions (Frost *et al.,* 1996). The most important cause of CVD and consequent

mortality is the phenomenon called atherosclerosis - narrowing of the arteries due to arterial lipid deposition (Lester *et al.,* 2006). Atherosclerosis is the major source of morbidity and mortality in the developed world. Atherosclerosis is the main underlying cause of heart disease that affects 60 million Americans and is the leading cause of death of adults in the United States. An estimated 1,100,000 new or recurrent heart attacks occur annually which translates into someone experiencing a heart attack every 20 seconds; one-third of these episodes will lead to death. At this moment, atherosclerosis places a great burden on society. According to the American Heart Association (AHA), 71.3 million Americans had some form of cardiovascular disease (CVD) in 2003. CVD was responsible for nearly 1 million deaths in 2003 and projected cost estimate in terms of direct and indirect health care in 2006 was 403.1 billion (Kleinschmidt, 2006). The magnitude of this problem is profound, as atherosclerosis claims more lives than all types of cancer combined and the economic costs are considerable. Although currently a problem of the developed world, the World Health Organization predicts that global economic prosperity could lead to an epidemic of atherosclerosis as developing countries take to more and more of Western habits (Stocker and Keaney, 2004). In urban China, the death rate from coronary disease rose by 53.4 per cent from 1988 to 1996 (Pande, 2004). Asian Indians have a have higher prevalence of premature CAD as compared to Europeans (Bahl *et al.,* 2001). In the last 40 years, India has witnessed a 10 folds increase in CAD (Mohan *et al.,* 2001) and the projected statistics assume that about 60% global cases of CADs will come from of Indian population (Pande, 2004).



Atherosclerosis is a chronic inflammatory disorder of intima of large arteries characterized by formation of fibro fatty plaques called atheroma. Accumulation of cholesterol-laden macrophages in large and medium-sized arteries characterizes the condition. This deposition leads to proliferation of certain cell types such as macrophages, lymphocytes, smooth muscle cells, micro vessels and collagen type within the arterial wall that gradually occludes the vessel lumen and impedes blood flow (Figure.3). This process may be quite insidious, lasting for decades until an atherosclerotic lesion is formed (Stocker and Keaney, 2003).

Figure.3 Induction of atherosclerosis

Epidemiological studies have revealed numerous environmental and genetic risk factors for the initiation of atherosclerosis. These include smoking, consuming a high fat diet, low blood antioxidant levels, hypertension, diabetes, systemic inflammation, male

gender, family history, and elevated blood lipid levels (Inoue et al 1996). High plasma total cholesterol and LDL levels show significant positive correlation with development of atherosclerosis and CVD (Lemieux *et al.,* 2001). Various studies have registered the relationship between high calorie diet and its effects on lipoprotein metabolism directly influencing the progression of the disease (Galassetti and Pondello, 2006). Diets rich in saturated fat such as palmitic, myristic, and lauric acids increase plasma cholesterol levels.

The local alteration in the vascular endothelium causes expression of surface adhesion molecules and cytokinesis causing adherence and subsequent migration of mononuclear cells (e.g. monocytes and lymphocytes) into the sub-endothelial space. These cells differentiate into macrophages and take up (modified) lipoproteins, forming foam cells (*fatty streak*) (Brown and Goldstein, 1986). Subsequently, smooth muscle cells proliferate and migrate to form a fibrotic cap, consisting of extracellular matrix, collagen, and proteoglycans, that covers the lipid or necrotic core (plaque) formed by excessive macrophage cell death (*advanced fibrous lesion*) (Gibbons, 1990). A thick fibrous cap covering the area makes the plaque stable but a thin and non uniform cap may lead to instability of the plaque leading to its rupture, secondary hemorrhage, thrombosis, and possible occlusion of the artery (Schmitz and Langmann, 2001).

Available treatments/therapy

Table. 1 available synthetic drug for the treatment of metabolic disorders

Drug	Mechanism of Action	Side Effects
Hyperlipidemia/hypercholesterolemia		
Statin (Lovastatin Pravastatin,Rosuvastatin Simvastatin, Atorvastatin, Fluvastatin)	Partially inhibits HMG-CoA reductase.	Abnormal LFTs yositis/myalgias.
Fibrates (Gemfibrozil Fenofibrate)	Reduces VLDL synthesis and induces lipoprotein lipase.	Nausea and skin rash.
Colestipol, Colesevelam hydrochloride	Interrupts bile acid reabsorption requiring bile acid synthesis from cholesterol	Taste/texture, bloating, heartburn, constipation, drug interaction and TG increase.
Niacin and lovastatin	Combination product of both extended-release niacin (niaspan) and statin (lovastatin).	Flushing, nausea, glucose intolerance, gout, elevated uric acid levels. Myositis/myalgias
Ezetimibe and simvastatin	Combination of intestinal absorption blocker and statin. Both selectively inhibit the intestinal absorption of cholesterol and partially inhibit HMG-CoA reductase.	Abnormal LFTs, myositis/myalgias
Niacin (Niaspan extended release®)	Reduces hepatic production of B-containing lipoproteins, increases HDL production.	Flushing, nausea, glucose intolerance, gout, LFT abnormalities, and elevated uric acid levels.
Omega-3 fatty acids	Inhibits hepatic TG synthesis and augments chylomicron TG clearance	Dyspepsia, nausea.
Obesity		
Orlistat (Xenical)	reduces intestinal fat absorption through inhibition of pancreatic lipase	increased blood pressure, dry mouth, constipation, headache, andinsomnia
Sibutramine (Reductil)	which is an anorectic, or appetite suppressant	

Radafaxine andoleoyl-estrone	centrally-acting drugs	
Cetilistat and AOD9604	drugs blocking fat absorption	
Insulin resistance		
Biguanides (Metformin,Phenformin,Buformin)	reduce hepatic glucose output and increase uptake of glucose by the periphery, including skeletal muscle	impaired liver or kidney function, lactic acidosis risk
Thiazolidinediones (rosiglitazone,pioglitazone,troglitazone)	bind to PPARγ, genes regulating glucose and fat metabolism.	withdrawn due to hepatitis and liver damage risk
Alpha-glucosidase inhibitors	These agents slow the digestion of starch in the small intestine, so that glucose from the starch of a meal enters the bloodstream more slowly, and can be matched more effectively by an impaired insulin response or sensitivity	effective only in the earliest stages of impaired glucose tolerance, associated with flatulence and bloating
Incretins(glucagon-like peptide-1 (GLP-1)	insulin secretagogues	cause a decrease in gastric motility, Nausea

Treatment/therapy for metabolic disorders

Treatment of hyperlipidemia requires major amendments in lifestyle that include avoidance of smoking, eating a heart-healthy diet, regular exercise and medication. However, a combination of dietary measures and drug treatment appears the most often employed schedule in high-risk individuals. Several modern drugs in vogue such as statins, fibrates, nicotinic acid and resins essentially find application as hypocholesterolemic agents (Satoskar *et al.*, 2003). Statins or 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) reductase inhibitors are the most common

medication for lowering LDL cholesterol. Usage of fibrates and niacin attempts to lower the level of triglycerides and increase HDL cholesterol. Bile acid sequestrants find common usage in clinical practice to lower LDL to recommended levels. The US National Cholesterol Education Program (NCEP) and Adult Treatment Panel (APT) III emphasize that patients with coronary heart disease (CHD) or risk factor of CHD >20% over 10 years and those with LDL cholesterol (LDL) levels >130 mg/dl should receive drug therapy with an aim of reducing LDL to <100 mg/dl (NCHS, 2006). Prevention of CHD and atherosclerosis includes reduced intake of saturated fat and cholesterol, increased activity and weight control (NCEP, 1994). A number of anti-obesity drugs are currently undergoing clinical development, including centrally-acting drugs (e.g. radafaxine and oleoyl-estrone), drugs targeting peripheral episodic satiety signals (e.g. rimonabant and APD356), drugs blocking fat absorption (e.g.cetilistat and AOD9604) and human growth hormone fragments (Yun, 2010; Halford, 2006; Melnikova and Wages, 2006).

However, these interventions are associated with undesirable side effects. The two main anti-obesity drugs, Orlistat and Sibutramine, have been associated with side effects such as increased blood pressure, dryness of mouth, constipation, headache, and insomnia. Surgical procedures for obesity include gastric bypass procedure, gastroplasty, Gastric banding, partial biliopancreatic bypass procedures, jejunoileal bypass. However, all these techniques are challenging and often manifest undesirable side effects.

Phytotherapeutics: A multipronged arrow for multiple targets

The available spectrum of drugs has failed to reduce the magnitude of the so-called lifestyle diseases. Therefore, the biggest challenge for the pharmaceutical industry is to find a drug with multiple therapeutic potentials to tackle this problem. Herbal medicines appear to be effective alternatives to synthetic medicines due to their cost effectiveness, efficiency and, relative lack of side effects. Herbal medicines find usage in about 80% of the world population, primarily in the developing countries, as primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and side effects. The chemical constituents present in them form part of the physiological *milieu* of living flora and hence believed to have better compatibility with the human body. World Health Organization (WHO) has recommended evaluation of the potentials of plants as effective therapeutic agents, especially in areas where we lack safe modern drugs (WHO, 1996).

India is blessed by a gold mine of well-recorded and well-practiced knowledge base of traditional herbal medicine. However, unlike China, India has failed to capitalize on this herbal wealth by promoting its use in the developed world despite the renewed interest in herbal medicines.

Ayurveda: A ray of hope

Ayurveda and Kabiraji (herbal medicine) are two important forms of alternative medicine of wide traditional practice in India. Ayurveda is a 5000-year old science of health care and herbal treatment. The existence of Ayurvedic medicines in India dates back to thousands of years. This field employs various techniques and parts of plants to heal or

provide relief to the ailing patients. Ayurveda, now strengthened by modern scientific research and technologies, can provide gentle healing touch to millions around the world. Many forms of alternative medicines are available in India for those who, either do not want conventional medicine or do not find succor by conventional medicines (Kamboj, 2000). The turnover of herbal medicines in India in the form of over-the-counter products, ethical and classical formulations or home remedies of Ayurveda, Unani and Siddha systems of medicine together accounts for about $ 1 billion with a meager export of about $ 80 million.

A variety of natural products, including crude extracts and isolated compounds from plants, can induce body weight reduction and prevent diet-induced obesity. Therefore, they have been widely used in treating obesity (Moro and Basile, 2000; Han *et al.,* 2005a; Rayalam *et al.,* 2008). A wealth of information supports the usage of numerous bioactive components from nature as potentially useful in the treatment of obesity. Hence, natural products for treating obesity are under exploration, and this may be an excellent alternative strategy for developing future effective and safe anti-obesity drugs (Park*et al.,* 2005; Nakayama *et al.,* 2007; Mayer *et al.,* 2009).

Phytocompounds and their therapeutic uses

Recent studies have shown effectiveness of herbal drugs with multipronged therapeutic potential as possible therapeutants against hyperlipidemia, obesity, NASH and atherosclerosis (Haddad *et al.,* 2009). Consumption of plant products as natural antioxidants has gained popularity in the last few decades mainly due to their cost effectiveness and minimal side effects (Verma and Singh, 2008). Consumption of

phenolics and flavonoids through diet appears to be inversely associated with morbidity and mortality from CHD (Hertog *et al.,* 1993, 1995; Muldoon and Kritchevsky, 1996). Different classes of flavonoids are present in fruits, vegetables and beverages and, polyphenolic flavonoids prevent coronary artery disease by reducing platelet aggregation, plasma cholesterol or by inhibiting LDL oxidation (Aviram, 1996; Aviram and Fuhrman, 2002; Middleton *et al.*, 2001).

Sida rhomboidea Roxb: The herb in question

Classification

Kingdom - *Plantae*

Division - *Angiospermae*

Class - *Eudicots*

Order - *Malvales*

Family - *Malvaceae*

Genus - *Sida*

Species – *Sida rhomboidea.* Roxb

Figure.4 A digital photograph of *S.rhomboidea*.Roxb

Common names

Hindi: Sahadeyi

Kannada: Muttavupulaga, Gubataba

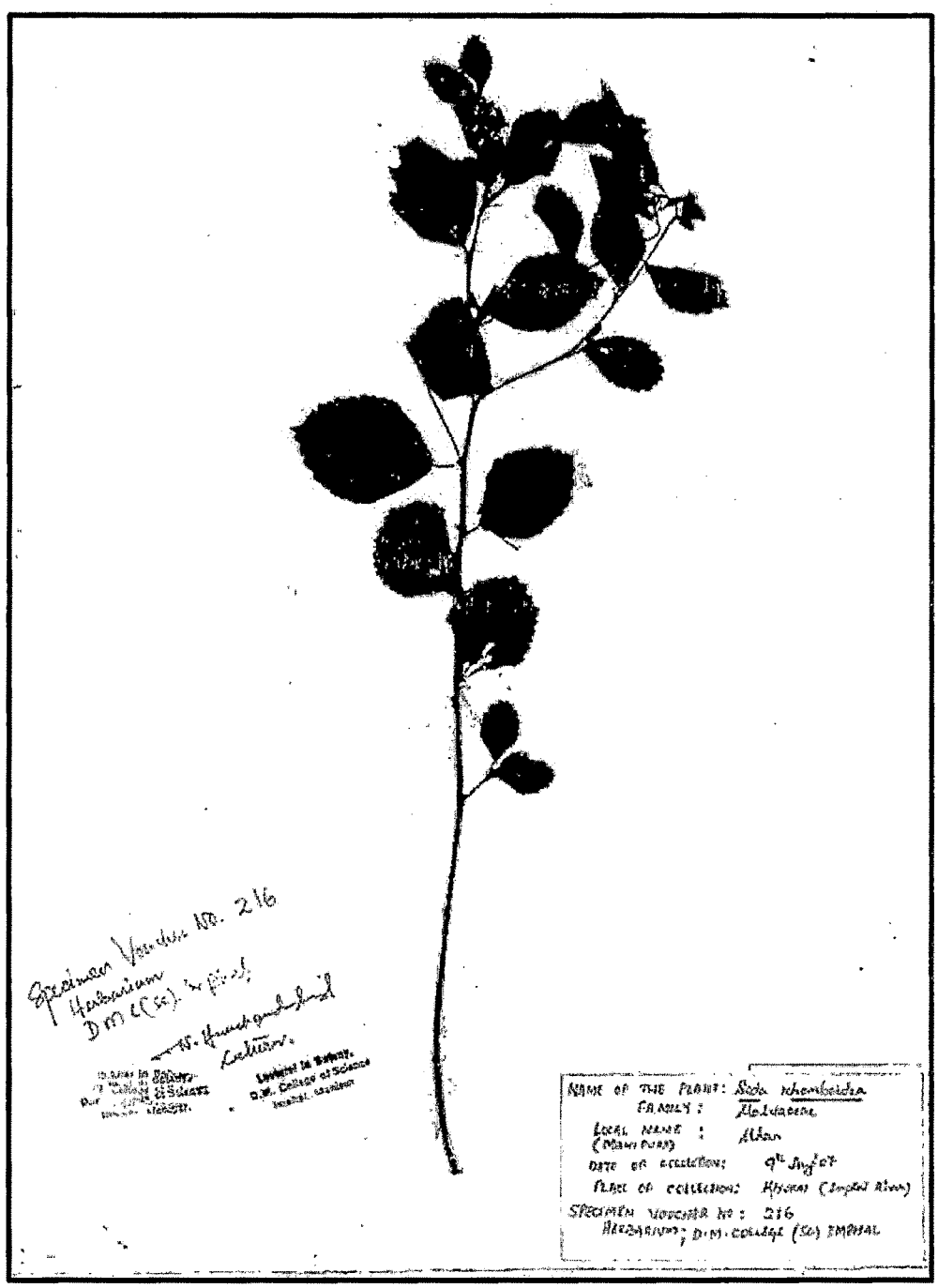
Malayalam: Vatturam

Manipuri: Uhan

Bengali: Bedela

Marathi: Bhaurdi

Figure.5 Voucher specimen of *S.Rhomboidea*.Roxb

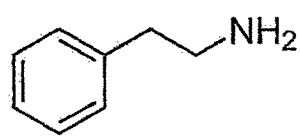


Botanical description

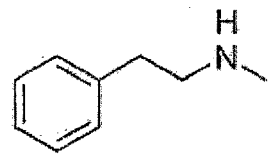
Sida rhomboidea. Roxb (*Sida rhombifolia.* L.var. rhomboidea) is present throughout India. It is sub shrub erect or prostrate; many branched, to ca. 1 m tall. Branchlets stellate. Stipules spine like, 3–5 mm; petiole 2–5(–8) mm, stellate puberulent; leaf blade rhombic to oblong lanceolateor obovate, rarely linear-lanceolate, 1–4.5 × 0.6–2cm, abaxially gray-white stellate pilose, adaxially sparsely stellatepilose to sub glabrous, base broadly cuneate, margin dentate, apex obtuse to acute. Flowers solitary, axillary. Pedicel 1-2.5 cm, densely stellate tomentose, articulate above middle. Calyxcup-shaped, 4–5 mm, abaxially stellate pubescent, lobes triangular, apices acute. Corolla ca. 1 cm in diam.; petals yellow, obovate, ca. 8 mm, base attenuate, apex rounded. Filament tube4–5 mm, glabrous. Style branches 8–10. Fruit semi globose tobroadly turbinate, 6–7 mm in diam.; mericarps 7–10, 2.5–3 mm excluding awn, shallowly grooved to near base, eventually dehiscent,side walls usually thin, not veined, stellate puberulent,apex usually (1 or)2-awned, awns to 1.5 mm. Seeds reniform,ca. 2 mm, blackish. Fl. autumn–winter (Anonymous, 2007).

Sida rhomboidea Roxb. (SR) is a weed of Malvaceae family found in marshy places throughout India (Puri, 2002) known in Ayurveda as "*Mahabala*". The root and leaves are used against fever, heart diseases, burning sensations, piles and all kinds of inflammation besides being used as an aphrodisiac (Pullaiah, 2006). In parts of North East India, a decoction prepared from leaves of SR is consumed by rural and urban populace to alleviate symptoms of obesity and diabetes. Its root is been used as antimicrobial and antipyretic agents (Alam *et al.*, 1991a,b)

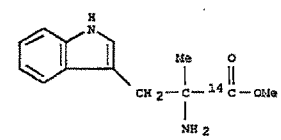
Phytochemical analysis of SR leaves (Prakash *et al.*, 1981; Goyal and Rani; 1988, 1989).



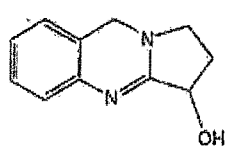
β-phenethylamine



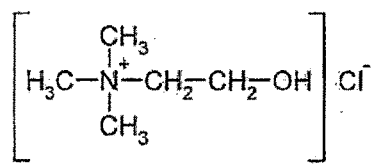
N-methyl-β phenethylamine



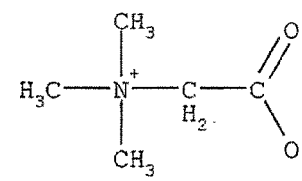
S-(+)-Nb-methyl tryptophan methylester



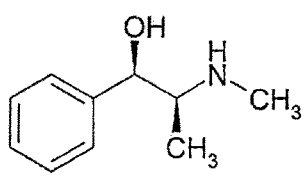
Vasicine



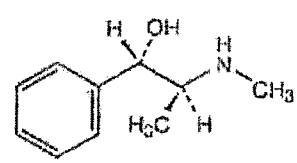
Choline



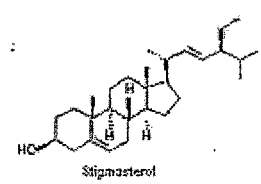
Betaine



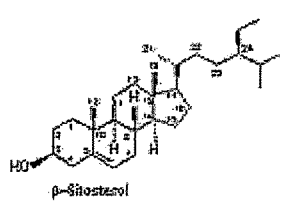
Ephedrine



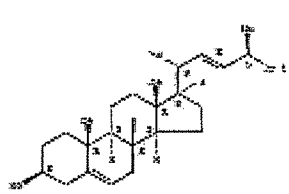
ψ-ephedrine



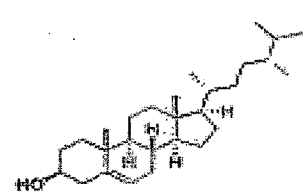
Sigmasterol



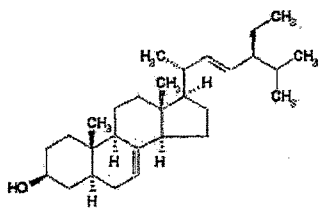
β-sitosterol



22-dehydrocampesterol



Campesterol



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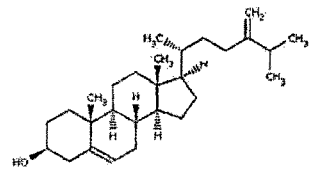
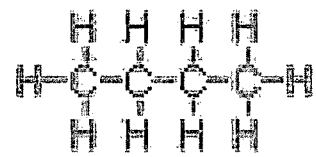


24-methylenecholesterol



n-alkanes

Pharmacological properties of SR leaf extract

Antinociceptive and anti-inflammatory activity

Various organic extracts of *Sida rhomboidea.* Roxb leaves were studied for antinociceptive and anti- inflammatory activity at a dose of 200 mg/kg. Ethyl acetate extract showed significant ($P<0.01$) antinociceptive activity in an experimental model of acetic acid induced writhing test. Percentage inhibition of carrageenin induced rat paw edema by butanolic extract (33.05, $P<0.001$) is comparable to that of phenylbutazone, 100 mg/kg inhibition (38.83%). Phytochemical screening of the extracts indicated the presence of alkaloids, steroids and/or triterpenoids and their glycosides, tannins, flavonoids and their glycosides, carbohydrates and absence of cardiac glycosides (Venkatesh *et al.*, 1994).

Nephroprotective potential

Nephroprotective potential of SR aqueous extract have been demonstrated against gentamicin (GM) induced nephrotoxicity and renal dysfunction in rats. Nephrotoxicity was induced in rats with GM (100 mg/kg bodyweight (i.p.) for 8 days) and were treated

with SR extract (200 and 400 mg/kg bodyweight (p.o.) for 8 days) or 0.5% arboxymethyl cellulose (vehicle). Plasma and urine urea and creatinine, renal enzymatic and non-enzymatic antioxidants along with lipid peroxidation were evaluated in various experimental groups. GM treatment induced significant elevation ($p < 0.05$) in plasma and urine urea, creatinine, renal lipid peroxidation along with significant decrement ($p < 0.05$) in renal enzymatic and non-enzymatic antioxidants. SR treatment to GM treated rats (GM + SR) recorded significant decrement ($p < 0.05$) in plasma and urine urea and creatinine, renal lipid peroxidation along with significant increment ($p < 0.05$) in renal enzymatic and non-enzymatic antioxidants. SR leaf extract ameliorates GM induced nephrotoxicity and renal dysfunction and thus validates its ethnomedicinal use (Thounaojam *et al.*, 2010a).

Antioxidant and free radical scavenging potential

Antioxidant potential of *Sida rhomboidea*.Roxb methanol extract (MESR) was evaluated using *in vitro* models such as lipid peroxidation, metal chelation and reducing potential. Its free radical scavenging ability was also examined using *in vitro* assays for 1, 1-diphenyl-2-picryl-hydrazil (DPPH), super oxide ($^{\cdot}O2$), hydrogen peroxide (H_2O_2), nitric oxide ($NO^{\cdot}$) and hydroxyl radical ($HO^{\cdot}$) radical scavenging. Wherein, MESR recorded dose dependent effect on inhibition of lipid peroxidation (IC50= 92.15±1.21µg/ml), effective metal chelation (IC_{50}=65.69±1.22µg/ml) and higher reducing potential (OD_{max}=1.20±0.27). Also, MESR could efficiently scavenged DPPH (IC_{50}=63.23±1.59µg/ml), $^{\cdot}O_2$ (IC_{50}=142.36±2.59µg/ml), H_2O_2 (IC_{50}=125.96±3.00µg/ml), $NO^{\cdot}$ (IC_{50}=85.36±2.01µg/ml) and $HO^{\cdot}$ (IC_{50}=90.45±1.88 µg/ml) radicals in a dose

dependent manner. Results obtained herein can be attributed to the presence of polyphenols (35.60±1.20 mg/ml gallic acid equivalent-polyphenols), flavonoids (26.94±0.94 mg/ml quercetin equivalent-flavonoids) and ascorbic acid (28.71±1.14 mg/ml ascorbic acid) in MESR (Thounaojam *et al.*, 2010b).

Prevention of insulin resistance

Protective effects of *Sida rhomboidea* Roxb on high fat diet (HFD) induced insulin resistance in was carried out in C57BL/6J mice. Changes in bodyweight, food intake, fasting blood glucose, plasma insulin, plasma and hepatic triglyceride (TG), total cholesterol (TC) and free fatty acids (FFAs) were investigated in various experimental groups. It was observed that feeding of SR extract to HFD fed mice (HFD+SR1 and SR3) reduced bodyweight ($p<0.05$), food intake ($p<0.05$) and feed efficiency ratio ($p<0.05$). Plasma and hepatic TC, TG and FFA were also significantly lowered ($p<0.05$) in HFD+SR groups. Efficient clearance of glucose in intraperitoneal glucose tolerance test (IPGTT), lowered area under curve ($AUC_{glucose}$) values, low plasma insulin and fasting insulin resistance index (FIRI) coupled with higher K_{ITT} values were observed in HFD+SR groups. These findings were further justified by significant reduction of adipocyte diameter ($p<0.05$) and surface area ($p<0.05$) in HFD+SR groups. This study is a first scientific report on protective role of *S. rhomboidea* Roxb extract against HFD induced insulin resistance in C57BL/6J mice (Thounaojam *et al.*, 2010c).

Toxicological evaluations

Safety evaluation of *S.rhomboidea*.Roxb leaf aqueous extract were carried out using acute and sub-chronic toxicity assays in Swiss albino mice as per Organization for Economic Co-operation and Development (OECD) guidelines. Possible behavioral changes and lethality were observed in mice administered a single dose [1 000, 2 000, 3 000, 4 000 or 5 000 mg/kg body weight (BW)] of SR. Plasma levels of metabolic, hepatic, cardiac and renal function markers, electrolytes, blood count and histopathology of major organs were monitored in mice chronically treated with EJSE (1 000, 2 000 or 3 000 mg/kg BW) for 28 days. Since no mortality was recorded in the acute toxicity evaluation up to a dose of 5 000 mg/kg bodyweight of SR, 50% lethal dose (LD_{50}) was assumed to be > 5 000 mg/kg BW. In the sub-chronic toxicity evaluation, no adverse observations were recorded in mice administered with 2 000 mg/kg BW of SR; however at 3 000 mg/kg BW dose, moderately significant increase in the plasma levels of urea and creatinine was observed. Hence, the lowest observable adverse effect level (LOAEL) for SR was found to be 3 000 mg/kg BW and the no observable adverse effect level (NOAEL) was adjudged as 2 000 mg/kg BW. It can be concluded from this study that, orally administered SR is safe up to a 10 fold higher dose than its reported therapeutic dose (Thounoajam *et al.*, 2010d).

Cardioprotective potential

Cardioprotective effect of SR leaf aqueous extract was evaluated against isoproterenol (IP) induced myocardial necrosis (MN) in male albino rats. Rats treated with IP (85 mg/kg, s.c.) recorded significant ($p<0.05$) increment in heart weight, plasma lipid

profile, plasma marker enzymes of cardiac damage, cardiac lipid peroxidation (LPO) and activity levels of Ca^{+2} ATPase whereas there was significant ($p<0.05$) decrease in plasma HDL, cardiac endogenous enzymatic and non-enzymatic antioxidants, Na^{+}–K^{+} ATPase and Mg^{+2} ATPase. Pre-treatment with SR extract (400 mg/kg per day, p.o.) for 30 consecutive days followed by IP injections on days 29th and 30th, showed significant ($p<0.05$) decrease in heart weight, plasma lipid profile, plasma marker enzymes of cardiac damage, cardiac lipid peroxidation, Ca^{+2} ATPase and significant increase in plasma HDL, cardiac endogenous enzymatic and non-enzymatic antioxidants, Na^{+}–K^{+} ATPase and Mg^{+2} ATPase compared to IP treated group (Thounaojam *et al.*, 2011).

The present study in a nutshell

The current work entitled "Protective role of *Sida rhomboidea*.Roxb leaf extract on high fat diet induced metabolic disorders: Biochemical and molecular evaluation in *in vivo* and *in vitro* experimental models" was undertaken to evaluate the efficacy of *S.rhomboidea*.Roxb extract; a pandemic herb of India, in controlling high fat diet (HFD) induced metabolic disorders in experimental models. Known as "*Mahabala*" in Ayurveda, it finds extensive use by the local populace of Manipur as a home remedy against diabetes and obesity. The attempted studies using the leaf extract of SR are:

1. The potential as hypolipidemic agent in normolipidemic and hyperlipidemic rats.
2. The potentials to inhibit absorption of dietary fat and cholesterol, control hepatic catabolism of fat and, conversion of cholesterol to bile acids.
3. Efficacy in prevention of experimentally induced steatohepatitis in HFD fed C57BL/6J mice.

4. Antioxidant potentials and mitochondrial antioxidant status and lipid accumulation in liver and structural deformities using *in vivo* HFD fed mice and *in vitro* HepG2 cell line.
5. Real time PCR analysis of PPARγ2, SREBP1c, CPT, FAS and Leptin in epididymal fat pad of HFD fed SR supplemented C57BL/6J mice.
6. *In vitro* evaluation of anti-adipogenic potential using 3T3L1 pre-adipocyte differentiation assay.
7. Assay of anti-atherogenic potential in terms of human LDL oxidation, ox-LDL induced macrophage oxidative stress and apoptosis, and monocyte-macrophage differentiation using *in vitro* experimental systems.
8. *In vivo* evaluation of high cholesterol induced plaque formation and expression of cell adhesion molecules in the thoracic aorta in atherogenic diet fed rats.

Chapter 1

Dysregulation of lipid and cholesterol metabolism in high fat diet fed hyperlipidemic rats: Protective effect of *Sida rhomboidea.roxb* leaf extract

The present study aims to evaluate the potential of *Sida rhomboidea*.Roxb in nullifying the dysregultion in lipid and cholesterol metabolisms in hyperlipidemic rats.

***Published as*: Thounaojam M**, Jadeja R, Ansarullah, Devkar R, Ramachandran AV. Dysregulation of lipid and cholesterol metabolism in high fat diet fed hyperlipidemic rats: Protective effect of *Sida rhomboidea.roxb* leaf extract. *Journal of Health Science* 2009; 55:413-420.

INTRODUCTION

Excessive dietary lipids and cholesterol are the major factors of relevance for the development of hypertriglyceridemia and hypercholesterolemia, two important cardiovascular risk factors (Hokanson, 2002; Tanasescu *et al.*, 2004). Hyperlipidemia, hypercholesterolemia, obesity, sedentary lifestyle etc are the key risk factors that precipitate cardiovascular disorders (CVD) (Reiner and Reiner, 2006) and the most common causes of death in developed as well as developing countries (Yokozawa *et al.*, 2003). Some of the major limitations in the effective pharmacological treatment of hyperlipidemia are the constraints imposed on healthcare resources, particularly in the low- and middle-income countries (Bergman *et al.*, 2005). There is urgent need to tackle this physiological problem as it is assuming grave proportions globally. In this scenario, natural agents may prove invaluable in tackling the problem due to their cost effectiveness and minimal side effects (Oluwatosin *et al.*, 2008). In recent times, much research interest remains focused on various herbs that possess hypolipidemic properties that may be useful in reducing the risk of cardiovascular diseases (Craig, 1999).

Hence, the present study aims to evaluate the potential of *Sida rhomboidea*.Roxb in nullifying the dysregulation in lipid and cholesterol metabolisms in hyperlipidemic rats.

MATERIALS AND METHODS

Plant material

SR leaves were collected from Imphal district India in the month of June and shade dried. The plant was identified at the Department of Botany, D.M.College of Science Manipur, Imphal and a sample (voucher specimen No.216) was deposited at the herbarium of the Department of Botany.

Preparation extract

Leaves of SR were shade dried and finely powdered. Hundred grams of the powder was boiled in distilled water at 100°C for 3 hours and the resulting filtrate was concentrated by heating to obtain a semisolid paste, that was later freeze dried resulting in 24% W/W yield. Different concentrations of freeze-dried extract (SR) were prepared by dissolving a known weight of dried paste in 0.5% Carboxy methylcellulose (CMC).

Phytochemical analysis

Preliminary qualitative analysis of SR leaves helped target specific quantitative analysis for saponins (Xi *et al.*, 2007), phytosterols (Goad and Akihisa, 1997), ascorbic acid (Barakat *et al.*, 1973), polyphenols (Yen and Hsieh, 1998) and flavanoids (Chang *et al.*, 2002).

HPLC fingerprinting of SR

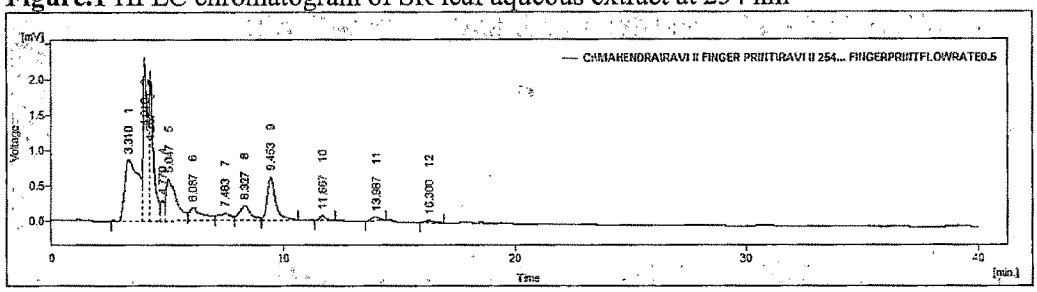
Solvent system: - methanol:water (50:50)

Flow rate:-0.5 ml/min

Total run time: - 40 min

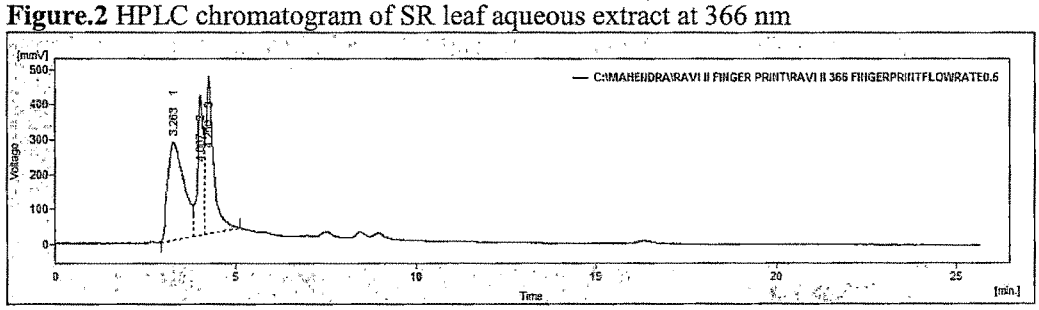
Scanning wavelengths: - 254nm, 366nm and 540 nm.

Figure.1 HPLC chromatogram of SR leaf aqueous extract at 254 nm



	Reten.Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	3.310	349.756	8.654	23.6	11.6	0.76
2	4.010	282.107	23.073	19.0	31.0	0.24
3	4.267	259.847	21.155	17.5	28.4	0.17
4	4.770	31.917	2.842	2.2	3.8	0.20
5	5.047	188.566	5.944	12.7	8.0	0.49
6	6.087	75.759	1.893	5.1	2.5	0.62
7	7.483	38.853	1.021	2.6	1.4	0.84
8	8.327	72.691	2.129	4.9	2.9	0.48
9	9.453	148.170	6.134	10.0	8.2	0.34
10	11.667	13.440	0.719	0.9	1.0	0.28
11	13.987	12.097	0.518	0.8	0.7	0.38
12	16.300	8.463	0.302	0.6	0.4	0.45
	Total	**1481.666**	**74.383**	**100.0**	**100.0**	

Figure.2 HPLC chromatogram of SR leaf aqueous extract at 366 nm



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	3.263	86.567	2.791	43.8	24.8	0.53
2	4.007	40.695	3.994	20.6	35.5	0.16
3	4.250	70.397	4.479	35.6	39.8	0.25
	Total	**197.658**	**11.264**	**100.0**	**100.0**	

Experimental Animals

Female *Charles foster* albino rats (180-220g) were maintained in clean polypropylene cages under controlled room temperature (22±2°C) and fed with normal diet (SLD) or high fat diet (HFD) (Rathi *et al.*, 1984) and provided with water *ad libitum*. Experiments on animals were performed in accordance with guidelines of the institutional animal ethical committee (Approval No.827/ac/04/CPCSEA).

Table. 1 Composition of experimental diets

Ingredients (g%)	Normal diet (SLD)	High fat diet (HFD)
Laboratory chow	35.0	48.9
Sucrose	20.0	20.0
Casein	20.0	20.0
Coconut oil	20.0	5.0
Salt mixture	4.0	4.0
Vitamin mixture	1.0	1.0
Cholesterol	-----	1.0
Cholic acid	-----	0.1

Experimental Design

Fifty-four animals were divided into 9 groups (n=6 per group). Groups I and V were fed with SLD and HFD respectively while, groups II, III, and IV were maintained on SLD and orally administered with 200,400 or 800 mg/kg SR extract daily. Groups VI, VII and VIII maintained on HFD received SR extract as mentioned above. Group IX fed with HFD received synthetic hypolipidemic agent, lovastatin (LVS; 5mg/kg BW). All the

animals maintained for a total of 42 days received oral administration of vehicle, extract or LVS by gastric intubation.

Lipid profile

Assay of total plasma total lipids was by the method of Frings *et al* (1972). Plasma total cholesterol (TC), triglycerides (TG) and high-density lipoprotein (HDL) were analyzed using commercially available kits (Reckon diagnostics, Baroda, India). Very low-density lipoproteins (VLDL) and low density lipoprotein (LDL) were calculated by Friedwald's formula (Friedwald *et al.*, 1972).

VLDL= TG/5 and LDL=TC-HDL+VLDL

Hepatic and fecal lipids were extracted in chloroform: methanol (2: 1) mixture and dried. Total lipids (TL) were estimated by gravimetric analysis (Folch *et al.*, 1957). Dried lipid extract was dissolved in 1% triton X 100 (Jong-Ho *et al.*, 2003) and TC and TG were analyzed as above.

Fecal cholic acid (CA) and deoxycholic acid (DCA)

Fecal samples from each experimental group were collected on every 3rd day between days 31 and 42 of study. Fecal samples were dried, eluted with absolute alcohol, filtered and processed for estimation of CA and DCA (Mosback *et al.*, 1954). The dried filtrate was hydrolysed with 2 ml of 5% NaOH at 15 LBS pressure in an autoclave for 1.5 hr, neutralized with ether and evaporated to dryness. Later, residues were dissolved in 1 ml acetone, centrifuged and the supernatant was dried. Then, 1.5 ml of 65% H_2SO_4 was added to the dried filtrate and heated at 60°C for 15 min, cooled at room temperature and the absorbance read at 320 nm for cholic acid and at 385 nm for deoxycholic acid.

Estimation of enzymes of lipid metabolism

- Activity levels of lipoprotein lipase (LPL; EC 3.1.1.34) were estimated in fresh tissue homogenates (Dole, 1955; Mays and Felts, 1968). A mixture of 0.5 ml of olive oil emulsion, 0.1ml of deoxycholate, 0.5ml of buffer and 0.5 ml of tissue homogenate was incubated at 37°C for 30 min along with control tubes to which enzymes were not added. The reaction was arrested by keeping the tubes at room temperature and 2.5 ml of chloroform and 5 ml of Doles mixtures were added and mixed well. Then 3 ml of hexane and 2ml of distilled water were added, shaken well and centrifuged. To 2 ml of the supernatant, 2 ml of chloroform and 2 ml of copper reagent were added. From this, 2ml aliquot was taken and 0.5 ml of diethyl thiocarbamate reagent was added. The color was read at 420 nm. Activity was expressed as μ moles of free fatty acids liberated /hr/mg protein.
- Heparin (50 units/rats) was injected intravenously two minutes before blood collection to facilitate release of endothelium bound LPL (Qi *et al.*, 2006). Separated plasma was assayed for post-heparin lipolytic activity (PHLA) as per Dole (1955) and Mays and Felts (1968).

Estimation of Cholesterol metabolizing enzymes

- An activity level of lecithin-cholesterol acyltransferase (LCAT, EC 2.3.1.43) was assayed in plasma by the method of Legraud *et al.* (1979) with modifications by Hitz *et al.* (1983). From incubation mixture containing 0.6 ml of substrate and 0.6 ml of tissue homogenate, 0.4 ml of the mixture was immediately transferred to a tube containing 1ml of isopropanol to arrest the reaction. This gave the cholesterol present at the beginning of the experiment. Incubation at 27°C was continued for 10 min and the reaction was arrested. After centrifugation 2 ml of

acetone and 1 ml of digitonin were added to the supernatant. It was left aside for 1 hr and centrifuged and cholesterol content was estimated. Plasma LCAT activity was expressed as moles of μmol of cholesterol esterified /hr/mg protein

- HMG CoA reductase (HMG-CoA reductase, EC 1.1.1.34) activity assayed by the method of Rao and Ramakrishnan (1975) was expressed as inversely proportional to the mevalonate content. 10% homogenate prepared in saline arsenate solution was mixed with equal volume of perchloric acid diluted (50 ml/l) and after 5 min; it was centrifuged for 10min at 2000rpm. One ml of supernatants was divided in two tubes, followed by addition of 0.5 ml of acidic (for mevalonate) or basic (for HMG Co A) hydroxylamine reagent and ferric chloride. The mixture was then incubated for 10 min at room temperature and absorbance was read at 540 nm.

Calculation:- ratio of absorbance = $\frac{\text{HMG Co A}}{\text{Mevalonate}}$

- Acetyl Co Acyl transferase (ACAT, EC 2.3.1.26) was assayed by the method of Kothari *et al.* (1973) in liver and intestine. An incubation mixture containing 0.5ml of acetate buffer, 0.2 ml substrate and 0.2 ml tissue homogenate was incubated at 37°C for 6 hr .The reaction was arrested by adding 5 ml acetone-ethanol mixture and centrifuged. Later, 1 ml digitonin was added to the supernatant, followed by 2 drops of acetic acid. After 16 hr of incubation in a dark chamber it was centrifuged and the precipitate was washed twice with acetone-ether mixture and finally with dry ether. The CES activity was expressed as moles of cholesterol esterified /hr/mg protein.
- Protein content of liver, adipose and intestine was analyzed by the method of Lowry *et al.* (1951).

Statistical analysis

Statistical analysis of the data was done by one-way ANOVA followed by Bonferroni's multiple comparison test. The results are expressed as mean ± S.E.M using Graph Pad Prism version 3.0 for Windows, Graph Pad Software, San Diego California, USA.

RESULTS

Phytoconstituents of S.rhomboidea.roxb

The quantitative phytochemical analysis of SR showed 4.5 mg/100mg saponins, 3.0 mg/100mg phytosterols, 1.9 mg/100mg ascorbic acid, 2.0 mg/100mg polyphenols, and 2.3 mg/100mg flavonoids.

Body weight and food intake

HFD fed rats recorded 16.56% increase in body weight while HFD+SR rats showed dose dependent reduction in weight gain with HFD+SR 800mg/kg recording the least increase of 8.35%. Body weight gain in SLD group was minimal within the range of 4-6%. There was no significant change in food intake of SLD+ SR fed rats but there was a significant reduction in food intake of HFD+SR 400mg/kg ($p<0.01$) and HFD+SR 800mg/kg ($p<0.001$) groups (Table.2).

Plasma and hepatic lipid profiles

Though SR *per se* did not have a significant effect in SLD fed rats, the extract had significant effect in reversing the altered serum and tissue lipid profiles induced by HFD, with the higher dose of 800 mg/kg being the most effective in normalizing the alterations. HFD+LVS group was also found to exert favorable effect but was not as effective as 800 mg dose of SR (Tables.3, 4 & 5 and Figures 3 & 4).

Lipid metabolizing enzymes

HFD rats recorded significant decrement in activity levels of PHLA, and LPL in adipose tissue and liver (P<0.001). HFD+SR (200mg/kg) did not record significant increment in PHLA and LPL activities. HFD+SR (400 and 800mg/kg) recorded significant increments in PHLA and LPL activities compared to HFD rats (P<0.001). HFD+LVS rats registered non-significant increment in PHLA and LPL activities compared to HFD group (Table.6 and Figure.5). The extract had no effect in control rats fed with SLD (Table.6 and Figure.5).

Cholesterol metabolizing enzymes

HFD rats recorded a significant decrement in plasma LCAT activity (P<0.001) and increment in hepatic and intestinal ACAT (Table.7 and figure.6). Hepatic HMG CoA reductase activity registered a non-significant alteration in HFD rats compared to SLD rats. Higher doses of SR (400 and 800 mg/kg) depicted dose dependent decrement in activity levels of HMG Co A reductase and increment in plasma LCAT activity (P<0.001). Hepatic and intestinal ACAT activity recorded significant decrement in HFD+SR rats with all three doses compared to HFD rats (Table.7 and Figure.6). HFD+LVS rats recorded highly significant decrease in HMG Co A reductase activity (P<0.001), a moderate decrement in Intestinal and hepatic ACAT activities (P<0.01) and a non-significant increment in plasma LCAT activity. The extract had no significant effect in hepatic HMG Co A reductase, plasma LCAT and ACAT activities in liver and Intestine when compared to SLD groups (Table.7 and Figure.6).

Fecal lipid profile and bile acid content

HFD rats did not show any significant changes in fecal TL, TC, TG, cholic acid and deoxycholic acid contents (Table.8 & 9 and Figure.7). The extract fed rats recorded dose

dependent increment in fecal TL, TC and TG contents compared to HFD rats (Table.8 & 9). Rats receiving SR (200mg/kg) showed no significant increment in fecal CA and DCA while, higher doses of SR (400 and 800 mg/kg) recorded a dose dependent increment in fecal CA and DCA compared to HFD groups ($P<0.001$). HFD+LVS rats recorded highly significant increment in fecal TL and TC ($P<0.001$) and a non-significant increment in fecal TG, CA and DCA but, definitely not as effective as 800mg of plant extract (Table.8 & 9 and figure.7).

Table. 2 Effect of *S.rhomboidea*.Roxb extract and lovastatin on body weight and food intake.

Treatments	Bodyweight (g)		Increase in Bodyweight (g)	Food intake (g/day)
	Initial	Final		
SLD	213±8.56	228±6.69	6.05%	10.23±0.59
SLD+200 mg SR	207±10.00	220±9.88	5.32%	9.66±0.98
SLD+400 mg SR	208±9.00	221±7.59	5.00%	9.60±0.77
SLD+800 mg SR	209±9.17	220±6.36	4.48%	9.15±0.96
HFD	205±8.09	250±5.23	16.56%	11.29 ± 0.86^{NS}
HFD+200 mg SR	200±7.00	231±8.97	12.55%	9.97 ± 0.64^{ns}
HFD+400 mg SR	196±6.11	220±5.53	9.68%	$8.36\pm0.65^{**}$
HFD+800 mg SR	204±6.32	225±4.99	8.53%	$7.39\pm0.49^{***}$
HFD+Lovastatin	207±5.96	233±7.12	10.23%	10.36 ± 0.78^{ns}

$^{\#}p<0.05$, $^{\#\#}p< 0.01$, $^{\#\#\#}p< 0.001$ and NS = non significant compared with SLD.

*p<0.05, **p< 0.01, ***p< 0.001and ns = non significant when compared with HFD.

Table. 3 Effect of *S.rhomboidea*.Roxb extract and lovastatin on plasma lipids.

Treatment	Total lipids (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
SLD	308.8±10.00	32.8±1.57	50.9±5.31
SLD+200mgSR	302.8±7.56	17.5±1.60	58.4±8.02
SLD+400mgSR	306.3±7.65	25.7±2.68	51.9±15.45
SLD+800mgSR	307.4±6.94	25.6±3.17	61.6±4.90
HFD	490.1±10.80$^{###}$	89.7±6.25$^{###}$	176.1±16.47$^{####}$
HFD+200mgSR	385.4±6.48***	39.6±4.30***	66.2±24.03**
HFD+400mgSR	360.6±6.91***	30.4±2.78***	75.4±21.25**
HFD+800mgSR	335.7±6.49***	26.6±2.67***	51.8±8.75***
HFD+Lovastatin	379.0±6.70***	36.4±4.60***	72.0±5.65**

$^{\#}p<0.05$, $^{\#\#}p< 0.01$, $^{\#\#\#}p< 0.001$ and NS = non significant compared with SLD.

*p<0.05, **p< 0.01, ***p< 0.001and ns = non significant when compared with HFD.

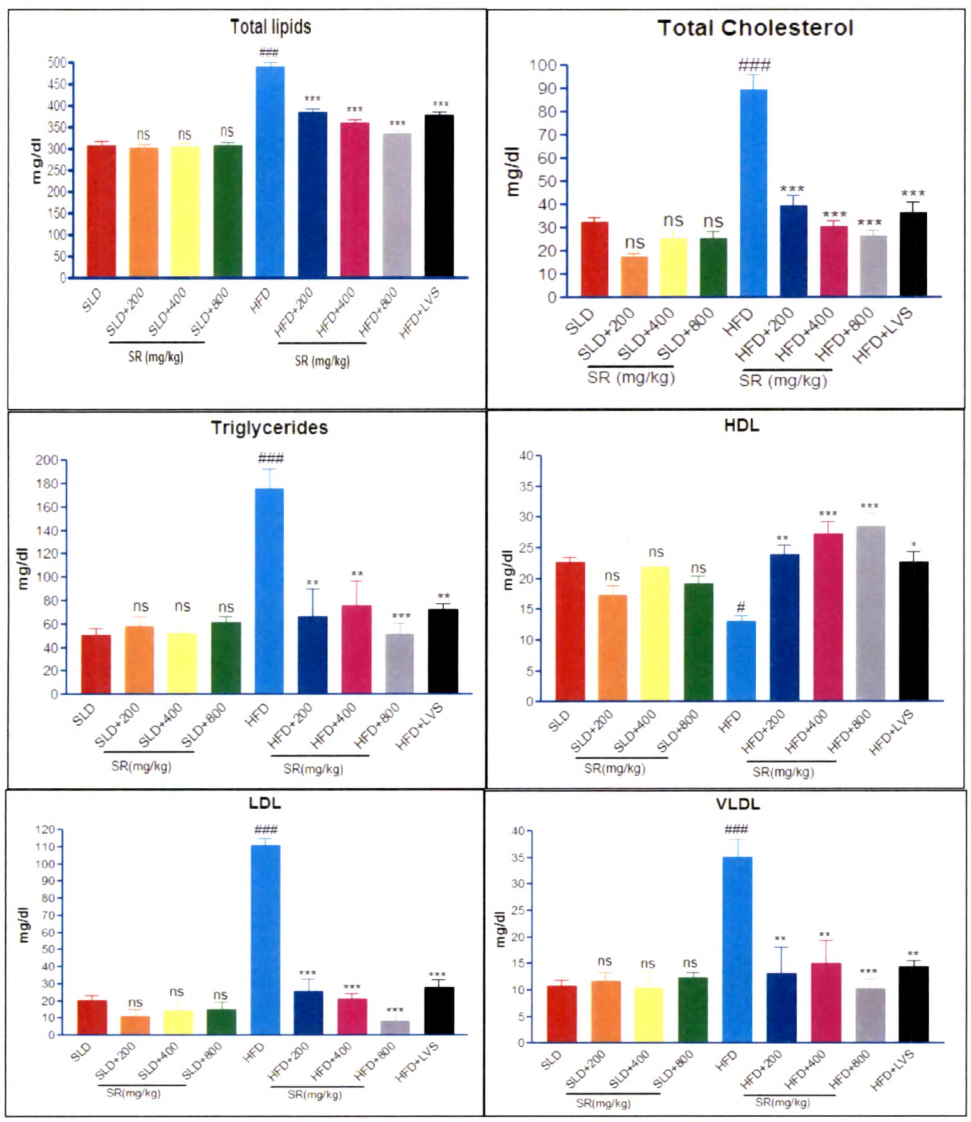
Table.4 Effect of *S.rhomboidea*.Roxb extract and lovastatin on plasma lipoproteins

Treatments	High density lipoprotein (mg/dl)	Low density lipoprotein (mg/dl)	Very low density lipoprotein (mg/dl)
SLD	22.7±0.71	20.3±2.76	10.8±1.06
SLD+200mgSR	17.3±1.50	11.1±4.05	11.7±1.60
SLD+400mgSR	22.0±0.73	14.7±5.92	10.4±3.09
SLD+800mgSR	19.8±1.20	14.8±4.56	12.3±0.98
HFD	13.1±0.81$^{\#}$	110.9±3.90$^{\#\#\#}$	35.2±3.29$^{\#\#\#}$
HFD+200mgSR	24.0±1.52**	25.5±7.23***	13.2±4.80**
HFD+400mgSR	27.3±1.93***	21.4±2.54***	15.1±4.24**
HFD+800mgSR	28.6±2.14***	8.3±2.19***	10.3±1.74***
HFD+Lovastatin	22.7±1.68*	28.2±3.96***	14.4±1.13**

$^{\#}p<0.05$, $^{\#\#}p< 0.01$, $^{\#\#\#}p< 0.001$ and NS = non significant compared with SLD.

*p<0.05, **p< 0.01, ***p< 0.001and ns = non significant when compared with HFD.

Figure.3 Effect of *S.rhomboidea*.Roxb extract and lovastatin on plasma lipid profile.



$^{\#}p<0.05$, $^{\#\#}p< 0.01$, $^{\#\#\#}p< 0.001$ and NS = non significant compared with SLD.

*p<0.05, **p< 0.01, ***p< 0.001and ns = non significant when compared with HFD.

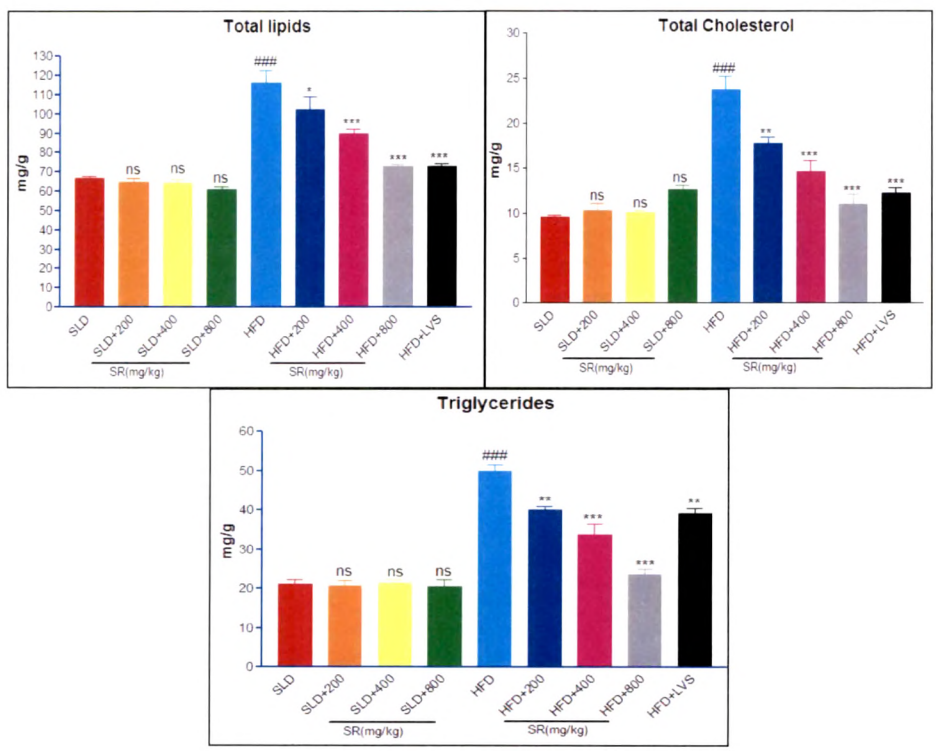
Table. 5 Effect of *S.rhomboidea*.Roxb extract and lovastatin on hepatic lipids.

Treatment	Total Lipids (mg/g)	Cholesterol (mg/g)	Triglycerides (mg/g)
SLD	66.8±0.94	9.6±0.22	21.1±1.10
SLD+200mgSR	64.9±1.74	10.3±0.82	20.8±1.31
SLD+400mgSR	64.4±1.77	10.1±0.17	21.3±1.01
SLD+800mgSR	60.9±1.60	12.6±0. 53	20.5±1.68
HFD	116.2±6.03$^{\#\#\#}$	23.7±1.53$^{\#\#\#}$	50.0±1.39$^{\#\#\#}$
HFD+200mgSR	102.4±6.76*	17.7±0.73**	40.1±0.93**
HFD+400mgSR	89.8±2.48***	14.6±1.18***	33.7±2.64***
HFD+800mgSR	72.8±1.39***	11.0±1.07***	23.7±1.24***
HFD+Lovastatin	72.9±1.77***	12.2±0.59***	39.1±1.31**

$^{\#}p<0.05$, $^{\#\#}p< 0.01$, $^{\#\#\#}p< 0.001$ and NS = non significant compared with SLD.

$^{*}p<0.05$, $^{**}p< 0.01$, $^{***}p< 0.001$and ns = non significant when compared with HFD.

Figure.4 Effect of *S.rhomboidea*.Roxb extract and lovastatin on hepatic lipids.



$^{\#}p<0.05$, $^{\#\#}p< 0.01$, $^{\#\#\#}p< 0.001$ and NS = non significant compared with SLD.

*p<0.05, **p< 0.01, ***p< 0.001and ns = non significant when compared with HFD.

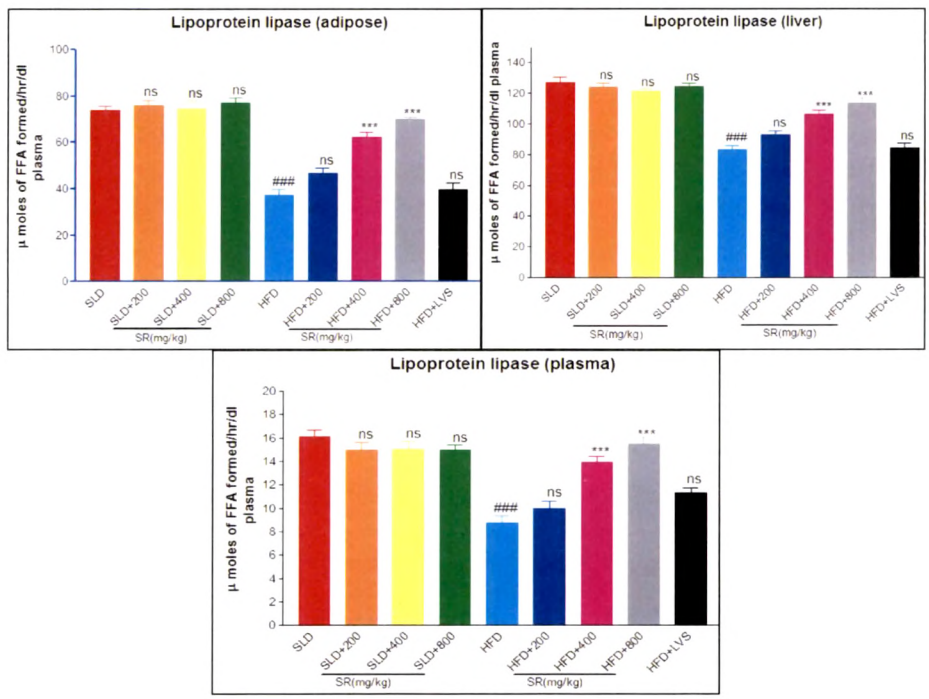
Table. 6 Effect of *S.rhomboidea*.Roxb extract and lovastatin on lipid metabolizing enzymes.

Treatment	Lipoprotein lipase (µmoles of FFA formed /hr/dl plasma or mg protein) (Plasma)	(Liver)	(Adipose)
SLD	16.1±0.61	127.2±3.19	73.8±1.79
SLD+200mgSR	15.0±0.69	124.0±2.33	76.1±2.08
SLD+400mgSR	15.1±0.59	121.8±1.79	74.7±2.97
SLD+800mgSR	15.0±0.39	124.3±2.00	77.0±2.22
HFD	8.8±0.51$^{###}$	83.6±2.05$^{###}$	37.2±2.61$^{###}$
HFD+200mgSR	10.0±0.63^{ns}	92.7±2.87^{ns}	46.6±2.14^{ns}
HFD+400mgSR	13.9±0.54***	106.6±2.53***	62.2±2.03***
HFD+800mgSR	15.5±0.55***	113.7±3.42***	70.1±0.69***
HFD+Lovastatin	11.3±0.43^{ns}	84.4±2.84^{ns}	39.5±2.80^{ns}

$^{\#}p<0.05$, $^{\#\#}p< 0.01$, $^{\#\#\#}p< 0.001$ and NS = non significant compared with SLD.

*p<0.05, **p< 0.01, ***p< 0.001and ns = non significant when compared with HFD.

Figure.5 Effect of *S.rhomboidea*.Roxb extract and lovastatin on lipid metabolizing enzymes.



$^{\#}p<0.05$, $^{\#\#}p< 0.01$, $^{\#\#\#}p< 0.001$ and NS = non significant compared with SLD.

*p<0.05, **p< 0.01, ***p< 0.001and ns = non significant when compared with HFD.

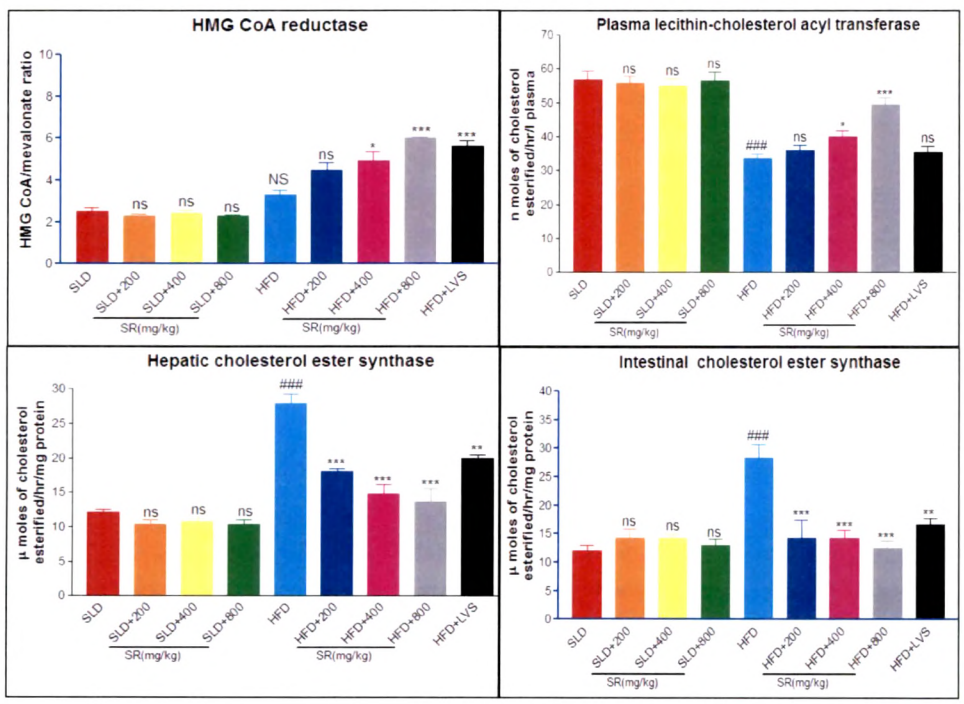
Table. 7 Effect of *S.rhomboidea*.Roxb extract and lovastatin on Cholesterol metabolizing enzymes,

Treatment	HMG CoA reductase (HMG CoA / mevalonate)	Plasma Lecithin-cholesterol acyl transferase (n moles of cholesterol esterified /hr/l plasma)	Hepatic Cholesterol ester synthase (µmoles of cholesterol esterified/hr/mg protein)	Intestinal Cholesterol ester synthase (µmoles of cholesterol esterified/hr/mg protein)
SLD	2.5±0.20	56.9±2.42	12.1±0.38	12.0±0.96
SLD+200mgSR	2.3±0.06	55.7±2.24	10.3±0.73	14.2±1.57
SLD+400mgSR	2.4±0.14	55.1±2.04	10.7±0.55	14.2±0.61
SLD+800mgSR	2.3±0.05	56.5±2.51	10.3±0.66	13.0±1.02
HFD	3.3±0.23^{NS}	33.6±1.31$^{###}$	27.9±1.40$^{###}$	28.4±2.23$^{###}$
HFD+200mgSR	4.5±0.34^{ns}	35.9±1.58^{ns}	18.1±0.36***	14.2±3.23***
HFD+400mgSR	4.9±0.45*	40.0±1.81*	14.8±1.37***	14.3±1.43***
HFD+800mgSR	6.0±0.08***	49.5±2.07***	13.6±1.91***	12.5±1.26***
HFD+ Lovastatin	5.6±0.29***	35.3±2.01^{ns}	20.0±0.39**	16.7±0.98**

$^{\#}p<0.05$, $^{\#\#}p< 0.01$, $^{\#\#\#}p< 0.001$ and NS = non significant compared with SLD.

*p<0.05, **p< 0.01, ***p< 0.001and ns = non significant when compared with HFD.

Figure.6 Effect of *S.rhomboidea*.Roxb extract and lovastatin on Cholesterol metabolizing enzymes,



$^{\#}p<0.05$, $^{\#\#}p< 0.01$, $^{\#\#\#}p< 0.001$ and NS = non significant compared with SLD.

*p<0.05, **p< 0.01, ***p< 0.001and ns = non significant when compared with HFD.

Table. 8 Effect of *S.rhomboidea*.Roxb extract and lovastatin on fecal lipids.

Treatment	Total lipids (mg/g feaces)	Cholesterol (mg/g feaces)	Triglycerides (mg/g feaces)
SLD	31.2±1.57	6.1±0.29	8.8±0.84
SLD+200mgSR	30.1±1.44	6.3±0.22	8.7±0.40
SLD+400mgSR	32.8±1.27	5.0±0.12	9.2±0.22
SLD+800mgSR	36.0±1.92	4.0±0.10	10.5±0.50
HFD	37.6±2.95 ^{NS}	6.5±0.27 ^{NS}	10.8±0.42^{NS}
HFD+200mgSR	48.4±1.99*	19.6±1.25***	22.8±1.18**
HFD+400mgSR	66.7±2.15***	24.3±1.02***	24.1±1.20**
HFD+800mgSR	77.6±1.48***	27.1±0.98***	27.4±2.88***
HFD+ Lovastatin	55.4±2.54***	14.1±1.32***	17.0±1.00^{ns}

$^{\#}p<0.05$, $^{\#\#}p< 0.01$, $^{\#\#\#}p< 0.001$ and NS = non significant compared with SLD.

*p<0.05, **p< 0.01, ***p< 0.001and ns = non significant when compared with HFD.

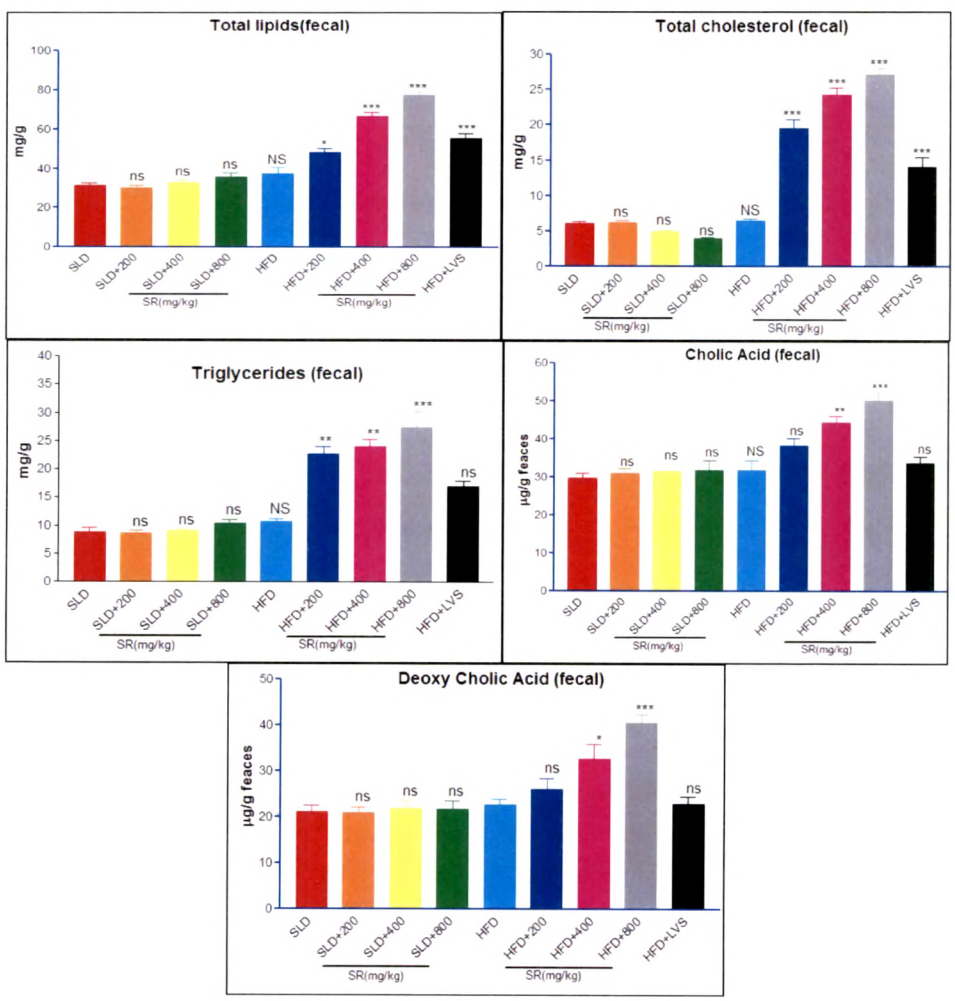
Table. 9 Effect of *S.rhomboidea*.Roxb extract and lovastatin on fecal bile acid content.

Treatment	Deoxycholic acid (µg/g feaces)	Deoxycholic acid (µg/g feaces)
SLD	29.9±1.23	21.3±1.27
SLD+200mgSR	31.0±1.43	21.1±1.16
SLD+400mgSR	31.7±1.80	21.9±1.57
SLD+800mgSR	32.0±2.31	21.8±1.71
HFD	31.9±2.44 ^{NS}	22.7±1.35 ^{NS}
HFD+200mgSR	38.4±1.76^{ns}	26.1±2.29^{ns}
HFD+400mgSR	44.5±1.68**	32.7±3.23*
HFD+800mgSR	50.2±2.21***	40.6±1.82***
HFD+ Lovastatin	33.8±1.74^{ns}	22.9±1.71^{ns}

$^{\#}p<0.05$, $^{\#\#}p< 0.01$, $^{\#\#\#}p< 0.001$ and NS = non significant compared with SLD.

*p<0.05, **p< 0.01, ***p< 0.001and ns = non significant when compared with HFD.

Figure.7 Effect of *S.rhomboidea*.Roxb extract and lovastatin on fecal lipids and bile acid content.



$^{\#}p<0.05$, $^{\#\#}p< 0.01$, $^{\#\#\#}p< 0.001$ and NS = non significant compared with SLD.

*p<0.05, **p< 0.01, ***p< 0.001and ns = non significant when compared with HFD.

DISCUSSION

A complex system regulates biosynthesis and transport of cholesterol between cells in the mammalian body. Cholesterol is transported through plasma by lipoproteins, which are complexes of lipids and proteins. With the exception of free cholesterol, the lipoprotein lipids consist of a hydrophobic core containing TG and cholesterol esters (CE) and a hydrophilic monolayered shell composed of phospholipids, free cholesterol, and specific proteins (apolipoproteins). Lipoproteins are classified according to their densities such as HDL, LDL, IDL, VLDL and chylomicrons (Table.10).

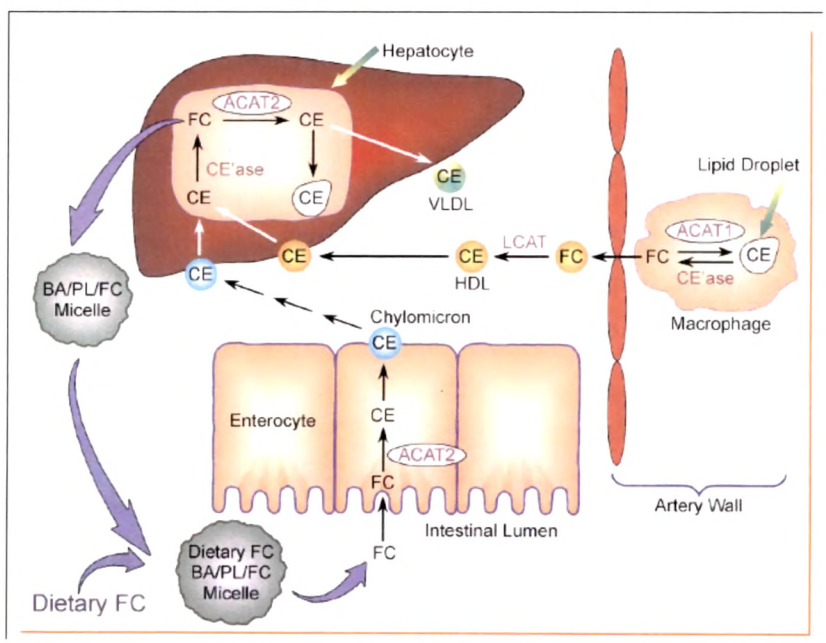
Table.10 Classes of lipoproteins

Class	Diameter (nm)	Source and function	Major apoliproteins
Chylomicrons (CM)	500	Intestine. Transport of *dietary* TAG	A, B48, C(I,II,III) E
Very low density lipoproteins (VLDL)	43	Liver. Transport of *endogenously* synthesised TAG	B100, C(I,II,III) , E
Low density lipoproteins (LDL)	22	Formed in circulation by partial breakdown of IDL. Delivers cholesterol to peripheral tissues	B100
High density lipoproteins (HDL)	8	Liver. Removes "used" cholesterol from tissues and takes it to liver. Donates apolipoproteins to CM and VLDL	A, C(I,II,III), D, E

The present investigation using HFD rats shows that SR extract (400 and 800 mg /kg) can significantly elevate plasma HDL level and reduce LDL, TC, TG and TL levels without significantly influencing the lipid profile of normolipidemic rats. Observations

on changes in body weight and daily food intake suggest no significant changes in body weight throughout the period of study in HFD+SR rats though they showed significant decrement in daily food intake.

Figure.8 Role of ACAT in cholesterol metabolism

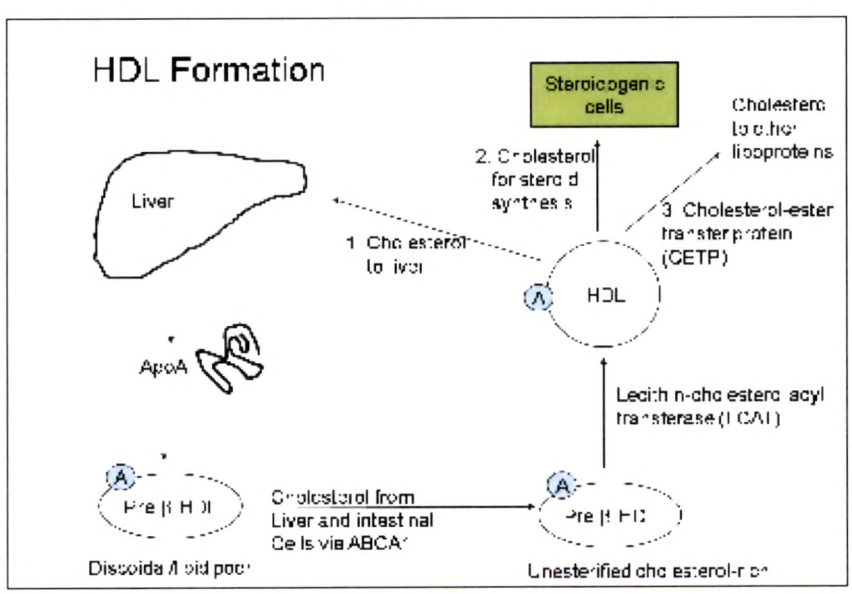


From: NATURE MEDICINE • VOLUME 6 • NUMBER 12 • DECEMBER 2000

Initial act of lipid digestion yields crude emulsions consisting of free cholesterol, TG, free fatty acids, and phospholipids, which are then mixed with bile salt micelles in intestine (Yao *et al.*, 2000). Acyl CoA:cholesterol acyltransferase/cholesterol ester synthase (ACAT/CES) converts free cholesterol to cholesterol esters using acyl coenzyme A (acyl-CoA) substrate. Most of the cholesterol absorbed during intestinal transport undergoes ACAT-mediated esterification before incorporation into chylomicrons. In the liver, ACAT2 is required for the production of cholesteryl esters that are packaged into intestinal apoB-containing lipoproteins (VLDL) (Figure.8; Ryan *et al.*, 2007). Cholesterol absorbed from diet is a major contributor to levels of cholesterol

in circulation. Phytosterols possess greater affinity for micelles than cholesterol and thereby reduces incorporation of cholesterol into micelles (Ikeda and Sugano, 1998), resulting in higher elimination of cholesterol through feaces. Further, low activity level of ACAT in intestine suggests poor esterification of dietary free cholesterol resulting in reduced absorption (Klein and Rudel, 1983). Hence, high fecal TC levels observed in HFD+SR rats could be attributed to the high content of phytosterols in the extract (3%) as well as to the low activity level of ACAT in the intestine. Low ACAT activity in the hepatic tissue as well, indicates poor esterification of free cholesterol and incorporation into VLDL (Lawrence and Gregory, 2000). Poor ACAT activity in liver and low VLDL content in plasma of HFD+SR rats is in accordance with other published reports (Song *et al.*, 2002).

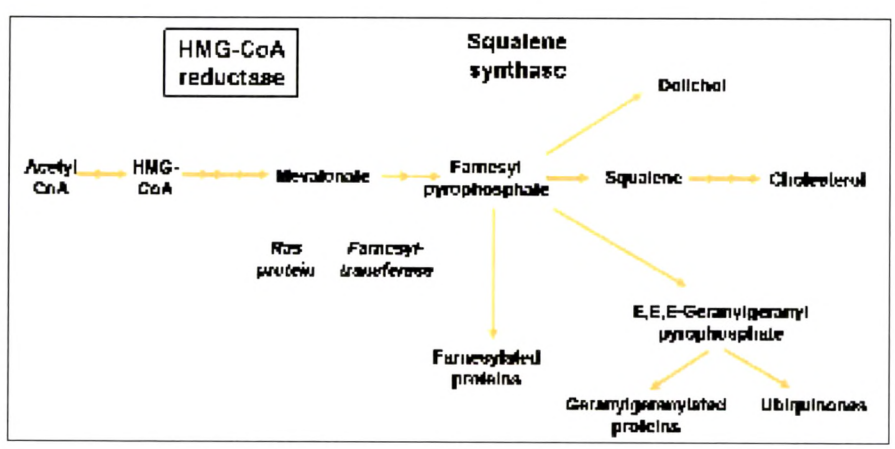
Figure.9 Synthesis of mature HDL.



HDL is mainly responsible for reverse cholesterol transport from the peripheral tissues back to liver. Higher HDL levels are documented to be physiologically beneficial (Wilson *et al.*, 1988). The nascent HDL particles take up free cholesterol from peripheral

cells via an ABC mediated efflux system (Rust *et al.*, 1999), which is then converted to cholesterol esters by LCAT, leading to the formation of small spherical HDL3 (Breslow, 1989). HDL3 is subsequently converted to large α-migrating mature HDL (Figure.9; Glass *et al.*, 1985). Flavanoids are reported to increase HDL concentration and lower LDL and VLDL in hypercholesteremic animals (Daniel *et al.*, 2003). Flavanoids (2.3 %) and polyphenols (2 %) found in SR presently could thus have contributed to the increase in HDL concentration in HFD+SR rats. High level of plasma LCAT in HFD+SR rats further justifies the increase in HDL as previous reports have established importance of LCAT in HDL metabolism (Asztalos *et al.*, 2007).

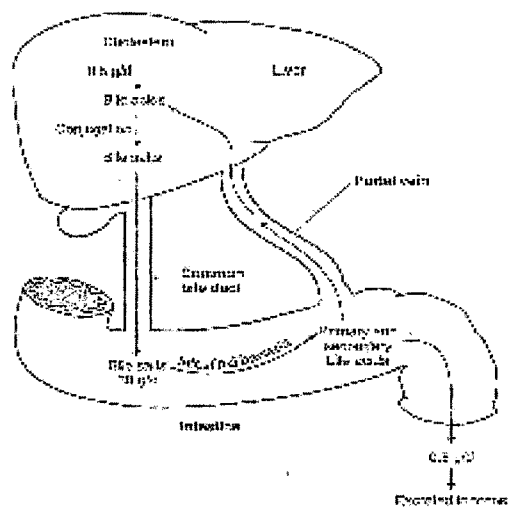
Figure.10 Role of HMG Co A in cholesterol metabolism.



In addition to the uptake of cholesterol from lipoproteins, the liver can also increase its intra-hepatic cholesterol level via *de novo* synthesis of cholesterol from acetyl coenzyme A (acetyl-CoA). The biosynthesis of cholesterol (C27) from acetyl-CoA involves the formation of several carbon intermediates, including 3-hydroxy-3-methyl-glutaryl CoA (HMG-CoA; C6), mevalonate (C6), isopentenyl phosphate (C5), and squalene (C30). The rate-limiting step in cholesterol biosynthesis is the formation of mevalonate from HMG-CoA by the cytosolic enzyme HMG-CoA reductase (Figure.10;

Vasu *et al.*, 2005). Low activity level of HMG CoA reductase in the liver of HFD+ SR rats indicates decreased *de novo* synthesis of cholesterol. Low cholesterol content in liver due to reduced HMG CoA reductase activity resulting in augmented LDL-R expression has been reported earlier (Ma *et al.*, 1986; Kovanen and Schneider, 1999). In this context, it is hypothesizable that, reduced hepatic cholesterol content in HFD+SR rats facilitates LDL uptake from circulation due to increased expression of LDL receptors. Decreased plasma LDL levels recorded in HFD +SR rats corroborate this hypothesis.

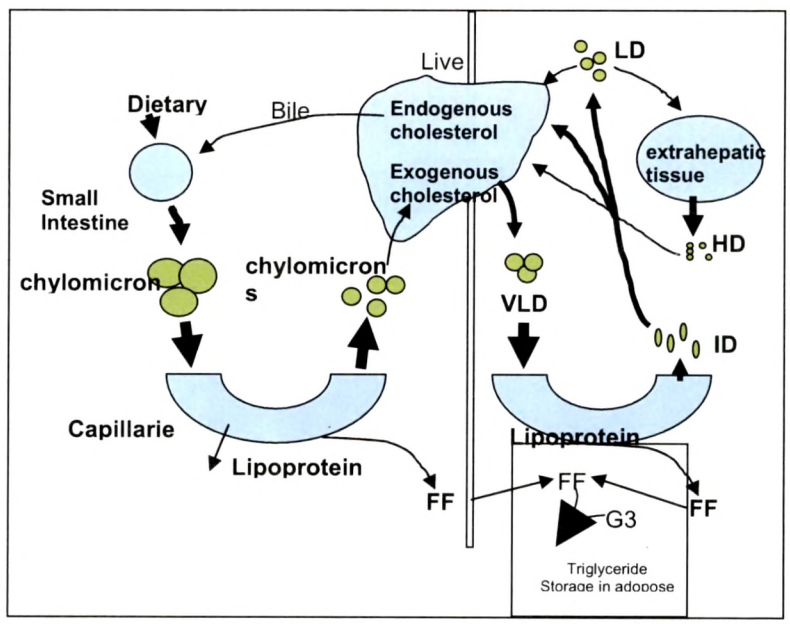
Figure. 11 Elimination of cholesterol as bile acids.



The intra-hepatic free cholesterol content decreases through catabolism of cholesterol to bile acids. It begins with the enzymatic modulation of hepatic cholesterol to7-α-hydroxycholesterol by cholesterol7-α-hydroxylase (CYP7A1) (Russell *et al.*, 1992). Total concentration of bile salt micelles stands positively correlated with cholesterol absorption (Ponz *et al.*, 1981) due to emulsification of lipids into smaller droplets, which interact more readily with lipase enzymes (Young and Hui, 1999). Bile salt emulsified triglycerides and cholesteryl esters are hydrolyzed by pancreatic lipase (PL) (Lowe, 1994) and carboxylester lipase (CEL) (Howles *et al.*, 1996) respectively.

Thus, hepatic cholesterol elimination may be either direct through bile or, indirect by conversion to oxysterol (Zhang *et al.*, 2001) that stimulates cholesterol 7α hydroxylase to form bile acids eliminable through feaces (Figure.11; Lehmann *et al.*, 1997). Reports of hypolipidemic activity of some natural products seem linked to higher fecal bile acid excretion (Khanna *et al.*, 1996). SR treatment of HFD rats seems to convert cholesterol into bile acids as noted by the higher fecal bile acid contents.

Figure. 12 Mechanism of dietary lipid metabolism.



Dietary triglycerides hydrolysed in the intestine by pancreatic lipase are absorbed by the intestinal epithelium and subsequently repackaged into chylomicrons. The products of pancreatic lipase, i.e. free fatty acids (FFA) and a mixture of monoacylglycerols (MG) and diacylglycerols (DG) from dietary TG diffuse into the intestinal epithelial cells where the re-synthesis of triacyglycerols occurs. Lipoprotein lipase (LPL) hydrolyses the core TG of the chylomicrons and, FFA then taken up by the underlying tissues or transported in the bloodstream bound to albumin (Auwerx *et al.*,

1992). Subsequently, smaller chylomicron remanant particles undergo hepatic uptake, which completes the exogenous lipoprotein pathway (Figure.12; Sattler *et al.*, 1999).

Phytocompounds like saponins apparently inhibit pancreatic lipase activity in HFD mice leading to greater fat excretion due to reduced intestinal absorption of dietary fats (Han *et al.*, 2002). Hence, increased elimination of TG through feaces of HFD +SR rats seen in the present study is correlatable with the high saponin content (4.5 %) in SR. HFD rats in the present study recorded suppressed PHLA while HFD +SR rats recorded significantly elevated levels. These observations are in accordance with the previous studies in hyperlipidemic rats fed with plant products (Khanna *et al.*, 1996). Similarly, LPL is related to hydrolysis of TG molecules and lipolytic activity in tissues. Elevated LPL activity in hepatic and adipose tissues of HFD+ SR rats could easily account for effective clearance of tissue lipid load.

In conclusion, results obtained in this study tend to suggest reduced absorption as the potent mode of action of SR in mediating its hypolipidemic and hypocholesterolemic effects while, effective elimination of lipids and cholesterol appears to be mainly due to the presence of flavanoids, saponins, polyphenols and phytosterols. The study also shows that SR (400 and 800 mg/kg) is more effective in elevating circulating HDL levels and lowering triglyceride and cholesterol levels and maintain tissue TG metabolism, even more effective than with LVS. Overall, the present study reveals the compeence of *Sida rhomboidea.Roxb* leaf extract to control dyslipidemia and hence, portends strong potential for use as a therapeutic agent against lipid disorders and obesity.

Summary

The present study was undertaken to evaluate the efficacy of freeze-dried extract of *Sida rhomboidea.Roxb* leaves (SR) on lipid and cholesterol metabolisms in high fat diet induced hyperlipidemic experimental rats. Plasma and hepatic lipid profiles, lipid and cholesterol metabolizing enzymes in target tissues and fecal total lipids and bile acid contents were evaluated in SR treated normolipidemic and hyperlipidemic rats. The results were compared with synthetic hypolipidemic drug lovastatin (LVS). Results indicate SR to be potent in positively regulating induced experimental hyperlipidemia by modulating plasma and tissue lipid profiles. The results seem related to the high content of saponin and phytosterols in SR, which could reduce absorption, elimination and catabolism of lipids and cholesterol. Apparently, SR extract appears to possess potent therapeutic value and its possible use as an effective hypolipidemic agent needs evaluation in depth.

Schematic summary