Chapter – 1

Introduction
1. INTRODUCTION

Hearing a physician say, “your lipid profile looks fine”, is reassuring. The blood lipid profile reveals the concentrations of various lipids in the blood, notably triglycerides and cholesterol and their lipoprotein carriers (VLDL, LDL and HDL) (The Lipids: 2004). Disorders of lipoprotein metabolism that result in elevated plasma concentrations of total cholesterol and LDL–C increase the risk of an individual developing coronary heart disease (CHD). In contrast, HDL-C confers protection against coronary heart disease, with the risk reducing as HDL-C increases. Such condition with abnormally high levels of LDL–C and low levels of HDL-C, as well as disorders in the composition of the various lipoprotein particles is hyperlipidemia or dyslipidemia (Walker & Whittlesea, 2007).

Non communicable diseases, Coronary artery disease (CAD) and diabetes top the list; have overtaken communicable diseases with respect to overall mortality, even in developing countries like India. High prevalence rates of diabetes, CAD, dyslipidemia and hypertriglyceridemia are seen not only in affluent migrant Indians, but also in those living within the subcontinent (Mukhopadhyay, et al, 2010). Various studies have reported the prevalence of CAD, dyslipidemia and hypertriglyceridemia in different urban and rural areas of all over India. The incidence of CAD was found to be 11%, which is 10 times more than 1970 (Reddy & Yusuf, 1998), 10 to 73% incidence of dyslipidemia in Asian Indians residing in India and among these, 28% of urban and 22% of rural had dyslipidemia. Also the incidence of hypertriglyceridemia was 61% in non - obese subjects and 73% in obese subjects (Mukhopadhyay et al, 2010). According to Global Burden of Disease Study, India is predicted to bear the greatest CAD burden and the overall cardiovascular mortality is predicted to rise by 103% in men and 90% in women between 1985 and 2015 (Mohan et al, 2010). Diabetes plays a contributory role for CAD in Indians by increasing the risk for hypertension, hypercholesterolemia, hypertriglyceridemia, low HDL cholesterol and increasing PAI-1 and fibrinogen levels which leads India to face double epidemic of diabetes and CAD (Mohan et al, 2010).
Oxidative stress leads to many biochemical changes and is an important contributing factor in several human chronic diseases, mutagenesis and degenerative diseases (Frei B, 1999). A large body of scientific evidence indicates that oxygen free radicals (OFR) play a significant role in the pathogenesis of many diseases such as atherosclerosis, cancers, hypertension and inflammation. Hypercholesterolemia, one of the major risk factors of atherosclerosis, enhances the free-radical generation in various ways. Free radicals in the presence of oxygen may cause peroxidation of lipids with in cellular and organelle membranes and cause damage to the cell. Free radicals can damage DNA and cause mutagenicity and cytotoxicity and thus play a key role in carcinogenesis. It is believed that Reactive Oxygen Species (ROS) can induce mutations and inhibit DNA repair process that results in inactivation of certain tumor repressor genes, leading to cancer. Oxidative damage, including those associated with lipid peroxidation is generally believed to be a significant factor in many pathogenic processes. Atherosclerosis is the focal point in the pathogenesis of these diseases; it is a condition involving arterial damage and is associated with some other vascular pathogenic states such as angina pectoris, myocardial infarction and cerebral thrombosis (Green Berg & Sporn, 1996; Jadhav et al, 2000).

The oxidation of LDL is a lipid peroxidation chain reaction that transforms the polyunsaturated fatty acid and cholesterol into lipid hydroperoxides and oxycholesterol, respectively, thereby causing damage of arterial endothelial cells and promoting the formation and deposition of atherosclerotic plaque. Hence, the hypothesis was formulated that antioxidants may at least in part prevent atherosclerosis and cardiovascular disease. The anti-oxidative activity of vegetable foods includes several mechanisms such as radical scavenging, metal chelation and quenching of singlet oxygen. An evaluation of the antioxidant capacity of a compound or group of compounds has to include this multifunctional acridity. Thus, to evaluate *in vitro* antioxidant potential it is necessary to assess using several assays based on different antioxidant mechanisms. The antioxidants may be exogenous or endogenous in nature. The endogenous antioxidants can be classified as enzymatic and non enzymatic. The antioxidant enzymes include Superoxide dismutase (SOD), Catalze (CAT), glutathione peroxidase (GPx), glutathione reductase (GRx).
On the other hand, to evaluate the potential antioxidant effect of plant origin in biological systems it is useful to measure, along with the total antioxidants activity of the plasma, several primary and secondary products of oxidative damage (Yusuf S. et al, 2004; Miliauskas et al, 2004; Halliwell, 1995).

Many phytomedicines exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a physiological process. This synergistic or additive pharmacological effect can be beneficial by eliminating the problematic side effects associated with the predominance of a single xenobiotic compound in the body.

Medicinal plants and their extracts have gained increasing importance over the past years, since they can be used as a source of herbal drugs. It is well known that medicinal plants like Allium sativum (Yeh & Liu, 2001), Apium graviolans, Achyranthes aspera, Terminalia arjuna, Zinziber officinalis, Phylanthus niruri, Momordica charantia, Ocimum sanctum, (Phadeke, 2007), Moringa oliefera (Ghasi et al, 2000; Arabshahi-Delouee et al, 2007; Urooj & Reddy, 2010). Solanum melongena. (Sudheesh et al, 1997), Azadiracta indica (Purohit et al, 1999), (Trigonella foenum graecum (Saleh et al, 2006) and many more plants are proven and supported scientifically in last few decades as nutraceuticals and lipid lowering agents. In Ayurveda, many plants are in use since ancient times and phytochemicals of such medicinal plants which act as antioxidants and lower the risk of degenerative diseases are need to be proven scientifically and promoted as therapeutic agent and as nutraceuticals. Before recommending or promoting the plant, its parts or its extracts as medicine or drug or as therapeutic agent, must be screened in depth using in vitro, ex vivo methods and animal models to establish their safety and also to validate the therapeutic effect.

In establishing the lipid lowering potential of a medicinal plant, many aspects have to be considered such as phytochemical composition, antioxidant contents and antioxidant activity which paly major role in influencing the lipid metabolism. Very few studies have established the mechanism of cholesterol reduction by medicinal plants i.e. whether the reduction in the cholesterol levels is by inhibiting cholessterogenesis or by
promoting excretion of cholesterol or bile acids or by inhibiting the activity of biosynthetic enzymes, especially HMG CoA reductase which is the rate limiting enzyme in the cholesterogenesis [3-Hydroxy-3-methylglutaryl CoA (HMG CoA) to Mevalonate] and squalene synthase. It is well known that the activity of hepatic HMG-CoA reductase is controlled by the nutritional and hormonal state of the animals. One of the nutritional factors controlling the activity of this enzyme in the liver is the amount and nature of the dietary fat. A number of plants with potent therapeutic components such as fibers, sterols, saponins, polyphenols, flavonoids, etc., have been investigated for their anti-hyperlipidemic, antioxidant and anti-atherosclerotic properties. These compounds are reported to be beneficial with great variation in magnitude and mechanism of action and hence have a potential therapeutic value in combating multifactorial atherosclerotic disorders (Nishant et al, 2009).

*Morus indica* of Family Moraceae, belongs to the genus Morus (Common name-Mulberry). It is a perennial woody plant having fast growth and short proliferation period (Pan & Lou, 2008; Yang et al, 2010). There are 24 species and sub species of Morus, with at least 100 known varieties (Ercisili & Orhan 2007) which is indigenous to China but has also been cultivated in the temperate and subtropical regions of the Northern Hemisphere for centuries because of its wide use for many purpose. The species found in India are *M Indica*, *M alba*, *M nigra*, *M rubra* etc. Mulberry is valued for its foliage which constitutes the chief feed for silk worms. Analysis of Mulberry leaves collected from different parts of India has revealed the presence of proteins carbohydrates, calcium, iron ascorbic acid and Vitamin D (Sastri BN, CSIR) Mulberry has been explored as a medicinal plant and its medicinal properties are testified in various scriptures. The leaves of Mulberry are nutritious, palatable, nontoxic and also enriched with different active principles (Sastri BN, CSIR). The plant is a good source of phytochemicals such as polyphenols, anthocyanins in particular and micronutrients. In epidemiological and clinical studies, these constituents have been associated with improved cardiovascular risk profiles (Andallu et al, 2001). Human intervention studies using mulberry as either fresh, or as juice or freeze dried or purified anthocyanin extracts have demonstrated significant improvements in LDL oxidation, lipid peroxidation, total plasma antioxidant capacity
dyslipidemia and glucose metabolism (Mahmoud, 2013). Different species of *Morus* have been explored for their strength against different degenerative diseases at biological level especially as hypoglycemic or antidiabetic agents. Few studies have evidenced their influence on lipid metabolism and antioxidant proficiency against oxidative stress in hyperglycemic condition (Devi & Uroj, 2009; Almeida, 20102).

Although, *Morus indica* is explored for its antioxidant and antidiabetic properties, complete information on its toxicological, hepatoprotective and influence on lipid metabolism at the biological level is not reported.

Literature survey carried out pertaining to the research topic revealed some grey areas in the area of lipid lowering potential of mulberry leaves. Hence, this investigation was designed to study the antioxidant potential using both food and biological substrates, establish its safety and finally confirm the lipid lowering effect in an animal model using three varieties of *Morus indica*.

The specific objectives are:

1. To screen the selected plant varieties for antioxidant activity by different *in vitro* methods.
2. To evaluate the hypocholesterolemic potential by *ex vivo* methods (Inhibition of HMG CoA reductase, squalene synthase).
3. To assess the safety of *Morus indica* against acute and CCl₄ toxicity.
4. To study the effect in animal model induced with hypercholesterolemia.