REVIEW OF LITERATURE
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Herbal medicines:

Plants as source of medicines and human sustenance have been in vogue since antiquity. Ancient Indian literature like Rigveda, Atharveda, Upanishad, Mahabharata, Puranas, Charak Samhita and Sushruta Samhita include medicinal plants used for drugs, essences, worships, food, fuels, poisons and agricultural tools. It can be said that herbs and herbal products occupied a prime place in all ages and in all civilizations on earth totally unconcerned with the development of synthetic and chemotherapeutic agents. All cultures have some definable plant knowledge that includes appropriate edible plants, medicinal plants and ceremonial plants. World Health Organization (WHO) has listed about 20,000 plant species in the world yielding drugs. In India, over 2,500 species are credited with medicinal values. Modern allopathic medicine is derived predominantly from the alchemical practice, but even here some well-known plants have become part of common practice. Cardiac glycosides, i.e. digitalis, from the foxglove is a well known example. Much of what we know about the nervous system function has been defined through the use of plant alkaloids i.e. muscarine, nicotine, atropine and ephedrine.

Plant-based drugs have created a sweeping resurgence in the west in their favor and demand for these has reached a scale never witnessed before. Plants synthesize thousands of metabolites that are used for their growth and development, reproduction, defense against attack by many different kinds of organisms and survival in often harsh and ever-changing environments.

Even in developed nations like United States, the use of herbal medicines has increased four folds within the last five years (Austin, 1998). A recent study (Johnson, 2000) has indicated that by the year
2000, approximately one in six patients in ambulatory clinic research settings has used herbal medicines in addition to modern medicines. A recent survey of a large adult population in the United States revealed that 42% of these consumers used complementary and alternative medicine. Currently more than 20,000 herbal products are sold for medication and dietary purposes. Sales of these products have been increasing at a rate of 20% per year since the early 1990s (Eiesenberg et al., 1993). Presently, the sales on these products are approximately $4 billion in the United States alone (Austin, 1998). These herbal products are being used for a variety of reasons, from the enhancement of general well being to more specific applications.

**Oxidative Stress and Disease:**

Oxygen is essential for life but reactive products of oxygen are amongst the most potent and omnipresent threats faced by any living organism. Its reduction to water provides the energy that allows the impressive complexity of higher organisms. However, oxygen reduction is a mixed blessing. Not only does it permit the biosphere’s versatility, but it is also life threatening. This is because incompletely reduced oxygen species, when uncontrolled, can nonspecifically oxidize, and thereby damage biological molecules. Oxygen’s nature as a double-edged sword manifests itself best in mitochondria, where most of the oxygen utilized by eucaryotic cells is reduced.

Oxidative stress has been defined as a disturbance in the pro-oxidant-antioxidant balance, resulting in cell damage. This leads to the increased production of reactive oxygen species which has been implicated in several biological and pathological processes like aging, inflammation, carcinogenesis, and ischaemia-reperfusion and in diseases including Acquired Immuno Deficiency Syndrome (AIDS), Parkinson’s disease Huntington’s disease, familial amyotrophic lateral sclerosis (ALS), and cataract formation in the eye (Ames et al., 1993;
Aruoma, 1998). Apoptosis has also been linked to these diseases, suggesting that both processes might be involved in these pathologies. It is now well established that oxidative cell injury generated by chemicals, during reoxygenation of hypoxic tissue, or as a result of acute or chronic inflammatory processes is associated with multiple alterations of cell structure and function. Among these perturbations are oxidation of intracellular thiols and pyridine nucleotides, impairment of signal transduction and ion homeostasis, modification of cytoskeletal organization, inhibition of glycolysis, infliction of DNA damage and activation of poly (ADP-ribose) polymerase, NAD+ depletion, ATP depletion and collapse of the mitochondrial membrane potential.

ROS is a collective term used to describe both free radicals (hydroxyl radical, OH°, superoxide anion, O2° ; peroxyl radical, ROO°), and non radical compounds (hydrogen peroxide, H2O2 singlet oxygen,1O2) (Aruoma, 1994; Diplock et al, 1998) that are produced in living cells as a result of normal cell metabolism. However, when ROS are not adequately removed or are formed additionally in the cells by exogenous sources, oxidative stress may occur (Halliwell, 1997). Molecular oxygen (O2) plays an essential role in a variety of metabolic processes invariably associated with an aerobic existence. Because of its oxidizing capacity, oxygen acts primarily as an electron acceptor, which leads to the formation of a variety of ROS. These substances comprise oxygen species with unpaired electrons such as superoxide anion (O2°) and *OH, or species with the ability to abstract electron from other molecules (eg: H2O2 and HOCI). (Halliwell and Gutteridge, 1990).

"Superoxide theory of oxygen toxicity" was postulated by Irwin Fridowich and Joe McCord in 1969 year, and states that oxygen is toxic because
some of it is metabolized to make super oxide radical. However it is not very reactive radical species, and it does not appear to react at significant rates with DNA, phospholipids or proteins. The toxicity of the superoxide radical ($O_2^-$) and hydrogen peroxide ($H_2O_2$) in living organisms is due to their conversion into $^\cdot$OH and into reactive radical metal complexes via either the iron-catalyzed Haber–Weiss reaction (Haber and Weiss, 1934) or the superoxide driven Fenton reaction (Fenton, 1894; Goldstein et al, 1993; Walling, 1975).

**Fenton Reaction:**

\[
H_2O_2 \xrightarrow{Fe^{2+}} OH^\cdot + OH^- + Fe^{3+}
\]

**Haber-Weiss Reaction:**

\[
O_2^- + H_2O_2 \rightarrow OH^o + OH^- + O_2^o
\]

$OH^o$ radical is because of its extreme reactivity the main factor of so-called oxygen toxicity. It reacts with all biological materials, oxidatively by hydrogen withdrawal, double bond addition, electron transfer and radical formation, and initiates autoxidation, polymerization and fragmentation. Thus nature of the damage done by excess formation of $H_2O_2$ and superoxide radical is affected by the location and concentrations of metal ion catalysts of reactions within the cells. This implies that if no catalytic metal ions are available, $O2^-$- and $H2O2$ will have limited, if any, damaging effects (Halliwell and Gutteridge, 1990).
Excessive generation of ROS and reactive oxygen intermediates (ROI) can modify and damage DNA, carbohydrates, proteins and polyunsaturated fatty acids in cells (Halliwell, 1997; Diplock et al, 1998). Oxidative injury has been reported to develop according to a general pattern that basically involves free thiol oxidation and appearance of protein disulfides, depletion of ATP pool (Schraufstatter et al, 1988), elevation of free cytosolic calcium, disassembly of cytoskeleton, increase in plasma membrane permeability and peroxidation, release of cytosolic components, and induction of DNA damage (Karczewski et al, 1999).

Factors that can cause oxidative damage:

Eversince Harman (1956) first proposed the free-radical theory of aging, the molecular basis of aging and the role of ROS in this process has attracted considerable attention in recent years. It is now generally agreed that aging and age-related diseases result from ROS-mediated oxidative damage of lipid, protein and nuclear and mitochondrial DNA molecules (Harman, 1981). The concentration of oxidatively damaged proteins, lipids, and DNA has been reported to increase with age (Sohal and Orr, 1995). Oxidative stress may also occur as a consequence of pollution (cigarette smoke, ozone and nitrogen oxides). Free radical formation may also be the side effect of certain drugs or disease treatments (radiation therapy) (Halliwell, 1997). An excess of free radicals may induce changes that ultimately lead to the development of various diseases.

Chromium (VI) as an oxidant:

Although chromium is an essential trace element involved in glucose and lipid metabolism (Anderson, 1990; Moris et al, 1988) it is toxic and carcinogenic when high exposures occur such as in industry (Antilla, 1990; Langard, 1990). Chromium (VI) compounds have been declared as potent occupational carcinogen among workers in chrome
plating, stainless steel and pigment industries. Epidemiologic studies have shown that industrial workers exposed to chromium (VI) had a higher incidence of respiratory cancer than the normal population (Leonard and Lawerys, 1980; Love, 1983; Norseth, 1986; Love, 1983; Norseth, 1986; Antilla 1990; Langard, 1990). Dermal, renal, and hepatic toxicity has been reported in workers exposed to chromium (VI) (Love, 1983; Leonard and Lawerys, 1980). Chromium (VI) compounds also induce tumors (Langard, 1988), nephrotoxicity (Appenroth and Braunlich, 1988; Laborda et al, 1986) and hepatotoxicity (Laborda et al, 1986) in experimental animals. Chromium (VI) compounds cause mutations in bacteria (Nishioka, 1975), chromosomal aberrations (Tsuda and Kato, 1977; Majone and Levis, 1979; Newbold et al, 1979; Umeda and Nihsimura, 1979; Sen et al, 1987), sister chromatid exchanges (Gainaldi et al, 1982; Sen and Costa, 1986;) as well as mutations (Bianchi et al, 1983; Paschin et al, 1983; Patierno et al, 1988) and transformation (Briggs and Briggs, 1988; Patierno et al, 1988; Fradkin et al, 1975) in cultured mammalian cells. Furthermore, they have been shown to produce DNA single-strand breaks (Sugiyama et al, 1986; Sugiyama et al, 1988), alkali labile sites (Cantoni and Costa, 1984), DNA-interstrand crosslinks ((Sugiyama et al, 1986) and DNA-protein crosslinks (Cantoni and Costa, 1984) in vivo and in cultured cells, as well as selectively inhibiting the activity of enzymes such as glutathione reductase in mammalian cells (Koutras et al, 1964; De Flora et al, 1984).

Chromium (VI) compounds are more toxic and carcinogenic than chromium (III) (Tsuda and Kato, 1977; Bianchi et al, 1983; De Flora and Wetterhahn, 1989; Bagchi et al, 2002), because chromium (VI), in contrast to chromium (III) readily enters cells by the sulfate anion transport system (Costa et al, 1984; De Flora and Wetterhahn, 1989). However, once inside, chromium (VI) is reduced through reactive intermediates such as chromium (V) and (IV) to more stable chromium (III) by cellular reductants (Connett and Wetterhahn, 1983; De Flora et
Therefore, the formation of trivalent chromium or other intermediate oxidation states may play an important role in the induction of chromium (VI) toxicity.

**Sodium Nitroprusside (SNP) as an oxidant:**

Nitric oxide (NO) plays a dual role in the inflammatory process and has been shown to be involved in numerous regulatory functions ranging from altering the cardiovascular system to modulating neuronal functions (Ignarro, 1989; Moncada *et al.*, 1991). In addition NO has been shown to be part of the oxidative war chest of the immune system by virtue of involvement in anti-tumor and anti-pathogen host response (Hibbs *et al.*, 1987; Stuehr and Nathan, 1989). These functions of NO are beneficial in maintaining physiological homeostasis. On the other hand, proinflammatory effects of NO are mediated by its exacerbated production and have been associated with a range of pathophysiological conditions such as atherosclerosis, cancer, diabetes, numerous degenerative neuronal diseases, stroke and myocardial infarction (Hibbs *et al.*, 1987; Stuehr and Nathan, 1989; Gross and Wolin, 1995). Sodium nitroprusside dissolves in water and releases NO slowly and has been extensively used for studying the nitric oxide induced cellular damage by many investigators (Bernabe *et al.*, 2001; Kaku *et al.*, 2001). Nitric oxide (NO*) is a reactive and unstable free radical that can cross cell membranes easily by diffusion without depending on any release or uptake mechanisms. NO* is involved in several signaling pathways related to a diverse array of cell functions. NO* produced by inducible Nitric Oxide Synthase (iNOS) mainly in macrophages and neutrophils, mediate cytotoxicity as the first line of host-defense against invading microorganisms (Adams *et al.*, 1990) or tumor cells (Stuehr and Nathan, 1989). NO may also
mediate apoptosis of macrophages (Albina et al, 1993; Sarih et al, 1993), pancreatic beta cell line (Ankarcrona et al, 1994) and mouse thymocytes (Fehsel et al, 1995). NO is relatively unreactive toward biomolecules, but in the presence of superoxides it forms the highly reactive peroxynitrite radical (Beckman et al, 1990). Nitric oxide injury takes place for the most part through the peroxynitrite route because peroxynitrite can directly oxidize low density lipoproteins resulting in irreversible damage to cell membrane.

**Targets of reactive oxygen species:**

**Lipid and protein oxidation:**

A lipid hydroperoxyl radical abstracts a hydrogen atom from the double bond of a neighbouring unsaturated lipid to form an alkyl radical, and the alkyl radical combines with oxygen to regenerate a lipid hydroperoxyl radical and initiate another round of oxidation. Ultimately, intramolecular reactions and decomposition yield cyclic endoperoxides and aldehydes, the latter of which are reactive and may act as mutagens (Frankel, 1980; Wiseman and Halliwell, 1996), inactivate enzymes (Szweda et al, 1993) or operate as endogenous fixatives, reacting with proteins and nucleic acids to form heterogenous cross-links (Chio and Tappel, 1969). Moreover, a primary effect of lipid peroxidation is decreased membrane fluidity, which alters membrane properties and can significantly disrupt membrane-bound properties (Tappel 1975). The oxidation of proteins is less well characterized, but several classes of damage have been documented, including oxidation of sulphhydryl groups or reduction of disulfides, oxidative adduction of amino acid residues close to metal-binding sites via metal- catalyzed oxidation, reaction with aldehydes, protein-protein cross-linking and peptide fragmentation (Starke and Oliver 1989; Stadtman and Oliver, 1991).
DNA damage:

Activated oxygen and agents that generate oxygen free radicals, such as ionizing radiation, induce numerous lesions in DNA that cause deletions, mutations and other lethal genetic effects. ROS can cause oxidative damage to both nuclear and mitochondrial DNA. The nature of damage includes mainly base modification, deoxyribose oxidation, strand breakage and DNA-protein cross-links. Characterization of this damage to DNA has indicated that both the sugar and the base moieties are susceptible to oxidation, causing base degradation, single strand breakage, and cross-linking to protein (Wiseman and Halliwell, 1996). Degradation of the base will produce numerous products, including 8-hydroxyguanine, hydroxymethyl urea, urea, thymine glycol, thymine and adenine ring-opened and -saturated products.

ROS mediated cell death can take place by two mechanisms: necrosis and apoptosis. Necrosis appears to be the result of acute cellular dysfunction in response to severe stress conditions or after exposure to toxic agents, and is a relatively passive process associated with rapid cellular ATP depletion. Morphologically, necrosis is characterized by a dramatic increase in cell volume and rupture of the plasma membrane, with spilling of the cellular contents into the intercellular milieu (Gores et al, 1990). This release of the dying cells' contents can cause further tissue damage by affecting neighboring cells or by attracting proinflammatory cells to the lesion (Haslett, 1992).

Apoptosis is a form of cell death that occurs during several pathological situations in multicellular organisms and constitutes a common mechanism of cell replacement, tissue remodeling, and removal of damaged cells (DeLong, 1998). Apoptosis is a complex process characterized by cell shrinkage, chromatin condensation,
internucleosomal DNA fragmentation (Kerr et al, 1972; Wyllie et al, 1980; McConkey et al, 1988), and formation of "apoptotic bodies". Several protease families have been implicated in apoptosis, the most prominent being caspases (Alnemri et al, 1996). Apoptosis is characterized by a variety of morphological features. Changes in plasma membrane are one of the earliest of these features. In apoptotic cells, the membrane phospholipid, phosphatidyl serine (PS) is translocated from the inner to the outer leaflet of the plasma membrane.

**Defenses against reactive oxygen species:**

The antioxidant defense system is essential for protection of cell structures and macromolecules from damage by free radicals, which normally result from metabolic processes. This defense system generally declines with age and can be compromised by various forms of oxidative stress resulting from exposure to smoke, drugs, environmental pollutants, radiation and physical exercise. Current understanding of the antioxidant defense system reveals a highly complex, interactive, multifactorial network which includes vitamin C and E; β-carotene and other carotenoids; enzyme antioxidants dependent on zinc, copper, selenium, or manganese such as superoxide dismutase, glutathione peroxidases; the tripeptide glutathione, alpha lipoic acid, and very likely, numerous phytonutrients. Complex recycling and regeneration reactions occur to optimize protection from free radicals. Moreover, deficiencies or imbalances may compromise these reactions as well.

Cells are equipped with an impressive endogenous enzymatic defense system against oxidative damage, and also many small-molecule antioxidants derived from dietary intake of fruit and vegetables (Ames et al, 1995; Smith et al, 1999). These include:

i) Enzymatic scavengers such as SOD, which hastens the dismutation of $O_2\cdot$ to $H_2O_2$ to water
ii) Hydrophilic radical scavengers such as ascorbate, urate and glutathione (GSH)

iii) Lipophilic radical scavengers such as Tocopherols (e.g., α-tocopherol, γ-tocopherol), flavonoids (e.g., quercetin, epigallocatechin gallate), carotenoids (β-carotene and lycopene) and ubiquinol

iv) Enzymes involved in the regeneration of oxidized forms of the small molecular antioxidants (GSH reductase, dehydroascorbate reductase) or responsible for the maintenance of protein thiols (thioredoxin reductase)

v) The cellular machinery that maintains a reducing environment (e.g., glucose-6P-dehydrogenase, which regenerates NADPH).

The complement of defenses deployed differs tremendously not only between organisms or tissues, but even between cellular compartments. For instance, GPx plays an important role in mammals but is absent in flies and nematodes (Vanfletern, 1993; Sohal et al., 1995), and there exists in humans three different forms of SOD (cytosolic Cu/Zn SOD, mitochondrial MnSOD, and extracellular SOD), each encoded and regulated independently (Fridovich, 1995) by different genes.

**Antioxidants in human health:**

The function and action of antioxidant defense system has been extensively studied both *in-vitro* and *in-vivo*. The therapeutic applications of natural and synthetic antioxidants are being explored. Epidemiological, *in vivo* and *in vitro* studies have suggested that diets rich in fruit and vegetables may exert protective effects against various stages of the cancer process and Coronary Heart Diseases (CHD) (Block *et al.*, 1992; Aruoma, 1994; Hollman *et al.*, 1996). These effects
have been contributed, in part, to bioactive components found in fruit and vegetables that possess antioxidant activities (Block et al, 1992; Hollman et al, 1996; Diplock et al, 1998). The most prominent representatives of these dietary antioxidants include ascorbate, carotenoids, flavonoids, and tocopherols (Diplock et al, 1991).

**Antioxidant vitamins:**

α-Tocopherol, biologically the most active form of vitamin E (Coquette et al, 1986; Kamal-Eldin and Appelqvist, 1996), is the major lipid-soluble antioxidant, which can function as a chain-breaking antioxidant (Burton and Ingold, 1981). α-tocopherol functions as an antioxidant by rapidly transferring its phenolic hydrogen atom to a lipid peroxyl radical, resulting in the formation of two molecules that are relatively unreactive towards polyunsaturated lipid, i.e., a lipid hydroperoxide and the α-tocopheroxy! radical.

β-carotene, is another lipid soluble antioxidant, which attracts widespread attention because of its anti-cancer activity. β--carotene is an excellent quencher of singlet oxygen and also a chain-breaking antioxidant (Sies and Stahl, 1995).

Ascorbic acid derives its antioxidant functions from its ability to reduce other compounds. Ascorbic acid has two electrons that it donates to radical species thus stabilizing them and quenching their reactivity. Because of its water solubility and its ability to enter cells via a Na⁺/ascorbic acid co-transporter, vitamin C is ubiquitous in all body fluids. Due to its prevalence in both intra- and extracellular fluids, ascorbic acid can scavenge many kinds of ROS produced from endogenous sources as well as those absorbed from the environment (Panayiotidis and Collins, 1997; Sweetman et al, 1997). Another way in which vitamin C is thought to prevent oxidative damage is through its interactions with
vitamin E. Which, because of its hydrophobic nature, forms an important component of cellular membrane where it protects against lipid peroxidation. In this process α-tocopherol is oxidized to an inactive state and thus cannot provide further protection against attacks by ROS. Vitamin C recycles vitamin E by reducing it to its active state and in this way reestablishes the antioxidant protection of the cell membrane (May, 1999).

Although both vitamin C and vitamin E are noted for their antioxidant capabilities, it has been known that ascorbate has the ability to generate free radicals in the presence of metal ions in vitro. Indeed, a classical reaction used in the laboratory uses Fe (III), ascorbate and H₂O₂ to create hydroxyl radicals. Vitamin E can also produce superoxide radicals in the presence of Cu (II) via reduction of Cu(II) to Cu (I) and the production of H₂O₂ from molecular oxygen(Yamashita et al, 1998). In-vitro these radicals induce extensive damage to DNA, lipids and proteins.

Fortunately nature has provided us with mechanisms found predominantly in plants to defend against oxidative injury.

**Phytonutrients associated with health promotion:**

Plants produce a number of phytonutrients which when present in an appropriate concentration act in synergistic fashion having therapeutic activity. Plants are rich source of vitamins, minerals, and various other bioactive components. Major classes of phytonutrients include carotenoids, polyphenols, anthocyanins, flavonoids, isothiocyanates, sulfides, and phytosterols (Harborne and Williams 2000; Harborne and Williams 2001; Bravo, 1998). This broad range of natural compounds appears to have dozens of biological functions, which may be overlapping and complementary. Interest in phytonutrients has
resulted in identifying mechanism of action at the cellular or molecular level for many of these compounds.

Plants are very good source for nearly all the important antioxidants and in addition contain thousands of phytonutrients. The most widely studied nutrients in plants are β-carotene, vitamin C and vitamin E. Their role as antioxidants for exerting a protective effect in vivo as well as a preventive role in cardiovascular diseases (CVD) and cancer has been well established. When disease outcomes of five large studies were correlated with plasma levels of these nutrients, the quintiles with the highest level of β-carotene (>0.22μg/ml), α-tocopherol (>12.9μg/ml), and vitamin C (>8.8μg/ml) showed the lowest risk for both cancer and cardiovascular diseases (CVD) (Stahelin et al, 1991; Gey, 1995). Many of these compounds are known to be good antioxidants in vitro and are thought to be protective in vivo against oxidative damage to cellular components (Cao et al, 1996; Wang et al, 1996; Cao et al, 1998). Flavonoids and other phenolic compounds appear to be antioxidants that contribute to the high antioxidant capacity observed in certain fruits and vegetables (Aruoma, 1994). Further, some flavonoids have been shown to have many times greater antioxidant capacity than vitamins C and E (Cao et al, 1997). The effect of feeding elderly women strawberries, spinach, red wine, or vitamin C was determined by three methods, which measure the antioxidant capacity in serum (Cao et al, 1998). A single meal including strawberries, spinach, or red wine increased serum antioxidant capacity to an extent equivalent or greater than a large dose of vitamin C (1250mg). These increases were attributed to the antioxidant properties of flavonoids and polyphenols, which were effectively absorbed from the single serving. Antioxidant of plant origin have been demonstrated to prevent or curtail progression of many diseases like cardiovascular disease, cancer, aging and immune disorders.
i) Cardiovascular diseases (CVD)

Several cohort studies have been performed in which the relationship between flavonoid intake and the risk of coronary heart disease (CHD) has been investigated. The studies show that the mortality from CHD is inversely correlated with the intake of flavonoids in the diet. Cohort and cross cultural studies have demonstrated that flavonoids from the flavonol and flavone subgroups have a protective role against cardiovascular diseases (Hollman and Katan 1998). The protective effect of the flavonoids is partly explained by the inhibition of LDL oxidation and by reduced platelet aggregability. As reviewed by Cook and Samman (1996), there are several possible routes as to how LDL is oxidized by free radicals generated in the cells and how the oxidized LDL initiates and promotes atherosclerosis in the human body.

The occurrence of plaques is accepted as an intermediate end point in the development of CHD (Biersalski, 1999). The blood platelets aggregate and adhere to blood vessels, thus favouring the development of thrombosis and atherosclerosis (Cook and Samman, 1996). Special flavonoids have been shown to inhibit the development of blood platelet aggregation and blood vessel adhesion, thereby reducing the risk of these thrombotic reactions. The mechanisms of flavonoid actions appear to be very complex. The flavonoids bind to the platelet membranes and scavenge free radicals from this position. The inhibitory effect of flavonoids on platelet function is linked to structural features of the flavonoid molecule, and flavonoids may thus vary in their efficiency in preventing conditions favorable for development of CHD.

ii) Cancer

The antioxidant properties and the inhibitory role in various stages of tumor development in animal studies make flavonoids interesting as
cancer-preventing agents (Hollman and Katan, 1998). Several reviews have been published with the conclusions that fruits and vegetables have preventive effects on various types of cancer, although the actual chemical components remain to be identified (Block et al, 1992; Steinmetz and Potter, 1996).

iii) Aging

Recent emphasis has focused on the role of antioxidants in the general aging of humans. As pointed out earlier by Stahelin (1999), key factors in the development of the aging processes are associated with a species capacity of repairing DNA and of the antioxidant defense of the body. Various organs of the body may respond to the oxidative stress at different rates and with different defense mechanisms. Atherosclerosis, cancer and degenerative brain diseases may result from specific processes in an organ or a cell system and at the same time may be the result of the universal aging process. Damage to DNA by radicals may be a significant contributor to the age dependent development of cancer (Halliwell, 1994). It is not yet clear, however, whether the oxidative stress is the primary cause of the diseases or whether formation of radicals is a secondary effect of tissue damage caused by the disease.

iv) Immune Functions

The human immune system plays an indispensable role in protecting against various disease-causing agents as well as mutant or malignant cells. It functions through a complex system of highly interactive cells and bioactive compounds produced by these cells. Many factors can adversely affect immune function including stress, environmental exposures, aging and nutritional deficiencies. In fact, essential nutrients and an array of phytonutrients have been shown to affect almost every aspect of the immune system (Kubena and McMurray, 1996). Two studies examined the effects of a multivitamin
and mineral supplement on immune functions in healthy elderly individuals. Both studies were double blind and placebo controlled, with a duration of twelve months. Chandra (1992) demonstrated that the supplement group had increased levels of certain T-cell subsets and natural killer cells, enhanced B-cell activity, increased IL-2 production and higher antibody response and natural killer cell activity. These subjects also had significantly less illness due to infections during the year than the placebo group.

v) Reduction in DNA damage

Damage to DNA occurs mainly through free-radical attacks on DNA bases, resulting in base alteration and strand breaks, and possible mutations. Antioxidants have been shown to provide protection against damage in a variety of human studies. In a double blind, placebo-controlled trial in smokers and nonsmokers, a significant decrease in cellular base damage in lymphocyte DNA was demonstrated using a daily supplement of β-carotene (25mg), vitamin C (100mg), and α-tocopherol (280mg) (Duthie et al., 1996). In another study, twenty three healthy men consumed a low carotenoid diet for two weeks while supplementing with carrot and tomato juices (33ml/day) and spinach powder (10g/day). All subjects demonstrated decreased lymphocyte DNA strand breaks (Pool-Zobel et al., 1997).

Plants with antioxidant activity:

Several studies have revealed that plants produce potent antioxidants to control the oxidative stress caused by sunbeams and oxygen and represent a source of new compounds with antioxidant activity (Scartezzini and Speroni, 2000).
Epidemiological studies indicate that populations that have a high soy intake have a lower incidence of breast and prostate, as well as other carcinomas. Genistein is an isoflavone found in high quantities in soybean products. Genistein-containing soy diets have been shown to decrease incidence and number of tumors and to increase latency in animal models of cancer (Barnes, 1995). Much work has been done on cell-culture models, which demonstrate that genistein inhibits proliferation of some types of cancer cells (Peterson, 1995). Turmeric (Curcuma longa, family: Zingiberaceae) has been used as a coloring agent and food additive in Indian culinary preparations from time immemorial (Huang et al, 1994). The active principle in Curcuma longa has been identified as curcumin, which has many pharmacological activities such as anti-inflammatory (Srimal and Dhawan, 1973), anti-cancer (Huang et al 1994) and anti-oxidant properties (Soudamini et al, 1992). Tinospora cordifolia is widely used in Indian Ayurvedic medicine for the treatment of diabetic mellitus. The stem of this plant has been demonstrated to have antioxidant (Prince and Menon, 2001) and immunomodulatory (Atal et al, 1986) activities.

Plants with immunomodulatory activity:

Modulation of immune responses to alleviate disease has been of interest for many years and the concept of 'Rasayana' in Ayurveda is based on related principles (Charak Samhita, 1949). In recent years, immunostimulatory activity has been reported in a number of plants (Burger et al, 2001; Gracious et al, 2001; Zhao et al, 2001; Lily et al, 2003). The immune system is involved in the etiology as well as pathophysiologic mechanisms of many diseases. The function and efficiency of the immune system is influenced by many exogenous and endogenous factors like food, pharmaceuticals, physical and psychological stress, hormones etc, resulting in either
immunosuppression or immunostimulation. Apart from being specifically stimulatory or suppressive, certain agents have been shown to possess activity to normalize or modulate pathophysiological processes and are hence called immunomodulatory agents (Patwardhan et al, 1991).

Indian herbs have been shown to possess anti-inflammatory (Singh and Atal, 1986) anti-stress and anti-cancer (Rege et al, 1999) effects by modulating the immune system. Asparagus racemosus, Withania somnifera, Tinospora cordifolia and Picrorhiza kurrooa have been found to enhance host resistance and reduce the side effects of other toxic agents. Withania somnifera has been used as relaxant, anti-spasmodic, anti-ulcerative and in arthopathies. A number of withanolides have so far been isolated from this plant and reported to possess both immunosuppression and immunostimulatory properties (Buddhiraja and Sudhir, 1987). Tinospora cordifolia has been used as a tonic and also as hepatoprotective agent (Peer and Sharma, 1989; Rege et al, 1999). It contains the glucosides and gilosterol, which are claimed to possess potent immunomodulatory activity (Bhattacharya et al, 1991).

Fruits of Emblica officinalis, commonly known as amla, have also been shown to possess antioxidant (Bhattacharya et al, 1999), adaptogenic (Rege et al, 1999), hepatoprotective and immunomodulatory activities (Jeena et al, 1999; Lily et al 2003). The fruit extracts have also been found to inhibit clastogenicity and mutagenicity induced by various metals (Dhir et al, 1991; Roy et al, 1992). Besides fruits, leaves have also been shown to have anti-inflammatory activity (Asmawi et al, 1993; Ihantola-Vormisto et al, 1997).

Ginko biloba is frequently used to enhance memory and mental function, primarily through its action in dilating capillaries and inhibiting platelet aggregation. It also scavenges free radicals, which has a protective effect on vascular walls. Ginko's antioxidant properties result
from synergistic actions of bioflavonoids, terpenoids and organic acids. These antioxidant compounds can ameliorate the cell damage and excessive lipid peroxidation seen in Alzheimer’s disease (Kanowski et al, 1996).

Himalayan plants:

India has a rich heritage of use of plants as medicines. There are over 2500 plant species in India having documented medicinal value, majority of them growing in a wild state; whereas only a few are cultivated. In recent years, a few developments in the drug industry have brought Indian Medical wealth and their Ecosystem into world focus. In particular, the clinical reports on many plants like Ruovolfia, Tylophora, Guggulu, Chirayita, Cinchona, Valeriana, Taxus, Arjuna have supported their claim of containing specific pharmacological activity for which they are used in the Indian System of Medicines (Chauhan, 1999). The wide range of altitude, topography and climatic conditions have endowed Himalayas with a rich and diversified flora. Out of around 3500 known plant species, there are around five hundred medicinal, one hundred and fifty aromatic and quite a good number of potent alternative and substitute drug plant sources available for being harnessed at present. In dry Himalayan temperate forests the predominant species occurring are: Pinus gerardiana, Cedrus deodara, Pinus wallichiana, Picea smithiana, Abies spectabilis, Juniperus macropoda, Populus ciliata, Salix viminalis, Quercus baloot, Ephedra gerardiana, Artemisia brevifoia, Hippophae salicifolia and Hippophae rhamnoides. Keeping in view the recent, growing popularity of herbal remedies, the Indian medicinal plants, more so the Himalayan herbs have received unprecedented attention. Even though we have a rich heritage of plant resource, India has still not exploited the full potential of the available resources. Seabuckthorn (Hippophae rhamnoides), is one such plant, which grows wildly in
Himalayas, medicinal activity of this plant still remains to be scientifically investigated.

SEABUCKTHORN (*Hippophae rhamnoides* L.)

Biological and genetic diversity:

*Hippophae* (Chinese: Shaji, literally: sand thorn, the English common name is sea buckthorn) is a member of the Elaeagnaceae family, a family that has very few medicinal herbs, mainly *Hippophae*, *Eleaegnus*, and *Elaeocarpus*. *Hippophae* is usually found at an altitude of 2000-4500m in cold climates, though it can grow at both higher and lower altitudes, in sandy soils. It has recently been planted in temperate zones worldwide to prevent soil erosion and to serve as a source of food and medicine (Li and Schroder, 1996). The genus *Hippophae* belongs to the Elaeagnaceae family. According to recent taxonomical studies, it includes three species, *H. rhamnoides*, *H. salicifolia* and *H. tibetana* of which *H. rhamnoides* has been further divided into eight subspecies (Rousi, 1971; Lian, 1988). However of all the species in the genus only *H. rhamnoides* has an extremely wide distribution in Europe and Asia; it grows on hills and hill sides, in valleys and river beds, along sea coasts and islands, in small isolated or large continuous pure stands or in mixed stands, with other shrub or tree species. In India seabuckthorn grows in high altitude areas of Himachal Pradesh, Jammu and Kashmir and Uttar Pradesh. In Himachal Pradesh, seabuckthorn grown on river sides and sun facing slopes in Lahaul-Spiti, parts of Chamba, Kinnaur, Kullu, Shimla and Kangra. Lahaul-Spiti has a very rich source of seabuckthorn at 2,500-4,300m above mean sea level. Seabuckthorn, locally known as ‘Tsermang’ is the most commonly used plant for the purpose of fuel, medicine and fruit by the local people.
Seabuckthorn (*Hippophae rhamnoides* L.)
Besides India, seabuckthorn grows naturally in Russia, Britain, Germany, Finland, Romania, France, Central Asia, China, Nepal, Pakistan, and Bhutan. Seabuckthorn also called Sallow thorn is a deciduous shrub with yellow or orange fruits (Li and Schroeder, 1996). It rapidly develops an extensive root system and is therefore, an ideal plant for preventing soil erosion (Yao and Tigerstedt, 1994).

History of application of seabuckthorn can be traced back to 8th century, when Tibetan doctors from Tang dynasty discovered medicinal properties of seabuckthorn fruit and leaves, which have been well documented in the Tibetan medical classic ‘The rGud bzi’ which records the application of seabuckthorn in curing diseases of cardiovascular and digestive systems. Chinese were the first to use seabuckthorn as a drug. Russians discovered the rich vitamin values of seabuckthorn fruit during the late 1940s and now the Russian astronauts are using seabuckthorn health foods and medicines for the improvement of body immunity during oxygen deficiency in their space flights.

Botany:

The seabuckthorn plants are spinescent shrub or a small tree up to 10m in height with rough brown bark. Leaves are small, liner-lanceolate, covered on both sides with silvery scales. Flowers very small, greenish or yellowish, appear with new leaves; male in axillary clusters, female solitary. Fruits are small, ovoid, round in shape with a diameter of six mm, orange-yellow or scarlet in color and sour to highly acidic in taste. Seeds single, oblong with shiny testa. The root system makes it suitable even in fragile slopes. A five year-old plant will have a taproot about five meters deep, with horizontal roots spreading six to ten meters.
Cultivation and distribution in India:

Seabuckthorn plant naturally growing in ladakh region of Jammu and Kashmir and is locally known as Tsermang or tasru- wonder plant. It has the potential of greening Ladakh by large scale plantation. The plant grows on a wide range of soil conditions and is distributed wild over the four of the five valleys of Ladakh i.e. Nubra, Indus, Suru and Zanskar, in a large area. Besides Ladakh, it is also found in Lahaul and Spiti, Kinnaur and Chamba region in Himachal Pradesh, Uttaranchal and in Sikkim. Flowering generally occurs in May-June and ripening sets in by late August –September. The Field Research Laboratory, Defence Research and Development Organization, Leh has initiated an extensive study on the propagation and utilization of this wonder plant. They have found that seabuckthorn can be propagated by sexual (seeds) as well as asexual (suckers and cuttings) methods. Asexual method is preferred since desired sex plants can be propagated.

The plant:

It is thorny, nitrogen fixing deciduous shrub. The *Hippophae rhamnoides*, subspecies turkestanica is the most dominant and widely distributed plant (2-4m tall), followed by *H.salicifolia* (4-9m tall) and *H.tibetana* (0.2-0.6m tall). Seabuckthorn remains alive from 60-350 years. It is a dioecious species with male and female flowers on separate trees. Plant bears foliage from April to November, flowers in June or July for a week and fruit from mid-August to April. Female plants bear red, yellow or orange colored fruits. A wild plant bears one - two Kg of fruits every year. Seeds are light to dark brown in color with the exception of white seeds of *H.tibetana*. The strong and well-developed root system grows vertically up to ten meter, producing up to ten meter, producing 30-40 new sister plants for several generations.
Seabuckthorn is easy to propagate by seed or cuttings. It can grow in arid to very wet conditions and tolerates cold winters. Though it prefers sandy and neutral soil, SBT survives in soils with pH values from 5 to 9 and tolerates sea water flooding. Like other members of the Elaeagnaceae family SBT is also a nitrogen fixer. Frankia, a symbiotic bacteria, fixes atmospheric nitrogen, improves soil properties and helps the plant in rapid regeneration even in poor soils (Akkermans et al, 1983). Genetic diversity is the basis for plant adaptation, evolution and breeding. Seabuckthorn is extremely variable in height, from a small bush less than 50cm to a tree more than 20m high (Rousi, 1971; Xu, et al, 1989; Yao and Tigerstedt, 1994). Phonological studies show a clear variation in growth, rhythm, hardiness and height according to the geographic distribution, i.e., the higher the latitude, the shorter the growth period and plant height (Yao and Tigerstedt, 1995).

Fruit:

Chemical composition:

Seabuckthorn fruits are rich in carbohydrates, protein, organic acids, amino acids and vitamins (Bernath and Foldesi, 1992). These components vary with fruit maturity, fruit size (Chen et al, 1991), species (Lu, 1992) and geographic locations (Wang, 1990).
<table>
<thead>
<tr>
<th>Components</th>
<th>Content</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotene and carotenoid</td>
<td>16-28mg/100g fruit</td>
<td>Kudritskaya et al (1989)</td>
</tr>
<tr>
<td>Flavonoids (Fruit)</td>
<td>120-2100mg/100g fruit</td>
<td>Chen et al (1991)</td>
</tr>
<tr>
<td>Flavonoids (leaves)</td>
<td>310-2100mg/100g leave</td>
<td>Chen et al (1991)</td>
</tr>
<tr>
<td>Dry matter</td>
<td>24.6-33.8%</td>
<td>Yang and Kallio (2001)</td>
</tr>
<tr>
<td>Volatile oil</td>
<td>3.6mg/100g fruit</td>
<td>Yang and Kallio (2001)</td>
</tr>
<tr>
<td>Oil (seed)</td>
<td>8.0-12.0%</td>
<td>Lu (1992)</td>
</tr>
<tr>
<td>Saturated fatty acid (fruit)</td>
<td>47.0%</td>
<td>Franke and Muller (1983)</td>
</tr>
<tr>
<td>Saturated fatty acid (seed)</td>
<td>21.0%</td>
<td>Franke and Muller (1983)</td>
</tr>
<tr>
<td>Unsaturated fatty acid (fruit)</td>
<td>53.0%</td>
<td>Franke and Muller (1983)</td>
</tr>
<tr>
<td>Unsaturated fatty acid</td>
<td>39.0%</td>
<td>Franke and Muller (1983)</td>
</tr>
</tbody>
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The fruit is the main part used as food and medicine. It is one of the richest sources of vitamin C 300-1600 mg/100g (Yao and Tigerstedt, 1992). Medicinally, the flavonoids of the fruit and the oil of the seed are deemed the main active constituents. The flavonoids are present in the range of 1-10mg/gram of fresh fruit (Zhong, 1989), which are considered to improve non-specific immunity. In addition, the flavonoids promote bone marrow production of red blood cells, and reduce allergy reactions (Diao Jingli, 1999).

**Seed oil:**

*Hippophae* seed oil is rich in vitamin E and essential fatty acids, including those that inhibit inflammation (Fu et al, 1993). One
ingredient, palmitoleic acid, is a component of skin that is considered very valuable in treating burns and healing wounds (Fushman, 1987). The oil is used alone or in various preparations topically applied for a wide range of skin ailments, including burns, scalds, ulcerations, including burns, scalds, ulcers (Jiang et al, 1989).

**Fruit oil:**

The oil obtained from its fruits acts as a raw material for the pharmaceutical industries. In fresh fruits, the oil content varies from 4 to 8%, in fruit pulp 2 to 4% and seed 10 to 20%. Unsaturated fatty acids constitute about 86% of the total oil, linoleic acid being the main component carrying several bioactive substances (Fushman, 1987). Its pharmaceutical functions involve properties of anti-bacterial, analgesic, pain-reliever and regenerating tissues. The fruit oil is quite rich in vitamin E and K, carotenoids, flavonoids, and steroids. Vitamin E (200mg/100g) has anti-cancer activity. It is much higher than many other oils (Fu et al, 1993). Vitamin K (59-64 mg/100g in pulp oil and 110-230mg/100g in seed oil plays a catalytic role in forming blood prothrombin.

The β-carotene, an important bioactive compound is present in sufficient amount (250mg/100g) in its fruit oil. Lycopene, another important carotenoids, which prevents vitamin A deficiency, makes it vital for human beings. A rich source of flavonoids (0.2% in juice and 0.6% in dry fruits), eating its fruits helps cure high blood fat content, coronary heart problems and angina.
Leaves:

Polyphenolic substances of *H. rhamnoides*, including tannins, catechins and flavonoids, are accumulated preferably in green organs of the plants (leaves) where the photosynthetic process take place. A systematic chemical investigation of active fractions from the leaves of this plant have led to the discovery of a new phytochemical drug-Hiporamin, possessing a wide spectrum of antiviral activity. Hiporamin is a purified fraction of polyphenolic substances (Shipulina, 2001), the principal component of which is shown to be hydrolysable gallo- and ellagi-tannins of monomeric type: strictinin, isostrictinin, casuarinin, casuaricin. Some amounts of flavonoid glucosides and cyclic polyol quebrachitol [2-O-methyl-(-)-inositol]. $C_7H_{14}O_6$ are accompanied polyphenolic compounds in the plant. The polyphenolic compounds in the leaves are represented by catechins, flavonols and leucoanthocyanidins. Leaves are a rich source of flavonoids such as (-)epicatechin, (+)gallocatechin, (-)epigallocatechin and gallic acid. Leaf drugs, containing flavonoids, increase the wound healing after chemical burns and wounds (Sheichenko *et al*, 2001). The main constituents of hydrophilic part of the leaf extracts are monomeric type of hydrolysable tannins (gallo-ellagi-tannins) identified with earlier known strictinin (I), isostrictinin (II) and others.

Pharmacological activities of seabuckthorn

The most important pharmacological activities attributed to seabuckthorn oil or pulp oil includes: anti-inflammatory, antimicrobial, and promotion of tissue regeneration. Seabuckthorn oil is also recommended as a treatment for oral mucositis, cervical erosion, radiation damage, heat burns, scalds, duodenal ulcers caused by malnutrition, poorly healing wounds and other skin damage (Lebedava, 1989; Jian *et al*, 1989; Chen, 1991; Gupta *et
More than ten different drugs have been developed from seabuckthorn in Asia and Europe and are available in different forms such as liquids, powders, plasters, films, pastes, pills, ointments, suppositories and aerosols (Li, 1996). Seabuckthorn oil is also used as a dietary supplement to improve conditions of mucous membranes and as a natural source of carotenes, phytosterols and essential fatty acids. In addition to the medicinal use, the fruits of seabuckthorn can be processed to make juice and jam or to be used for flavoring dairy products because of the unique taste of seabuckthorn berries. Fruit pulp/peel oil contains a high level of palmitoleic acid (16:1n-7, up to 43%) (Zadernowski et al, 1997; Ul’chenko et al, 1995) which is very uncommon in the plant kingdom. The oil is attracting more and more attention because of the increasing interest in the physiological role of the monounsaturated fatty acids (Perz-Jimenez et al, 1999).

Recently, interest in seabuckthorn has also developed in Europe, Japan, Canada and the United States as more information on the chemical composition and physiological effects of the berries became available. Earlier studies also show that seabuckthorn has potent antioxidant activities. Eccleston et al, 2002 had showed that seabuckthorn juice supplementation decreased the susceptibility of LDL to oxidation. Seabuckthorn has also been documented to have radioprotective properties (Goel et al, 2002), protecting the mice from whole body lethal irradiation and provide protection against nicotine induced oxidative stress (Suleyman et al, 2002).

Value added Products:

Numerous products have been made from seabuckthorn including tea from leaves; beverages, jam and pickle from fruits; fermented products from pulp; and animal feeds from leaves, pulp.
and seed residues (Beveridge et al, 1999; Chauhan et al, 2001). The fruit pulp remaining after juice removal provides for extraction of "seabuckthorn yellow", a pigment that has potential use as a food coloring material (Chen et al, 1995; Liu et al, 1989). Defence Research and Development Organisation (DRDO), India has developed technologies for extracting juice from seabuckthorn and converting the same into a ready to serve health drink (beverage), sauce, jam as well as squash. The technology has already been transferred and the products are commercially available under the name Leh berry.

Seabuckthorn leaves are nutritive fodder for the cattle as they are a rich source of proteins (18-22%), fat (4-5%) and other micronutrients. The flavonoid content of leaves ranges from 310-2100mg/100g of air-dried leaves. Numerous products are made from the leaves such as leaf extract, tea, tea powder and animal feed (Singh et al, 2001). Apart from this, 18 tonnes of fuel wood are produced from a hectare of forest.

Although many clinical benefits of seabuckthorn have been claimed, systematic evidence based investigations have not been carried out and there is paucity of information as cytoprotective, antioxidant and immunomodulatory activity of seabuckthorn growing at high altitude in cold desert region. Also, the research on seabuckthorn is mainly in countries like Russia, China and Germany, which are largely unavailable to western readers. Since, seabuckthorn is considered to be a rich source of various antioxidants, the present study was undertaken to evaluate the antioxidant and cytoprotective activity of seabuckthorn in-vitro and in vivo using immune cells as a model system.
**Immune cells as model:**

The immune system is a remarkably adaptive defense system that has evolved in vertebrates to protect them from invading pathogenic microorganisms and cancer. It is able to generate an enormous variety of cells and molecules capable of specifically recognizing and eliminating an apparently limitless variety of foreign invaders.

Generation of an effective immune response involves two major groups of cells: lymphocytes and macrophages, which are the first line of defence against invading organisms. These cells are sensitive to changes in oxidant-antioxidant balance because of higher percentage of polyunsaturated fatty acid (PUFA) in their membrane. Immune cells are also exposed to changes in this balance because of the high number of reactive oxygen species (ROS) produced as part of their normal physiological function. Membrane related signaling and gene expression, which are sensitive to oxidative stress, are critical in maintaining normal function of immune cells and their ability to defend against the wide range of foreign antigens they are exposed to. These cells offer an excellent model system to study the antioxidant and immunomodulatory activity of herbs (Sai Ram *et al*, 2003).

Therefore, the present study was undertaken to evaluate the antioxidant and immunomodulatory properties of seabuckthorn using immune cells (*in-vitro*) and male albino rats (*in-vivo*) under normal and immunosuppressive conditions like chromium-induced cytotoxicity and multiple environment stress.