CHAPTER 2

INTRODUCTION

Many medical and scientific researchers have become increasingly concerned that the presence of uncontrolled free radicals in the body is the direct cause of a number of health problems. Free radical reactions have been implicated in the pathology of many human diseases including atherosclerosis, ischemic heart disease, the aging process, inflammation, diabetes, immunodepression, the neurodegenerative condition and other disease conditions (Maxwell, 1995).

The startling fact that almost no one has understood until now is that along with the health-building processes we have millions of potentially deadly weapons within the cells and fluids of our bodies. These "weapons" are "loose cannon" molecules, which have come to be known as free radicals and which are making us sick, cause us to age and eventually kill us. There is an ongoing, potentially deadly battle which is being waged in our bodies every second of our lives, in which billions of free radicals are out to destroy our cells and alter our genetic material. Free radical scavengers (anti-oxidants) are key elements in the defense system, which the body uses in order to neutralize the activity of these dangerous and over the long-term, deadly free radical enemies.

The world of antioxidants is thus a battlefield. It's not just a war going on inside our cells, but also a battle between the conventional scientific community, (who have yet to be convinced about the benefits of antioxidant supplements), and the commercial pirates, who have hijacked the concept, to plug a myriad of anti-oxidant remedies (In the States, as many as 1 in 3 of the population take some form of antioxidant supplement). Somewhere, in the middle is a genuine phenomenon, which might have important implications for preventing or treating a range of age-related, degenerative diseases.

Interest in oxidant research has soared during the last two decades. Initially oxidants were mostly studied as inducers of oxidative damage. As we started learning that oxidative damage dependent mechanisms are implicated in the etiology of a wide variety of commonly occurring diseases and that key physiological processes such as aging are influenced by oxidants, interest in oxidant research increased tremendously. Antioxidants were studied for their ability to control oxidative damage.

Overwhelming evidence has accumulated showing that antioxidants may prevent oxidative damage and thus protect against the adverse effects of oxidants. Based on these findings the role of nutritional and pharmacological antioxidants turned into a subject of intense research. During the last decade, and particularly during the last 5 years or so, oxidant and antioxidant research has taken a new turn.

2.1 FREE RADICALS

Living systems have evolved to survive in the presence of molecular oxygen and for most biological systems life depends upon its presence. The oxidative properties of oxygen play a vital role in diverse biological phenomena, including use of nutrient foods, electron transport to produce ATP and the removal of xenobiotics. However oxygen has double edged properties, i.e. it is essential for life but it can also provoke damaging oxidative events within cells (Halliwell, 1994). Aerobic organisms thus exist in a perpetual catch-22 situation. Oxygen sustains them, but it also poisons them through reactive intermediates produced during respiration. The powerful oxidants produced in this process - including superoxide anions, hydroxyl radicals and hydrogen peroxide - are known as free radicals. These highly reactive molecules are capable of independent existence with one or more unpaired electrons. The unpaired electrons are highly energetic and seek out other electrons to pair from another molecule, thus changing it and possibly damaging it and atleast causing the second molecule to now be a reactive species resulting in damage to as yet third molecule, and so on, beginning a chain reaction (Pillai and Pillai, 2002). Once the process is started, it can cascade, finally resulting in the disruption of a living cell. Thus, free radicals are molecular thugs on the biochemical scene, indiscriminately stealing electrons from other molecules and converting them to potentially harmful forms.

The culprits are free radicals or reactive species of oxygen, nitrogen or chlorine. Superoxide, hydroxyl ions, hydrogen peroxide, and nitric oxide are examples of free radicals.

Free radicals are constantly being generated in the body, as a result of the normal metabolic processes. They are naturally occurring and an

important part of biological functions such as immunity, inflammation, growth and repair. Environmental insults, infections, smoking, radiation and sunlight can also cause the formation of free radicals. Free radicals can have negative effects when they damage proteins, lipids and nucleic acids. They are normally held in balance in biological systems by antioxidant defense mechanisms. Under physiological conditions, damage due to free radicals is countered by antioxidants. Sometimes, excessive free radical formation occurs in the body and the antioxidant systems in the body cannot cope with the situations, i.e., the pro-oxidants overwhelm the antioxidants (Sies, 1991). This situation is known as oxidative stress. Thus, oxidative stress is a general term used to describe a state of potential oxidative damage caused by free radicals. This oxidative stress can attack lipids, which constitute the cellular membranes, bases of the DNA, and amino acids of proteins.

2.2 ANTIOXIDANTS

Antioxidants and other phytochemicals are Mother Nature's protection from assaults by free radicals. Antioxidants (Free radical scavengers) fight free radicals, and therefore may be able to help prevent the diseases that free radicals promote.

Inside the cells themselves, antioxidant defense is provided largely by specific enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase. These preventive antioxidant enzymes are the first line of defense against reactive oxygen species. Reduced glutathione (GSH) is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated lipid peroxidation (Halliwell, 1995). Although the body produces anti-oxidant defenses in the form of transferrin-lactoferrin, ceruloplasmin, albumin, haptoglobin-hemoplexin, urate, etc., it also makes use of nutrients and minerals, such as the well-known vitamins E, C and beta-carotene and the minerals selenium and zinc.

Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and well-being. The need for antioxidants becomes even more critical with increased

exposure to free radicals. It is thought that a shortage of antioxidants could cause, or assist in causing Alzheimer's disease, cancer, cardiovascular disease, cataracts, diabetes, hypertension, infertility, macular degeneration (eye lens degeneration) measles, mental illness, periodontal disease, respiratory tract infection as well as rheumatoid arthritis. As part of a healthy lifestyle and a well-balanced, wholesome diet, antioxidant supplementation is now being recognized as an important means of improving free radical protection.

Antioxidant compounds must be constantly replenished since they are used up in the process of neutralizing free radicals. Therefore these have to be continuously ingested in diet or be supplemented. Antioxidant supplements were once thought to be harmless but increasingly we are becoming aware of potential interactions and potential toxicity. As an example, in normal concentrations found in humans, vitamin C and beta-carotene are antioxidants; but at higher concentrations they are pro-oxidants and can be harmful. It is also possible that unforeseen metabolic disturbances may occur after prolonged use of highly bioavailable pure compounds; such effects may not be apparent when antioxidants are obtained from natural foods. Thus, supplementation of antioxidants using natural sources seems to be a safe approach (Pillai and Pillai, 2002).

2.3 ANTIOXIDANTS IN DISEASE PREVENTION

There is evidence concerning the participation of reactive oxygen species in the etiology and pathophysiology of human diseases, such as cancer, atherosclerosis, ischemic heart disease, the aging process, inflammation, diabetes, neurodegenerative disorders, viral infections, autoimmune pathologies and digestive system disorders such as gastrointestinal inflammation and gastric ulcer (Repetto and Llesuy, 2002).

Peptic ulcer is the most common gastrointestinal disorder in clinical practice. The drugs used in the treatment of ulcer include receptor-blocking drugs, drugs inhibiting the proton pump, drugs affecting the mucosal barrier and drugs that act on the central nervous system. Eventhough a range of drugs is available for the treatment of ulcer; many of these do not fulfill all the requirements and side effects such as

arrythmias, impotence, gynaecomastia and haematopoeitic changes are noted (Manonmani et al., 1995) Thus, there is still a need for safe and curative agents for gastrointestinal ulcers. Considering the drawbacks of modern medicine, indigenous drugs possessing fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer (Akhtar et al., 1992).

Free radical participation in the pathophysiological processes of gastrointestinal injury has been suggested (Mizui et al., 1987). Recently oxygen-derived free radicals have been postulated to play an important role in the pathogenesis of acute gastric mucosal injuries such as ischemia-reperfusion (Pery et al., 1986)-, stress (Cochran et al., 1982)-, ethanol (Pihan et al., 1987)-, anti-inflammatory drug (Del Soldato et al., 1985)- and pylorus-ligation (Rastogi et al., 1998)- induced gastric mucosal injuries in rats. Recent studies in the rat showed that oxygen-derived radicals are directly implicated in the mechanism of acute and chronic gastroduodenal ulceration and that scavenging them, stimulates the healing of ulcers (Salim, 1992). It has been demonstrated that many drugs or formulations possess potent antioxidant actions and are effective in healing experimentally induced gastric ulcers, e.g parenteral pretreatment with antiperoxidative drugs such as butylated hydroxytoluene, quercetin and quinacrine prevented lesion formation due to oral administration of absolute ethanol (Mizui et al., 1987). Melatonin and β-carotene showed protective effect against indomethacin induced gastric injury and this effect was found to be mediated by scavenging of oxygen derived free radicals (Singh et al., 2002).

Congestive heart failure is increasing in prevalence in developed countries. It is rapidly assuming similar trends in developing countries also. It has been predicted that cardiovascular diseases will be the most important cause of mortality in India by year 2015 (Mengi, 2003). Despite a decline in mortality from cardiovascular diseases over the last four decades, CHF remains a major cause of morbidity and mortality, afflicting 4 million patients and killing 400,000 patients each year, more than 1000 a day. It is the only cardiovascular disorder that is increasing in

prevalence and constitutes the most common cause for hospitalization to medical services in patients over 65 years of age (Cheng, 1991).

The last quarter of a century has witnessed several major advances in our understanding of the mechanisms and therapy of CHF due to all causes including coronary artery diseases. In the mid-1960s, potent new diuretics revolutionized the management of patients with CHF and fluid retention. In the mid-1970s, the concept of vasodilator therapy gained widespread acceptance. In the mid-1980s, newer inotropic agents and angiotensin-converting enzyme (ACE) inhibitors were introduced. Alone or in combination, the three Ds-diuretics, dilators and digitalis or digitalis-like drugs- are usually effective not only in relieving the symptoms of CHF but also in improving the hemodynamic status. Unfortunately, despite these advances, most patients with CHF remain disabled and die of their underlying disease. It is therefore obvious that strategy for future investigation of the management of CHF needs to incorporate a different approach based on some of the modern concepts (Cheng, 1991).

Reactive oxygen species (ROS) are implicated in many pathogenic processes including the cardiovascular system. Cardiovascular diseases including atherosclerosis and cardiac tissue injury after myocardial infarction are due to free radicals generated at the site of damage (Tappel, 1973). Detoxification of ROS by antioxidants therefore affords protection against such diseases. There is a growing body of evidence suggesting that antioxidants contribute to cardioprotection. A study involving 2000 men and women with angiographically defined coronary artery disease resulted in marked reduction in non-fatal myocardial infarction in subjects taking vitamin E compared with the placebo group (Stephens et al., 1996). Although antioxidant administration has been shown to reduce the severity of the myocardial injury, yet some properties of antioxidants such as cytotoxicity (Visweswaran et al., 1997), pro-oxidant activity (Edge and Truscott, 1997) or high molecular weight in case of SOD, limited their therapeutic application. Use of plant extracts (Bhattacharya et al., 1999), food supplement (Seneviratne et al., 1999) and even drugs (Cavanagh et al., 1997) which augment major cellular endogenous antioxidants following chronic administration have been identified as a promising

therapeutic approach to combat oxidative stress associated with ischemic heart disease. The property of augmenting cellular endogenous antioxidants has been defined as a major constituent of myocardial adaptation against oxidative stress (Das et al., 1995). Myocardial adaptation against oxidative stress is mediated through augmentation of a number of cellular antioxidants, such as SOD, catalase, glutathione peroxidase and glutathione (Das et al., 1995; Schaefer et al., 1998).

Isoproterenol-induced myocardial infarction has been used as a model to evaluate cardioprotective agents (Sheela and Shyamala Devi, 2000). Isoproterenol is a β-adrenergic agonist and has been found to cause a severe stress in the myocardium resulting in infarct like necrosis of the heart muscle. The pathogenesis and repairs of the lesions in rats are reminiscent of the classical description of human myocardial infarction. Isoproterenol has also been reported to increase lipid peroxidation through free radical formation. This increased concentration of myocardial lipid peroxides can result in the structural changes and cause myocardial necrosis. Carnitine (Sushmakumari et al., 1989), vitamin E (Ithayarasi and Shyamala Devi, 1997), curcumin (Nirmala and Puvanakrishnan, 1996) and AO-8, a herbal formulation (Mitra et al., 1999) were found to help the myocardium to survive from the oxidative stress induced by isoproterenol.

Nephrotoxicity is an inherent adverse effect of certain anticancer drugs. Therefore, renal failure in cancer patients is a common problem in oncology. Previous renal impairment as well as combinations with other nephrotoxic drugs may increase the risk of nephrotoxicity during administration of chemotherapy (Ries and Klastersky, 1986). Several pathways have been shown to contribute to the toxicity including formation of reactive oxygen species, direct damage to renal tubule cells and activation of systemic inflammatory cells, which infiltrate and damage the kidney. Cell injury by oxidative stress has been implicated in renal epithelial cell destruction during the progression of kidney diseases. Cisplatin [cis-diamminedichloroplatinum (II): CDDP] is a widely used anti-cancer drug whose clinical use is limited by its dose-related renal toxicity. Early clinical trials revealed that the incidence of nephrotoxicity may range from 25-33% and 50-75% following single and multiple course therapy

with cisplatin, respectively (Goldstein and Mayor, 1983). Although the mechanism of action of the renal toxicity of cisplatin is still not clear, it has been suggested that the oxygen free radicals play an important role. Levi *et al.* (1980) showed that SH group depletion may be a primary event leading to the renal failure. Ways of protecting SH groups and reducing the toxic effects caused by cisplatin have been reported (Daly, 1996).

Administration of substances containing sulphydryl groups, or with antioxidant properties, before cisplatin, significantly reversed the inhibitory effects caused by cisplatin alone, supporting the hypothesis that the cisplatin toxicity is associated with the reduction of free sulphydryl groups (Daly, 1996).

Many antioxidants such as sodium selenite, hydroxy ethyl rutoside etc., are known to reduce the renal toxicity caused by cisplatin (Rao and Rao, 1998). Also epidemiological observations show lower cancer rates in people whose diets are rich in fruits and vegetables. This has led to the theory that these diets, containing antioxidants, protect against the development of cancer (Hennekens et al., 1984). Thus supplementation of antioxidants can, not only reduce the dose-related toxicity of cisplatin but can also prevent the oxidative stress generated in cancer.

Liver disease is a worldwide problem. Liver is an organ of paramount importance as it plays an essential role in maintaining the biological equilibrium of vertebrates. The spectrum of its functions includes: metabolism and disposition of chemicals (xenobiotics) to which the organ is exposed directly or indirectly; metabolism of lipids, carbohydrates and proteins; blood coagulation and immunomodulation (Venkateswaran et al., 1997; Latha et al., 1999; Mitra et al., 2000).

Treatment options for common liver diseases such as cirrhosis, fatty liver and chronic hepatitis are problematic. The effectiveness of treatments such as interferon, colchicine, penicillamine and corticosteroids are inconsistent at best and the incidence of side effects is profound. All too often the treatment is worse than the disease. Conservative physicians often counsel watchful waiting for many of their patients, waiting in fact for the time when the disease has progressed to the point that warrants the use of heroic measures (Scott Luper, 1998).

Conventional or synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Physicians and patients are in need of effective therapeutic agents with a low incidence of side effects. Plants potentially constitute such a group. So there is a worldwide trend to go back to traditional medicinal plants. Many natural products of herbal origin are in use for the treatment of liver ailments (Venkateswaran et al., 1997; Latha et al., 1999; Dhuley and Naik, 1997). In most cases, research has confirmed traditional experience and wisdom by discovering the mechanisms and modes of action of these plants as well as reaffirming the therapeutic effectiveness of certain plants or plant extracts in clinical studies (Scott Luper, 1998).

The hepatotoxin generally used to study the liver protective effect of drugs is CCl_4 because CCl_4-induced liver dysfunction in rats simulates liver cirrhosis in man (Pérez-Tamayo, 1983; Wensing et al., 1990). It has been stated that one of the principal causes of carbon tetrachloride (CCl_4)-induced hepatopathy is lipid peroxidation by $CCl_3^{\bullet}$, a free radical derivative of the toxin. The antioxidant activity or the inhibition of the generation of free radicals is important in providing protection against such hepatic damage. An antioxidant effect has been reported to play a crucial role in the hepatoprotective ability of many plants. Search for crude drugs of plant origin with antioxidant activity has thus become a central focus for study of hepatoprotection today. This may ensure a possible solution for the increasing incidence of tissue damages seen in organisms as a consequence of exposure to toxins of extrinsic or intrinsic origin (Venukumar and Latha, 2002). The impairment in hepatic antioxidant status and the associated hepatocellular damage were found to be attenuated by vitamin E, a lipid-soluble antioxidant; or multiple doses of *Polygonum* or *Cassia* extracts; and preparations reported to possess antioxidant properties. This supports the involvement of uncontrolled free radical-mediated processes in the pathogenesis of CCl_4 hepatotoxicity (Ko et al., 1993).

Coronary heart diseases resulting from progressive atherosclerosis, remains the most common cause of death in our societies. Atherosclerosis is a primarily a lipid disorder that affects the arteries. Increased

intracellular generation of ROS has also been proposed as a mechanism of tissue injury in atherosclerosis. Hence hyperlipidaemia and excess of free radicals formed are the important risk factors in the initiation and progression of atherosclerosis. As a result there is increased awareness for the need to lower the elevated plasma lipoproteins, especially LDL and the oxidative stress due to excess of free radicals. Many plant products are increasingly recognized as having protective role in coronary artery diseases through several mechanisms including antioxidant and hypocholesterolemic properties. The antioxidant and hypocholesterolemic properties of a drug can be studied against diet-induced hypercholesterolemia in rats (Mary et al., 2002). The results from several animal studies suggest that increasing the intake of dietary antioxidants inhibits the progress of vascular diseases. Initial trials of antioxidant vitamins and drugs such as probucol in animals suggested that the atherogenic process could indeed be retarded. These observations were strengthened by epidemiological surveys in humans linking low plasma levels or dietary intake of antioxidant vitamins (ascorbate, tocopherol, and β-carotene) with ischaemic heart disease.

It's a scientifically accepted fact that cholesterol (itself a fat-soluble, lipid-like sterol) and other dietary fats and oils only become harmful after they are damaged by oxygen and oxygen radicals. The resulting oxidized and peroxidized lipids are not only toxic in their own right, but are also precursors for further chain reactions of free radical propagation. This potential for disease-causing effects can be avoided in two ways: 1. fats and body tissues can be protected by antioxidants, or 2. fats and cholesterol can be restricted, starving the oxidative process of fuel. Unfortunately, it also deprives the body of essential fatty acid nutrients and cholesterol, which are necessary for many vital functions of the body's antioxidant defenses. Lipids and cholesterol are also the raw material from which a large number of hormones and vitamin D are produced within the body. In recent years, a sizeable body of scientific evidence has accumulated to show that antioxidant deficiency is much more important than an excess of fat and cholesterol as a cause of disease. Low blood levels of vitamin E were shown in a World Health Organization study to be

100 times more significant relative to atherosclerosis and cardiovascular disease than high blood levels of cholesterol. The daily intake of a broad spectrum of multivitamin preparation was shown in a study by the University of California to increase life expectancy by as much as six years. To use a metaphor, if the fire in a furnace is breaking through the walls and threatening to burn the house down, it can be combated in two ways: put out the fire by depriving it of fuel, which is analogous to the low-fat, low-cholesterol approach to nutrition, including the use of cholesterol-lowering drugs, or fireproof the house and make the furnace walls more resistant, protecting the structure from damage while continuing to benefit from a steady supply of fuel.

2.4 HERBAL DRUGS AS ANTIOXIDANTS

A large section of the world's population relies on traditional remedies to treat a plethora of diseases. Medicinal herbs are an indispensable part of the traditional medicine practiced all over the world due to low costs, easy access and ancestral experience (Marini-Bettolo, 1980).

In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants. This interest in drugs of plant origin is due to several reasons, namely, conventional medicine can be inefficient (e.g. side effects and ineffective therapy), abusive and/or incorrect use of synthetic drugs results in side effects and other problems, a large percentage of the world's population does not have access to conventional pharmacological treatment, and folk medicine and ecological awareness suggest that "natural" products are harmless (Rates, 2001).

Today, the market is flooded with herbal medicines. A number of companies are entering into the arena of herbal medicines. These medicines are available for each and every disorder ranging from diabetes to rejuvenators (Bhutani, 2003).

Generation of reactive oxidants in living cells is an inevitable process of normal metabolism, and many anti-oxidant systems in organisms are able to control the toxic levels of reactive oxidants and

protect against damages inflicted by the reactive oxidants (Hall and Braughler, 1986). In condition of severe oxidative stress, however, these defenses do not provide complete protection from attack of reactive oxidants, such as superoxide ($O_2^{\bullet-}$) and hydroxyl radical ($OH^{\bullet}$), which are the most toxigenic and mutagenic. Therefore, many scientists have suggested that dietary antioxidants such as ascorbate, tocopherol, and carotenoids from fruits and vegetables could help to protect cells from damages caused by oxidative stress and to fortify the defense systems against degenerative diseases (Ames, 1983). Recently, intensive researches on biological function of natural antioxidants have been carried out with numerous plant materials worldwide, including those used as foods (Aruoma et al., 1997). It is also well known that natural antioxidants could protect against damages caused by reactive oxidants through dietary supplement in various biological mechanisms of living cells (Ozer et al., 1993). Crude drugs or natural diet food which possess anti-oxidant or free radical scavenging activity has become a central focus for research designed to prevent or ameliorate tissue injury and may have a significant role in maintaining health (Jer-Min et al., 1995).

Even the most conservative medical fields nowadays accept the importance of antioxidants and people find great benefits from these nutritional ingredients in achieving optimum health.

As plants produce a lot of antioxidants to control the oxidative stress, they can represent a source of new compounds with antioxidant activity. Natural antioxidants strengthen the endogenous antioxidant defenses from reactive oxygen species (ROS) ravage and restore the optimal balance by neutralizing the reactive species. Thus, herbal drugs containing antioxidants are gaining immense importance by virtue of their critical role in disease prevention.

2.5 AIMS AND OBJECTIVES

The role of reactive oxygen species in several diseases and the potential antioxidant protective effect of natural compounds on affected tissues are topics of high current interest. A number of plants and plant

isolates have been reported to protect free-radical induced damage in humans and animals.

DHC-1 (manufactured by Himalaya Drug Company, Bangalore, India) contains extracts of *Bacopa monnieri, Emblica officinalis, Glycyrrhiza glabra, Mangifera indica* and *Syzygium aromaticum.*

Activit (manufactured by Ayur Herbals Pvt. Ltd., Baroda, India), contains extracts derived from *Mucuna pruriens, Withania somnifera, Argyreia speciosa, Centella asiatica, Tribulus terrestris, Asparagus racemosus, Piper longum, Anacyclus pyrethrum, Nux vomica, Tinospora cordifolia* and shring bhasma.

Pepticare (manufactured by Ayur Herbals Pvt. Ltd., Baroda, India) contains powders of *Tinospora cordifolia, Emblica officinalis, Glycyrrhiza glabra* alongwith Sootshekhar ras, Praval bhasma, Swarnabhasma and Kapardi bhasma.

Normacid (manufactured by Ayur Herbals Pvt. Ltd., Baroda, India) contains Bhunimbadi kwath, *Solanum nigrum*, Mouktika bhasma, Shuddha Gairika bhasma, Kapardi bhasma, Swarnabhasma, Praval bhasma and Shankh bhasma.

DHC-1, Activit, Pepticare and Normacid, are herbal/herbomineral formulations formulated by combining plant ingredients, which are individually known or proved to exhibit antioxidant properties in various *in vitro* and *in vivo* experimental models. But these as formulations have not been evaluated for their antioxidant effect with reference to various disease conditions such as ulcers, myocardial infarction, nephrotoxicity and liver damage. It has been reported that these diseases are associated with the generation of free radicals or reactive oxygen species (ROS). Thus, in the present study DHC-1, Activit, Pepticare and Normacid were tested for their antioxidant activity using models such as pylorus-ligation and ethanol-induced ulcers, isoproterenol-induced myocardial infarction, cisplatin-induced nephrotoxicity and carbon tetrachloride-induced hepatotoxicity which characterize the most common diseases/disorders prevailing in the society. The study would prove whether these formulations can ameliorate the tissue injuries

inflicted by the release of free radicals as a result of the disease process or toxic actions of chemicals. Quantitative data obtained from this study will also be useful to carry out clinical studies and then launch these products in the market to make it available for the benefit of mankind.

To consider a natural compound or a drug as an antioxidant substance it is necessary to investigate its antioxidant properties *in vitro* and then to evaluate its antioxidant functions in biological systems.

Thus the aim of the study was to evaluate the herbal/herbomineral formulations DHC-1, Activit, Pepticare and Normacid for antioxidant activity by using some common *in vitro* methods, namely:

1. **Radical scavenging activity by DPPH reduction (DPPH assay) and**
2. **Superoxide radical scavenging activity in riboflavin/light/NBT system.**

and *in vivo* models, namely:

1. **Pylorus-ligation induced ulcers,**
2. **Ethanol-induced gastric mucosal injury,**
3. **Isoproterenol-induced myocardial infarction,**
4. **Cisplatin-induced nephrotoxicity and**
5. **Carbon tetrachloride-induced hepatotoxicity.**

These *in vivo* models were selected for the study as they involve the common diseases/disorders related to the important organs of our body, namely, stomach, heart, kidneys and liver. Also as these methods involve the generation of free radicals, they serve good standardized models to study the beneficial antioxidant effects of many drugs.

Drug-X, a herbal formulation, consists of the powder of fruit of *Moringa oleifera*. *Moringa pterygosperma* or *Moringa oleifera* Lam (family: Moringaceae) is a small or middle-sized tree, which is cultivated throughout India and Myanmar (Burma). The fruit of this tree is edible

and constitutes one of the common vegetable dishes in India The leaves of *M.oleifera* were used by the Indians in their herbal medicine as a hypocholesterolemic agent in obese patients. The scientific basis for their use in hypercholesterolemia was examined by Ghasi et al. (2000). They tested the crude extract of leaves of *M oleifera* and showed that it possessed hypocholesterolemic activity.

As the fruit of *M.oleifera* is widely consumed by the people of India, it was considered worthwhile to study it for its hypocholesterolemic activity. Thus the aim of the study was to evaluate Drug-X for its effect on the lipid profile in normal and hypercholesterolemic rabbits and also to study whether the hypocholesterolemic activity is due to its antioxidant effect.

Thus the aims and objectives of the present investigation were

- **To study the antioxidant activity of herbal/herbomineral formulations like DHC-1, Activit, Pepticare and Normacid by using *in vitro* methods, like DPPH assay and NBT reduction method.**

- **To study the anti-ulcer effect of herbal/herbomineral formulations like DHC-1, Activit, Pepticare and Normacid in pylorus-ligation induced ulcers and ethanol-induced gastric mucosal injury and also to study the effect of these formulations on various antioxidant parameters to justify whether the gastroprotective effect is due to the antioxidant mechanism of action of these formulations.**

- **To study the cardioprotective effect of herbal/herbomineral formulations like DHC-1, Activit, Pepticare and Normacid by using isoproterenol-induced myocardial infarction method and also to study the effect of these formulations on various antioxidant parameters to justify whether the cardioprotective effect is due to the antioxidant mechanism of action of these formulations.**

- **To study the nephroprotective effect of herbal/herbomineral formulations like DHC-1, Activit, Pepticare and Normacid by using cisplatin-induced nephrotoxicity model and also to study the effect of these formulations on various antioxidant parameters to justify whether the nephroprotective effect is due to the antioxidant mechanism of action of these formulations.**

- **To study the hepatoprotective effect of herbal/herbomineral formulations like DHC-1, Activit, Pepticare and Normacid using carbon tetrachloride-induced hepatotoxicity model and also to study the effect of these formulations on various antioxidant parameters to justify whether the hepatoprotective effect is due to the antioxidant mechanism of action of these formulations.**

- **To study the hypocholesterolemic effect of Drug X, a formulation consisting of *Moringa oleifera* in hypercholesterolemic rabbits and also to study the effect of Drug 'X on various antioxidant parameters to justify whether the hepatoprotective effect is due to its antioxidant mechanism of action.**