Introduction

INTRODUCTION

Hyperlipidemia

Hyperlipidemia is a condition, which reflects high levels of lipids in the blood. It can be further classified into 1) hypercholesterolemia - elevated levels of cholesterol in the blood, 2) hypertriglyceridemia - elevated levels of triglycerides in the blood and 3) hyperlipoproteinemia or dyslipidemia - elevated levels of LDL and VLDL along with lower level of HDL. Hyperlipidemia is a major cause of atherosclerosis and related conditions like coronary heart disease (CHD), ischemic cerebrovascular disease, peripheral vascular disease and pancreatitis (Gupta *et al.*, 2011).

Types of hyperlipidemia:

Hyperlipidemia can be broadly divided into:

Primary:- Monogenic defect: It is familial and called as monogenic or genetic.

Polygenic defect: Multiple genetic defects, dietary patterns and physical activity

Secondary: - Mainly induced by lifestyle habits and higher intake of dietary fat and cholesterol. It is also associated with diabetes, nephritic syndrome, chronic alcoholism and use of drugs like corticosteroids, oral contraceptives and Beta-blockers.

Table 1: Types of primary hyperlipidemia (Tripathi, 2008)

Type	Disorder	Causes	Occurrence	Markers
I	Familial lipoprotein lipase deficiency	Genetic	Very rare	↑ Chylomicrons
IIa	Familial hypercholesterolemia	Genetic	Less common	↑ LDL
IIb	Polygenic hypercholesterolemia	Multi factorial	Commonest	↑ LDL
III	Familial dysbetalipoprotenemia	Genetic	Rare	↑IDL, Chylomicrons remnants
IV	Hypertriglyceridemia	Multifactorial, genetic	Common	↑ VLDL
V	Familial combined hyperlipidmia	Genetic	Less common	↑ VLDL and VLDL

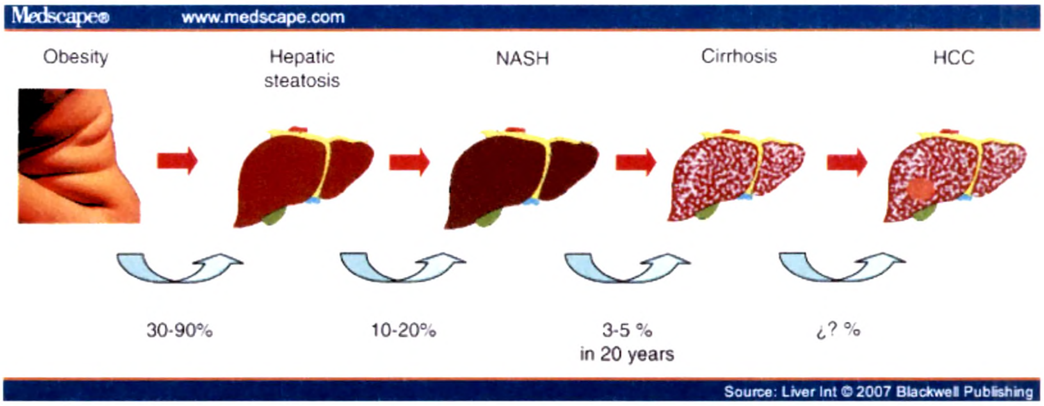
Table 2: Types of secondary hyperlipidemia (Joseph, 2005)

Type	Reasons
Hypercholesterolemia	Hypothyroidism, anorexia nervosa, acute intermittent porphyria, obstructive liver disease, nephritic syndrome, drugs like progestins, thiazide, diuretics, glucocorticoids, β-blockers, cyclosporine etc.
Hypertriglyceridemia	Obesity, pregnancy, lipodystrophy, acute hepatitis, diabetes mellitus, ileal bypass surgery, glucogen storage disease, estrogen, alcohol, Drugs like isotretinoin, glucocorticoids, β-blockers, thiazides, antifungals, anabolic steroids etc.
Hyperlipoprotenemia/dyslipidemia	Obesity, medications, hypothyroidism, a high-fat diet, inactivity, and smoking.

Non-alcoholic steatohepatitis

Ludwig and colleagues (Ludwig *et al.*, 1980) were the first to introduce this condition in 1980 to describe a series of patients with hepatic cirrhosis similar to alcoholic cirrhosis who did not have a history of alcohol consumption. Non-alcoholic fatty liver disease (NAFLD) is a broader category of hepatic disorder, manifesting despite non-alcoholism, encompassing a spectrum of alterations ranging from simple fatty liver/hepatic steatosis (accumulation of fat in liver upward of 5-10% of liver mass) to non-alcoholic steatohepatitis (NASH) and increasing fibrosis leading to cirrhosis, liver failure and hepatocellular carcinoma. Non-alcoholic steatohepatitis (NASH) is rapidly becoming a worldwide public health problem. Despite the high prevalence of NASH, the underlying etiological factors that determine disease progression through fibrosis to cirrhosis remain poorly understood. Moreover, the available non-invasive techniques to study hepatic metabolism in humans are limited. Further, liver biopsies are required to identify individuals with NASH (Erickson, 2009). According to the population-based studies on NASH, it is histologically similar to alcohol-induced steatohepatitis. Moreover, many of the factors implicated in the development of alcoholic steatohepatitis are also associated with NASH (Matteoni *et al.*, 1999). Estimates of global prevalence of NAFLD/NASH, leading cause of hepatic dysfunction and cirrhosis, among the general populace suggest a prevalence rate of 20-30% in the western and U.S population but with a higher incidence of 75-100% in obese (BMI≥30) and morbidly obese (BMI≥40) subjects respectively (Fan *et al.*, 2010). In United States, NASH is the third most common liver disease after hepatitis C and alcoholic fatty liver (Patel and Lee, 2001) with a current estimate of 20%. Recent estimates suggest 6 million individuals of the general populace in U.S to have progressed to NASH and 0.6 million to NAFLD related cirrhosis (Erickson, 2009). The most commonly identified risk factors for NASH are high-fat diet, high-calorific diet, sedentary lifestyle, insulin resistance, metabolic syndrome and its components like obesity, hypertension, dyslipidemia and T2D (Adams and Feldstein, 2010) (Figure 1)

Figure 1: Stages of NASH



Due to the many caveats associated with this technique apart from its invasiveness, reliable alternate non-invasive methods need to be pursued for effective diagnosis and staging of NASH. Though many techniques are proposed, none has met clinical acceptance to-date (Erickson, 2009). Diagnosis of NASH usually requires testing for liver biochemistry, as most such cases stand diagnosed subsequent to an evaluation of abnormal liver function tests and/or ultrasound or computed tomography scans indicating a fatty liver status. Ultrasonography, computed tomography, magnetic resonance imaging, and radionucleotide techniques, are routine techniques employed to characterize hepatic steatosis. Ironically, none of these techniques helps distinguish between simple steatosis and steatohepatitis with progressive fibrosis. NASH being essentially a clinicohistologic entity, histology is of prime importance to confirm the diagnosis (Lewis and Mohanty, 2010).

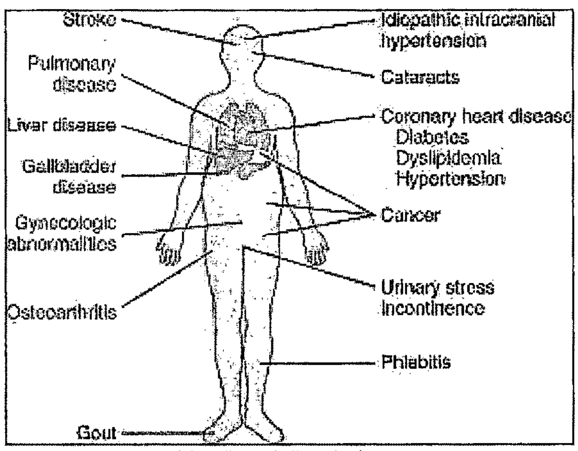
The first and foremost treatment for NASH is induced weight loss, along with other lifestyle modifications. It stands well documented that, rapid weight loss (very low calorie diet or starving) has a negative impact on NASH contributing to increased risk of cirrohsis of liver (Grattagliano *et al.*, 2000; Neuschwander-Tetri and Caldwell, 2003). Therefore, instead of inducing rapid weight loss, one should aim at a controlled weight loss of less than 10% body weight over a period of 6-12 months (Okita *et al.*, 2001). It is advisable to consume more vegetables and fruits rich in fiber and complex carbohydrates with a low glycemic index and, avoid meat, saturated fat and products with less complex carbohydrates. Apart from lifestyle modifications, one also needs to avoid consumption of alcohol. Liver biopsy is highly recommended in patients with diabetes, dyslipidemia or glucose intolerance also diagnosed with NASH. The general diet recommendations individualized to achieve energy deficit of 500 to 1000 kcal per day depending on the patient's BMI involve consumption of reduced saturated and total fat less than 30% of the total energy intake, reduced refined sugars and increased soluble fiber intake. Physical activities for 60 minutes per day for at least 3 days a week and progressive increase of exercise regimen to five times a week form part of ameliorative program. In the present scenario, life style modifications and dietary restrictions are the only available therapeutic approaches as no specific drugs are available to-date for treatment of NASH.

Obesity

Obesity is a medical condition in which excess body fat accumulates to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems (Haslam and James, 2005) (Figure 3). Body mass index (BMI), a measurement which compares weight and height, defines people as overweight (pre-obese) if their BMI is between 25 and 30 kg/m^2, and obese when it is greater than 30 kg/m^2.

Obesity increases the risk factor for various diseases such as coronary heart disease, type II diabetes, breathing difficulties during sleep, certain types of cancer, and osteoarthritis (Haslam and James, 2005). (Figure 3) Obesity is most commonly caused by a combination of excessive food energy intake, lack of physical activity, and genetic susceptibility, although a few cases are caused primarily by genes, endocrine disorders, medications or psychiatric illness. Evidence to support the view that some obese people eat little yet gain weight due to a slow metabolism is limited; on average obese people have a greater energy expenditure than their thin counterparts due to the energy required to maintain an increased body mass (Adams and Murphy, 2000).

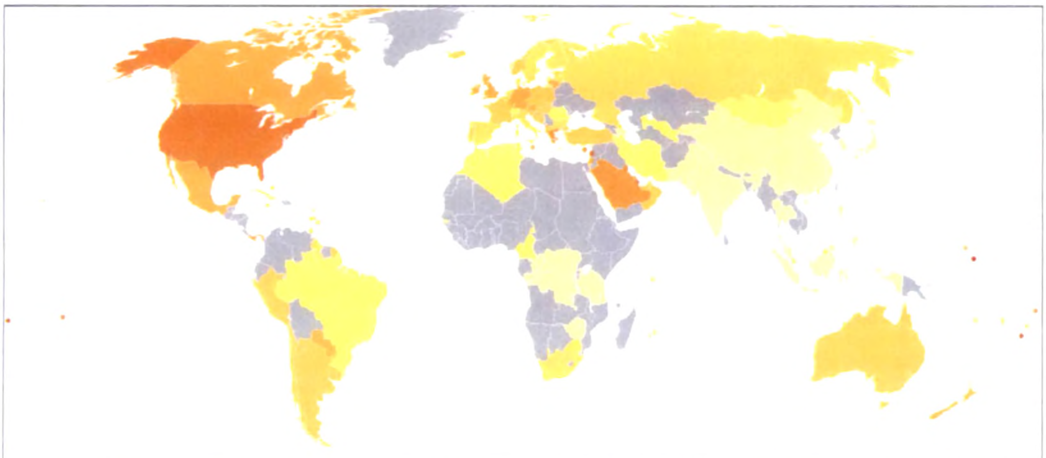
Figure 3: Medical conditions associated with obesity



From:- http://www.mdconsult.com/das/book/body/217997120-2/0/1555/216.html?printing=true

Obesity was rare before the 20th century but in 1997, WHO formally recognized obesity as a global epidemic (Caballero, 2007). As of 2005, WHO estimates indicate at least 400 million adults (9.8%) to be obese, with higher rates among women than men (WHO, 2009). The rate of obesity also increases with age at least up to 50 or 60 years old and severe obesity in the United States, Australia, and Canada is increasing faster than the overall rate of obesity (Sturm, 2007; Howard *et al.*, 2008). Once considered a problem only of high-income countries, obesity rates appear to be on the rise worldwide and affecting both the developed and developing world (Tsigos *et al.*, 2008) (Figure 2).

Figure 2: Global prevalence of obesity.



no data | <5% | 5-10% | 10-15% | 15-20% | 20-25% | 25-30% | 30-35% | 35-40% | 40-45% | 45-50% | 50-55% | >55%

From: http://en.wikipedia.org/wiki/File:World_map_of_Male_Obesity,_2008.svg

Dieting and physical exercise are the mainstays of treatment for obesity. Moreover, it is important to improve diet quality by reducing the consumption of energy-dense foods such as those high in fat and sugars, and by increasing the intake of dietary fiber. To supplement this, or in case of failure, anti-obesity drugs may be taken to reduce appetite or inhibit fat absorption. In severe cases, surgery or insertion of an intragastric balloon to reduce stomach volume and/or bowel length is the option, leading to earlier satiation and reduced ability to absorb nutrients from food (Imaz *et al.*, 2008).

BMI is calculated by dividing the subject's mass by the square of his or her height, typically expressed in metric or US "customary" units:

Metric: $BMI = kilograms / meters^2$

US customary and imperial: $BMI = lb \times 703 / in^2$

Where *lb* is the subject's weight in pounds and *in* is the subject's height in inches.

Table 3: Classification of BMI.

BMI Classification	
< 18.5	underweight
18.5–24.9	normal weight
25.0–29.9	overweight
30.0–34.9	class I obesity
35.0–39.9	class II obesity
≥ 40.0	class III obesity

Obesity is one of the leading preventable causes of death worldwide (Allison *et al.*, 1999; Mokdad *et al.*, 2004). A BMI above 32 has been associated with a doubled mortality rate among women over a 16-year period (Manson *et al.*, 1995). In the United States, obesity is estimated to cause an excess 111,909 to 365,000 deaths per year (Allison *et al.*, 1999) while, 1 million (7.7%) of deaths in the European Union are attributed to excess weight (Fried *et al.*, 2007). On average, obesity reduces life expectancy by six to seven years: a BMI of 30–35 reduces life expectancy by two to four years while, severe obesity (BMI > 40) reduces life expectancy by 10 years (Whitlock *et al.*, 2009).

At an individual level, a combination of excessive food (energy) intake and a lack of physical activity expectantly explain most cases of obesity. A sedentary lifestyle plays a significant role in obesity. Worldwide, there has been a large shift towards less physically demanding work (Ness-Abramof and Apovian, 2006) and, currently at least 60% of the world's population gets insufficient exercise (WHO, 2008). This is primarily due to increasing use of mechanized transportation and a greater prevalence of labour-saving technology at home (Ness-Abramof and Apovian, 2006; WHO, 2008). Children also appear to be victims of increasingly low levels of physical activity due to minimal walking and lack of physical education (Salmon and Timperio, 2007). Like many other medical conditions, obesity is the result of interplay between genetic and environmental factors. Polymorphism in various genes controlling appetite and metabolism predisposes to obesity in a background of plentiful energy loaded food. As of 2006, more than 41 of these sites stand related to the development of obesity in a favourable environment (Poirier *et al.*, 2006).

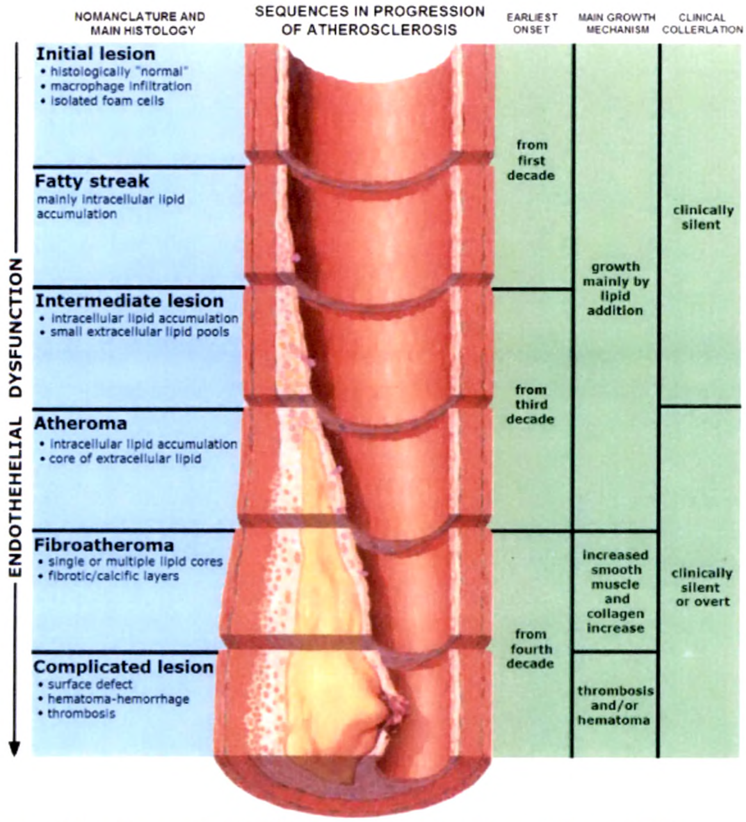
Atherosclerosis

Accumulation of cholesterol deposits in macrophages in large and medium-sized arteries characterizes atherosclerosis. This deposition leads to a proliferation of certain cell types within the arterial wall that gradually impinge on the vessel lumen and impede blood flow. This process may be quite insidious lasting for decades until an atherosclerotic lesion appears. Impaired heart and brain function due to reduced blood flow (termed heart attack and stroke respectively) represent clinical manifestations of atherosclerosis

(Stocker and Keaney, 2004). Atherosclerosis is the major source of morbidity and mortality in the developed world. 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 WHO predicts that global economic prosperity could lead to an epidemic of atherosclerosis with increasing acquisition of western habits by developing countries.

According to United States data for the year 2004, heart attack or sudden cardiac death (death within one hour of onset of the symptom) constitutes the first symptom of atherosclerotic cardiovascular disease for about 65% of men and 47% of women. Most events of disrupted artery flow occur at locations with *less* than 50% lumen narrowing (~20% stenosis is average). In India about 25 % of deaths in the age group of 25- 69 years occur because of heart diseases. In urban areas, 32.8 % deaths occur because of heart ailments, while 22.9 % in rural. It is the leading cause of death among males as well as females (Sharma, 2010).

Figure 4: Classification and stages of atherosclerosis



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Risk factors

Age: - It is the most important risk factor for predicting incidents of cardiovascular disease.

Gender: - Numerous observational studies indicate excessive risk for cardiovascular disease in males compared to age-matched women (Barrett-Connor and Bush, 1991).

Dyslipidemia: - Elevated levels of cholesterol, LDL and VLDL along with low level of HDL constitute the major factor for development of atherosclerosis.

Smoking:- A series of studies unequivocally link smoking with incidence of myocardial infarction (English *et al.*, 1940; Doll and Hill, 1956; Hammond and Horn, 1958).

Hypertension:- There exists a linear relation between blood pressure elevation and the increased incidence of atherosclerotic vascular disease (MacMahon *et al.*, 1990).

Diabetes mellitus:- The risk of coronary atherosclerosis is three- to five fold greater in patients with diabetes than in non-diabetics (Pyorala *et al.*, 1987; Bierman, 1992;).

Modern drugs against hyperlipidemia: merits and demerits

Statins: - Popularly used synthetic drugs for treatment of hypercholesterolemia are the HMG Co A reductase inhibitors called statins (lovastatin, pravastatin, simvastatin etc.), Though known to lower cholesterol level, statins however induce side effects on prolonged use. However, they are easy to administer, have high patient acceptance and have minimal chance of drug-drug interactions. Most common side effects are gastrointestinal, including constipation and abdominal pain and cramps. These symptoms are usually mild to severe and generally subside as therapy continues. However, patients are advised to avoid statins during pregnancy, chronic liver disorder or in cases of advent of allergy towards statins.

Bile acid sequestrants: - These drugs (cholestyramine, colestipol, nicotinic acid etc.) have been shown to reduce the risk for coronary heart disease in controlled clinical trials. Nicotinic acid is preferred in patients with triglyceride levels > 250 mg/dl because bile acid sequestrants tend to raise triglyceride levels.

Other available drugs:- Gemfibrozil and clofibrate are most effective for lowering high triglyceride levels but are only moderately effective in reducing LDL levels in hypercholesterolemic patients. If a patient does not respond adequately to single drug therapy, combination therapy becomes necessary to lower LDL levels. In cases of severe hypercholesterolemia, a combination of bile acid sequestrant and either nicotinic acid or lovastatin has been found to be effective. Nicotinic acid or gemfibrozil needs consideration as one of the agents in hypercholesterolemic patients with elevated triglycerides. Nevertheless, these synthetic drugs possess side effects such as bloating, constipation, muscle pain, progression of cataract, cutaneous flushing, skin disorders nausea, gastrointestinal, hepatobiliary neoplasms and cardiac arrhythmias.

Use of herbal medicines

Herbal medication has a traditional history stretching back to ancient times as part of harmonious co-existence with nature by early human civilizations. Either by a Divine gift or by closer observation and understanding of nature, ancient humans including seers in India had a clear perception of availability of remedial measures in the nature itself. The development of Indian Ayurvedic system starting from Charak Samhita somewhere between 400-200 B.C has served as its basis. The earliest literatures on Indian medicinal practice appear during the Vedic period (Anonymous). Out of the 17,000 species of higher plants in India, 7500 of them find medicinal uses (Shiva, 1996). This proportion of medicinal plants is the highest ever known for their medical properties in any part of the world from the existing flora. Ayurveda, the oldest medical system in Indian sub-continent, has alone reported approximately 2000 medicinal plant species, followed by Siddha and Unani. The Charak Samhita, an age-old written document on herbal therapy, reports on the production of 340 herbal drugs and their indigenous uses (Prajapati *et al.*, 2003). Currently, approximately 25% of drugs are derived from plants, and many others are synthetic analogues built on prototype compounds isolated from plant species in modern pharmacopoeia (Rao *et al.*, 2004).These along with traditional folklore practices in other parts of the world have played a major role in the development of modern civilization. Primitive human has great appreciation for the great diversity of plants in their immediate environ and understanding of medicinal uses of plants as medicines/curatives came not only by their trial and error methods but also by close observations of wild animals. With the passage of time, newer and newer herbs were added to the knowledge base of tribals and their methodological collection of information led to well defined herbal pharmacopeia. These have essentially served as the basis for much of the modern pharmacopeia of scientific medicine. Potentially, many of the modern drugs in common use have a herbal origin and indeed at least 25% of the prescribed drugs in the world contain at least one active ingredient of herbal origin. It is of interest that about 11% of essential medicines in the list of WHO are exclusively of plant origin (Rates, 2001). Primary health care of more than 80% of African and Asian populations is essentially based on traditional medicines and more than 80% of the rural population of India uses medicinal herbs as part of their indigenous system of medicine (Mukherjee and Wahile, 2006; WHO, 2008). Near about a 1000 species find application in Indian herbal industries, of which nearly 180 of them are of high volume exceeding 100 metric ton a year (Sahoo *et al.*, 2010). Based on the nature of usage, herbal preparations seem to fall into three categories 1) those used in the crude from, as is the practice of tribals and traditional healers, 2) isolated and purified active compounds from plant extracts and 3) use after scientific validation through animal experimentation (Iwu *et al.*, 1999). According to a WHO categorization, four groups of herbal drugs such as indigenous herbal medicine, herbal medicine in system, modified herbal medicines and imported product with a herbal medicine base have been recognized (WHO, 2003). Secondary metabolites seem to constitute the active principles or chemicals of herbal preparation that affect physiological function and hence could exhibit better compatibility with human body. The world health organization has recommended screening and evaluation of potential herbs for application as effective therapeutants, especially in remote areas that lack advanced health care and availability of safe modern drugs (WHO, 1996). In this context, the turn of the century has witnessed an up spring in the use of herbals in the developed world (Kamboj, 2000). Though various synthetic drugs are available for treatment of hyperlipidemia/hypercholesterolemia and related disorders such as obesity, steatohepatitis and atherosclerosis, ironically manifestation of side effects limits their usage greately. Further, synthetic antidiabetic and hypolipidemic drugs are also unable to alleviate the pleiotropic effects of these metabolic disorders. The pharmaceutical industries therefore face a serious challenge to develop herbal alternatives or even a combination therapy against development of NASH in diabetic, obese and IR individuals. Because of their minimal side effects and their multiple modes of action, herbal medicines are gaining increasing popularity and recognition in the management of hyperlipidemia, obesity and IR.

Ethnomedicinal diversity of North-East India

North-eastern states of India comprise of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram Nagaland and Tripura representing a land area of about 2,65,037 km^2 with hills and plains that extends from sea level to snow line and is known for its rich flora and fauna (Nath, 2000). This region of India is also the homeland of people belonging to more than 150 ethnic groups with double the number of sub-groups. This region is also a home to a number of primitive societies like Abor, Garo, Dafla, Khasi, Kuki, Mishi, Rabha, Naga, Apatani, etc. Although the tribal population in this zone is less than 12% of the total tribal population of the country, it constitutes the bulk of the population in this region. This wide geographical, climatic and cultural diversity provide a repository of wealthy traditional knowledge in the region. The North Eastern states (NES) comprising 8 states harbor more than 180 major tribal communities of the total 427 tribal communities found in India (Sajem *et al.*, 2008). A large part of the NES India stands botanically under explored or even unexplored (Jamir *et al.*, 1999; Sharma *et al.*, 2001). About 90 per cent of the total population of the region has a rural background and most still live in remote, isolated areas, maintaining individual identity and primitive economic life (Begum and Nath, 2000). Traditional agriculture is the sole mean of livelihood of these people and they depend mostly upon their surrounding plant communities for their day-to-day need including medicament for healthcare. Over the past few decades, a large number of ethnobotanic investigations conducted in the region have collected information on the local usage of a large number of plants. However, this information has not found any scientific validation that successfully merits preclinical studies.

***Clerodenron glandulsoum*.Coleb**

The genus *Clerodendrum* L. (Verbenaceae) is very widely distributed in tropical and sub-tropical regions of the world and is comprised of small trees, shrub sand herbs. This genus, with about 560 taxa, is the largest in the Verbenaceae and is taxonomically complex. Term *Clerodendrum* was named by Linnaeus in Species Plantarum in 1753

(Anonymous, 1753). The name is derived from two Greek words, *kleros*, meaning "chance or fate" and *dendron*, "a tree" (Quattrocchi, 2000). It refers to the considerable variation in reports of the usefulness of *Clerodendrum* in medicine (Mabberley, 2008). After a decade later in 1763, Adanson changed the Latin name "*Clerodendrum*" to its Greek form "*Clerodendron*"; in Greek *Klero* means "chance" and *dendron* means "tree" i.e. chance tree which means the tree which does not bring good luck. The reason for the application of the name to the genus is obscure, but it may well come from the ancient belief that some species had healing properties, while others acted in exactly the opposite way. Later on, after a span of about two centuries in 1942, Moldenke readopted the Latinized name '*Clerodendrum*', which is now commonly used by taxonomists for the classification and description of the genus and species (Moldenke 1985; Rueda 1993; Hsiao and Lin 1995; Steane *et al.* 1999).

Figure 5: Classification and digital photograph of *Clerodendron glandulosum*.Coleb taken from Imphal district, Manipur, India.

Classification

Kingdom: *Plantae*

Phylum: *Magnoliophyta*

Class: *Magnoliopsida*

Order: *Lamiales*

Family: *Verbenaceae*

Genus: *Clerodendron*

Species: *Clerodendron glandulosum*.Coleb

Botanical description

It is a shrub or small tree, perennial, wild or cultivated. Stem quadrangular, branches robust, sparstly pubescent with corky internodes. Young stem is shiny but turns light gray on maturity. Leaves are (1.5-28 cm) opposable, decussate, petiolate, long with prominent scars. Each leaf is broadly ovate, sub truncate or chordate at base, apex acute to acuminate, simple margin entire to slightly undulate, disagreeable small lateral veins (6-9) with few glands clustered at the petiole and scattered beneath. Inflorescence is terminal, compact and corymbose cymes (30-60 cm long and up to 5 cm in diameter). Peduncles is robust, cylindrical (5-20 cm long), minutely pubescent and hollow. Flowers (0.4-2cm) are pediculate, numerous, bracteates, white in colour and pedicelate (2-4cm). Bracts are lanceolate or narrowly ovate, caduceus at the time of flowering. One bract is present for each flower and glands are present on the lower surface. Calyx are gamosepalous, persistent, sepaloid, pubersent, campanulate with several peltate glands, sepals 5 (up to 0.4 cm long) and glandular. Teeth triangular, less than 0.1 cm, fruiting calyx, reddish purple (0.5-1cm), corolla gamopealous, hypocrateri forms tube slander. Petals 5, white in colour, limps oblong or obovbate, acute at apex [0.5cm x (0.25-0.3 cm)]. Tube nearly glabrous (2.5-3cm), stamen 4, didynamous, filiform, epipetalous exerted, glabrous and white (upto 1.5 cm). Anther are reddish or maroon, introse and 0.2cm long. Gynoecium (1.5 cm long) has exerted style, shorter than stamen with 4 loculi (due to the presence of false partitions). One locule is ovate in each loculus. Fruit is drupe, subglobose, glossy, bluish green in colour that turns black on drying.

Traditional medicinal uses

The term "Kuthab" is a combination of "Ku" meaning a "wooden coffin" and "Thap", "distant", which literally means that a person using the plant as food or medicine is believed to be away from ills and ailments. It has a long association with the culture and tradition of Manipur since early A.D. The plant is used in many occasions, by the people living in both valley and hills. One that can be mentioned is the sajibu cheiraoba, the New Year day in Manipur calendar, in which the "kuthap" is an important ingredient used in the preparation of a special menu called "Uti", a naturally carbonated mixed vegetables pourice. Here, it may be stressed that one taking uti on the first day of a year will endure long without any ailments until the end of the year. At the time of childbirth, the tribals use kuthap along with another plant called "Kokan" to insure easy parturition for mother. The decoction of kuthap is soaked in a piece of cotton and inserted into the rectum of child to expel worms out of the body. In other cases, "kuthap" finds usage in the preparation of natural hair care remedy' locally known as "chinghi", to kill lice. From the ancient times, belief has it that, a twig of kuthap hung on the wall of room can protect from bugs carried in by bats. Traditionally, kuthap finds usage as medicine for lowering hypertension and for curing many skin diseases. Now, local markets sell kuthab for various purposes including household consumption as a food.

Leaves of CG are used by *Apatani* and *Nyishi* tribes of North-East India as a therapeutic agent against hypertension (Kala, 2005; Deb *et al.*, 2009) while, the tender shoots are used by the Debaru tribe for antipyresis (Jubilee et *al.*, 2005). Rural and urban people of Manipur (India) routinely grow CG in kitchen garden with the leaves often sold in the market. Traditionally, cross sections of people across Manipur consume decoction of CG leaves for treating diabetes, obesity and Hypertension (Jadeja *et al.*, 2009).

Figure 6: Digital photograph of *chingmeirong* market, Imphal, Manipur, India, where leaves of *C.glandulosum*.Coleb is being sold with regular vegetables.

Phytochemical constituents

Quantitative and qualitative phytochemical analysis (Jadeja *et al.*, 2009b)

Table.5. Qualitative (A) and Quantitative (B) phytochemical analysis of *C.glandulosum.* Coleb methanolic extract.

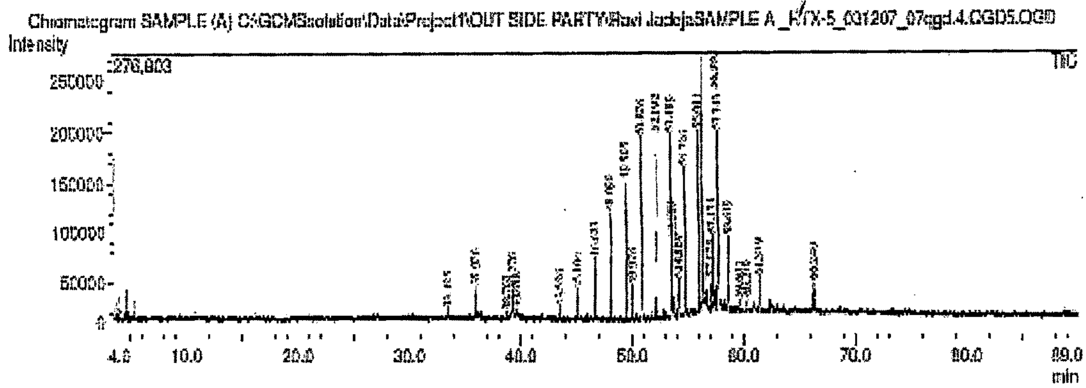
A. Qualitative analysis			
No.	**Tested for**	**Test performed**	**Presence/absence***
I	Phenols	Phosphomolybdic acid test	++
II	Tannins	Braemer's test	-
III	Steroids	Liebermann-	++
IV	Terpenoids	Burchardt test	-
V	Alkaloids	Dragendorff's test	
VI	Anthraquinones	Bornträger test	+
VII	Flavonoids	Shinoda's test	++
VIII	Saponins	Frothing test	++

* – Absent; + Traces; ++ Abundant

B. Quantitative analysis		
No.	**Phytocompounds**	**mg/ml**
I	Polyphenols*	34.3±0.89
II	Flavanoids#	46.1±1.00
III	Ascorbic acid@	53.36±0.93

*Gallic acid equivalent per 100mg plant extract, # Quercetin equivalent per 100mg plant extract, @ mg/ml ascorbic acid per 100mg plant extract

GC-MS analysis of hexane extract



Peak no.	Retention time	Name	Area %
1	33.425	Neophytadiene	0.41
2	35.975	Palmitic acid	1.94
3	38.767	Phytol	0.37
4	39.275	2-Pentadecyn-1-ol	1.26
5	39.617	Estra-1,3,5(10)-trien-17.beta	0.10
6	43.525	Eicosane	0.48
7	45.108	Octacosane	1.02
8	46.625	Hexacosane	2.29
9	48.092	Hentriacontane	3.85
10	49.505	Heptacosane	5.49
11	49.508	Hentriacontane	1.26
12	50.876	Hexatriacontane	7.17
13	52.192	Hexacosane	7.11
14	53.489	n-Hentriacontane	8.30
15	53.580	n-Eicosanol	3.10
16	54.109	Vitamin E	1.64
17	54.185	Isoquinolinone	1.46
18	54.726	n-Hentriacontane	5.88
19	55.941	Hentriacontane	7.49
20	56.380	Strongyloster	13.81
21	57.078	Norolean-12-ene	1.51
22	57.174	Hentriacontane	3.14
23	57.743	Lupeol	9.24
24	58.619	Hentriacontane	4.28
25	59.601	Oxirane, hexadecyl-	0.74
26	59.601	Octacosane	0.90
27	61.379	Nonadecan	2.39
28	66.240	Oxirane, hexadecyl-	3.36

Previously reported pharmacological activities from our laboratory

- *Antioxidant and free radical scavenging potential* (Jadeja *et al.*, 2009b)

This study evaluated the antioxidant and free radical scavenging properties of *Clerodendron glandulosum*.Coleb leaf extract. Methanolic extract of *C.glandulosum*.Coleb (MECG) was assayed for its qualitative and quantitative phytochemical constituents as well as free radical scavenging potential using different *in vitro* assays for hydrogen peroxide, hydroxyl, superoxide, DPPH, nitric oxide, peroxynitrite, singlet oxygen and hypochlorous acid radicals. Its lipid peroxidation inhibitory activity, metal chelating activity and reducing power were also assayed. Qualitative phytochemical screening of MECG showed presence of polyphenols, steroids, flavanoids and saponins. Quantitative phytochemical analysis revealed 34.3±0.89 mg/ml gallic acid equivalent-polyphenols, 46.1±1.00 mg/ml quercetin equivalent-flavanoids and 53.36±0.93 mg/ml ascorbic acid per 100 mg MECG. The MECG scavenges hydrogen peroxide (IC_{50} 120.23±1.53 μg/ml), hydroxyl (IC_{50} 70.23±1.36 μg/ml) superoxide (IC_{50} 80.36±1.36 μg/ml), DPPH (IC_{50} 50.11±1.36 μg/ml) and nitric oxide (IC_{50} 90.11±1.55 μg/ml) radicals in a dose dependent manner. The IC_{50} values for peroxynitrite, singlet oxygen and hypochlorous acid were 48.41±1.72, 62.15±1.69 and 136.69±2.01μg/ml respectively. MECG also inhibited lipid peroxidation (IC_{50} 150.56±3.02 μg/ml) and promoted metal chelation (IC_{50} 50.29±2.00 μg/ml) in dose dependent manner. Assay of reducing capacity of MECG showed a dose dependent response.The results suggest that a methanolic extract of *Clerodendron glandulosum*.Coleb possesses strong antioxidant activity against all known radicals and qualifies consideration as a natural antioxidant.

❖ *Antihypertensive and anti-diabetic activities* (Jadeja *et al.*, 2010)

This study investigated protective effect of *Cleordendron glandulosum* Coleb (Verbenaceae) aqueous leaf extract (CG) against fructose induced insulin resistance and hypertension in male *Charles foster* rats. Changes in bodyweight, food and fluid intake, plasma glucose, insulin, fasting insulin resistance index (FIRI), plasma total lipid profile, free fatty acids (FFA), oral glucose tolerance test (OGTT), blood pressure and vascular reactivity have been investigated in Control; rate fed with laboratory chow for 8 weeks, Fructose; rats fed with fructose rich diet for 8 weeks and Fructose+CG; rats fed with fructose rich diet and orally administered with 200 or 400 mg/kg of CG extract for 8 weeks. Fructose+CG groups recorded significant decrement (P<0.05) in plasma glucose, insulin, FIRI, lipid profile whereas, plasma HDL level was significantly increased (P<0.05) along with an efficient clearance of glucose during OGTT and lowered area under curve values. FRU+CG groups also recorded significantly decreased (P<0.05) mean arterial blood pressure along with decreased vasoconstriction and increased vasorelaxation in response to administration of various pharmacological agents. These results were comparable with metformin treated rats. It was concluded that, CG leaf extract ameliorates experimentally induced insulin resistance and hypertension. This study provides the first pharmacological evidence for protective role of CG leaves against experimentally induced metabolic syndrome.

❖ *Toxicological evaluation and hepatoprotective potential* (Jadeja *et al.*, 2011)

This inventory evaluated toxicological effects and hepatoprotective potential of *Clerodendron glandulosum*.Coleb (CG) aqueous extract. Acute (single administration of 1000, 2000, 3000, 4000 or 5000 mg/kg) and sub-chronic (750, 1500 or 3000 mg/kg for 28 days) toxicity tests were performed using *Swiss* albino mice as per the guideline of Organisation for Economic Cooperation and Development (OECD). Further, hepatoprotective potential of CG extract was also evaluated in experimental model of CCl_4 induced hepatotoxicity. Acute and sub-chronic toxicity tests revealed that CG extract to be non-toxic and LD_{50} value > 5000mg/kg bodyweight with no significant alterations in the biochemical profile. Moreover, rats pre-treated with CG extract (400 mg/kg for 15 days) followed by administration of CCl_4 (0.5 ml/kg on 16^{th} day) recorded significant decrement in plasma marker enzymes of hepatic damage, total bilirubin content and hepatic lipid peroxidation. In addition, contents of hepatic reduced glutathione, ascorbic acid and plasma total protein and activity levels of superoxide dismutase and catalase showed significant increase in the same animals. Microscopic examination of liver showed that pre-treatment with CG extract prevented CCl_4 induced hepatic damage in CG + CCl_4 group. It was concluded that, CG extract imparted hepatoprotection by modulating activity levels of enzymes and metabolites governing liver function and by helping in maintaining cellular integrity of hepatocytes that is comparable with that of standard drug silymarin.

Objectives of the present study

1) To evaluate effect of *C.glandulosum*.Coleb leaf aqueous extract on normolipidemic and hyperlipidemic rats.
2) To evaluate the protective role of *C.glandulosum*.Coleb leaf aqueous extract against experimentally induced non-alcoholic steatohepatitis using *in vitro* and *in vivo* experimental models.
3) To decipher molecular mechanism/s involved in regulation of high fat diet induced obesity in C57BL/6J mice and in vitro 3T3L1 pre-adipocyte differentiation by *C.glandulosum*.Coleb leaf aqueous extract.
4) To evaluate anti-atherogenic potential of *C.glandulosum*.Coleb leaf aqueous extract in an experimental model of vitamin D3 + high cholesterol diet induced atherosclerosis in rats.
5) To evaluate effects of *C.glandulosum*.Coleb leaf aqueous extract on *in vitro* LDL oxidation, monocyte to macrophage differentiation, Ox-LDL induced macrophage foam cell formation and apoptosis.