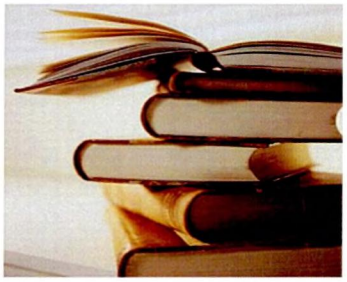

Review of Literature

2. REVIEW OF LITERATURE

Oxygen is very essential for aerobic organisms. During respiration, mammalian cells produce energy by reducing molecular oxygen to water. As a natural by-product of normal metabolism, reactive oxygen species (ROS) play a regulatory part in cellular function (Lubos *et al.*, 2009).

For organisms living in an aerobic environment, exposure to ROS is unavoidable. ROS encompass a variety of partially reduced metabolites of oxygen, possessing higher reactivity than molecular oxygen (Yuan *et al.*, 2009).

The deleterious effects of oxidative stress can be counteracted by the presence of molecules called antioxidants. Antioxidants derived from plants have received a great attention. But plant-derived medicines are not a new concept to India, where around 20,000 medicinal plants have been recorded. The medicinal plants are listed in various indigenous systems such as Ayurveda, Siddha, Amchi and Unani. The Ayurvedic concept appeared and developed between 2500 and 500 BC in India as a home medicine using natural plant extracts. The Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments (Deocaris *et al.*, 2008; Perumalsamy *et al.*, 2008).

FREE RADICALS

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbits. To this group belong reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical and hydrogen peroxide, as well as reactive nitrogen species (RNS), which include nitric oxide and peroxynitrite. Although structurally

different, free radicals share similar mechanisms to harm body's cells and tissues through damage on lipids, DNA and proteins (Pauwels *et al.*, 2007).

SOURCES OF FREE RADICALS

Free radicals are generally produced endogenously or also derived exogenously. Free radicals, like reactive oxygen, nitrogen and chlorine species, are normal by-products of metabolism, which is considered as the endogenous source for free radicals. Exogenously, these free radicals are introduced into the body from sources like harmful chemicals in the environment, unhealthy foods, stress, certain drugs and cigarette smoke (Ranjbar *et al.*, 2006).

REACTIVE OXYGEN SPECIES

ROS might function as dual effectors, modulating both pro-survival and anti-survival signals. ROS are unstable molecules, ions or radicals that are formed continuously as a consequence of both biochemical reactions, e.g., mitochondrial respiration, and external factors. ROS are involved in various normal cellular processes like gene expression, proliferation and differentiation. The overproduction of ROS may lead to damage of biomolecules like lipids, DNA and proteins. This, in turn, can induce cell cycle arrest and premature senescence, as well as activation of pathways leading to cell death (Fialkow *et al.*, 2007; Lukandu *et al.*, 2008; Ruder *et al.*, 2008).

ROS are responsible for oxidative stress-related diseases. In addition to being implicated in diseases and ageing, ROS are major culprits of food deterioration, loss of colour, flavor and nutritive value (Sachindra *et al.*, 2010).

Superoxide radical ($O_2^{\bullet-}$)

Among the ROS, superoxide anion radical ($O_2^{\bullet-}$) is the key radical because the generation of many ROS is derived from $O_2^{\bullet-}$ (Tanaka *et al.*, 2010). Superoxide anion reacts with other molecules to generate secondary ROS, either through enzyme- or metal-catalyzed processes. Superoxide anion radicals are constantly produced in the oxidation metabolic process of organisms, e.g., in the mitochandria by complexes I and II of the electron transport chain. They can attack biological molecules in living cells, leading to the onset of various diseases such as cancer, cardiovascular disorders and diabetes, and can lead to immune system decline (Afonso *et al.*, 2007; Ali *et al.*, 2008b; Ma *et al.*, 2010).

Hydroxyl radical ($^{\bullet}OH$)

The hydroxyl radical ($^{\bullet}OH$) has high reactivity, making it a very toxic radical with a short half-life. $^{\bullet}OH$ is mostly produced through Fenton reaction, which requires Fe^{2+} (Komagoe *et al.*, 2008; Singh *et al.*, 2008), as shown below.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$$

$$RH + {}^{\bullet}OH \rightarrow R^{\bullet} + H_2O$$

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$$

The hydroxyl radical is an extremely reactive oxidant. It is recognized as the most reactive of the reactive oxygen species. Cancer, arthritis and Parkinson's disease are a few of the ailments that are linked to hydroxyl radical. Despite its role in disease, $^{\bullet}OH$ is also a vital part of the body's natural defense mechanisms (Mitroka *et al.*, 2009).

Hydrogen peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) is a non-radical ROS produced from superoxide anions in several different cell types in the human body, including vascular endothelial and smooth muscle cells via enzymatic conversion by superoxide dismutase (Nacitarhan *et al.*, 2007). H_2O_2 is a versatile molecule that may be involved in several cell processes under normal and stress conditions (Quan *et al.*, 2008).

H_2O_2 is a small, diffusible molecule and has a longer lifespan than superoxide (Lee *et al.*, 2007a). H_2O_2 readily reacts with metal ion and generates more radicals like hydroxyl radicals, which are extremely harmful to all classes of biomolecules (Bystrova and Budanova, 2007). H_2O_2 also functions as a signaling molecule that mediates responses to various biotic and abiotic stimuli in both plant and animal cells (Li *et al.*, 2009a).

Singlet oxygen ($^1O_2$)

Singlet oxygen is a highly reactive molecule that is potentially damaging to biological systems. It will rapidly oxidize molecules containing carbon-carbon double bonds to form hydroperoxides or endoperoxides. In proteins, singlet oxygen will specifically oxidize cysteine, histidine, methionine and tryptophan residues. Interaction of singlet oxygen with lipids will lead to lipid peroxidation (Flors *et al.*, 2006).

REACTIVE NITROGEN SPECIES

Overproduction of reactive nitrogen species is called nitrosative stress. This may occur when the generation of reactive nitrogen species in a system exceeds the ability of the system to neutralise and eliminate them. Nitrosative

stress may lead to nitrosylation reactions that can alter the structure of proteins and so inhibit their normal function (Valko *et al.*, 2007).

Nitric oxide (NO)

Nitric oxide (NO) is a diatomic free-radical produced from L-arginine by constitutive and inducible nitric oxide synthases in numerous mammalian cells and tissues (Liao *et al.*, 2007). NO is the gaseous short-lived free pleiotropic signaling molecule involved in a large variety of physiological processes including bone cell functions, and initiates diverse cellular signaling cascades (Ozgocmen *et al.*, 2007; Huang *et al.*, 2009). NO reacts readily with oxygen to form nitrogen dioxide and is harmful to human health (Skalska *et al.*, 2010).

Peroxyl radical ($ONOO^-$)

Peroxynitrite, a potent electrophile, is generated from the bi-radical reaction of nitric oxide and superoxide at a diffusion-limited rate. $ONOO^-$ plays an important role in many pathological processes, chronic diseases and ageing, due to their involvement in the oxidative degradation of DNA and proteins and in the autooxidation of lipids within cell membranes. These species are also key intermediates in thermal and photochemical degradation of both natural and man-made materials (Jia *et al.*, 2009a; Chen *et al.*, 2010a).

HYPOCHLOROUS ACID

Hypochlorous acid ($HClO^-$) is a non-specific oxidizing and chlorinating agent that reacts rapidly with a variety of biological compounds, such as sulphydryls, polyunsaturated fatty acids, DNA, pyridine nucleotides, aliphatic and aromatic amino acids and nitrogen-containing compounds (Messner *et al.*, 2006; Brambilla *et al.*, 2008).

OXIDATIVE STRESS

Oxidative stress occurs due to the imbalance between the production and the elimination of a variety of oxygen species like superoxide, hydroxyl, alkoxyl radical and hydrogen peroxide. These ROS have the ability to degrade macromolecules such as lipids, nucleic acids, proteins and pigments, finally leading to cell death (Karuppanapandian *et al.*, 2008). However, some ROS, such as the superoxide radical and H_2O_2, at low concentrations, fulfill important roles in stress perception, photosynthesis regulation, pathogen recognition, programmed cell death and plant development (Matamoros *et al.*, 2010). The ROS-mediated cellular injury can be overcome by enhancing the endogenous defense capacity against oxidative stress through dietary or medicinal intake of antioxidants (Zhang *et al.*, 2009).

MOLECULAR DAMAGE INDUCED BY FREE RADICALS

In many cells, ROS cause DNA damage, peroxidation of lipids, oxidation of proteins and thus induce apoptosis. Growing research has given evidence that the deleterious effects of ROS are responsible for various chronic diseases. Therefore, therapeutic strategies aimed at preventing or delaying ROS production might be a reasonable choice for the treatment of these diseases (Lin *et al.*, 2009).

Oxidative damage to lipids

Lipids are highly susceptible to free radical attack. ROS cause peroxidation of polyunsaturated fatty acids in the membranes (Kim *et al.*, 2005). When reactive oxygen species attack polyunsaturated fatty acids on the cell membrane of living organisms in the presence of molecular oxygen, a

chemical cascade is triggered. This eventually leads to the disintegration of fatty acids and the formation of hydrocarbon gases (e.g., pentane) and aldehydes like malondialdehyde (MDA). This process is called as lipid peroxidation (Cemek *et al.*, 2006). MDA, the end product of lipid peroxidation, has also been demonstrated to be a mutagenic and genotoxic agent that can contribute to the development of human cancers (Ajith, 2010).

Oxidative damage to DNA

Normal cellular metabolism is well established as the source of endogenous ROS. Exogenously-derived components may also follow the cell metabolism, which leads to the generation of ROS. Irrespective of their origin, their increased production plays a central role in numerous pathologies, such as cancer, neurological disorders and ageing. An excess of ROS is toxic and damages cell components, including nucleic acids, proteins and lipids. In addition, they enhance apoptosis or necrosis. Moreover, ROS cause DNA damage, specifically DNA double strand breaks that are considered the most significant nuclear lesion (Pizarro *et al.*, 2009). The mitochondrial DNA is also highly susceptible to ROS compared to nuclear DNA and consequently is more prone to oxidative injury than the nuclear DNA (Cao *et al.*, 2006).

Of the ROS, $^{\bullet}OH$ attacks DNA, generating multiple mutagenic purine, pyrimidine and deoxyribose oxidation products. Hydroxyl radical, the extremely reactive free radical, could be involved in all the stages of radiation-induced carcinogenesis. Reactive chlorine, bromine, sulfur and nitrogen species can also attack DNA. RNS have been suggested to be especially important in causing DNA damage (Halliwell, 2007).

Oxidative damage to proteins

Proteins are considered to be one of the major targets of oxidative stress. Oxidative stress-induced modifications of proteins include carbonylation of various amino acids, tyrosine nitration and oxidation of cysteine residues. Protein oxidation is a complex process. It involves many different amino acids, and a variety of mechanisms, both reversible and irreversible. Some modifications may be considered reversible and beneficial against attack caused by ROS. Some modifications such as carbonylation are irreversible and may thus be destructive, resulting in alterations in the structure and function of proteins. Protein carbonylation is being implicated in the pathogenesis and progression of a variety of diseases (Korolainen *et al.*, 2007; Umstead *et al.*, 2009).

DISEASES ASSOCIATED WITH OXIDATIVE STRESS

The oxidative stress caused by ROS is significantly associated with ageing and numerous age-related pathologies (Roginsky *et al.*, 2009). For more than two decades, the free radical-mediated peroxidation of membrane lipids and oxidative damage of DNA have been thought to be associated with a variety of health problems, such as cancer, atherosclerosis, diabetes, stroke, neurodegenerative diseases and ageing (Shia *et al.*, 2009). Antioxidants derived from natural origin have attracted special interest because they can protect the human body from free radical mediated diseases (Sunilson *et al.*, 2008).

Cancer

Cancer is the second most life threatening disease in the world. Unifying property of cancer includes six canonical characteristics: self sufficiency in

growth signals, insensitivity to growth inhibitory signals (anti-growth), evasion of programmed cell death (apoptosis), unlimited proliferation of diseased cells, sustained angiogenesis, intrusion of adjacent cells and tissues and metastasis to distant niches in the body (Singh *et al.*, 2010). Cancer is affected by alterations in multiple physiological events including apoptosis, inflammation, differentiation and angiogenesis. Oxidative stress, resulting from the imbalance between antioxidants and pro-oxidants, has been recognized to play an important role in cancer development (Liu *et al.*, 2009a).

Approximately 35% of humans have cancer by age 85. This is due to life time attack by reactive species, especially $^{\bullet}OH$, which are generated endogenously or derived exogenously. DNA damage caused by reactive oxygen/nitrogen species is considered as the major cause for cancer development. Reactive species at higher level might promote carcinogenesis and contribute to inflammation (Halliwell, 2006). Reactive oxygen and nitrogen species can promote mutation in DNA, cause DNA damage and inhibit caspases, which leads to delaying apoptosis, the most important mechanism to kill cancer cells (Sawa and Ohshima, 2006). A research revealed that knockout mice lacking Cu/Zn-SOD have increased rates of liver cancer development later in life (Elchuri *et al.*, 2005), indicating the importance of antioxidants in cancer prevention.

Cardiovascular disease

Cardiac risk factors and cardiovascular disease impair endothelial function, oxidative stress being the major cause (Förstermann, 2010). Hypertensive vascular disease is a major risk factor for a variety of cardiovascular diseases including stroke, myocardial infarction, congestive

heart failure and renal microvascular disease. An increase in reactive oxygen species during hypertension plays a pivotal role in this process. In hypertension and other vascular diseases, there is an imbalance in oxidant-generation vs. oxidant-catalyzing systems, leading to the generation of endogenous superoxide anion and hydrogen peroxide (Haurani and Pagano, 2007).

Diabetes mellitus

One of the onsets of diabetic complications is a diabetes-induced increase in oxidative stress in the absence of the counterbalancing effects of endogenous antioxidants. Reactive oxygen species (superoxide) generated by high glucose concentrations are also considered a causal link between hyperglycaemia and other metabolic abnormalities in the development of many complications of diabetes mellitus (Mariappan *et al.*, 2010). Metal ions, act as the co-factors for antioxidant enzymes; metabolism of trace elements may be impaired in diabetic patients (Yildirim *et al.*, 2009).

Retina, a tissue rich in polyunsaturated fatty acids with high glucose oxidation and oxygen uptake, experiences increased oxidative stress in diabetes. Increased oxidative stress in diabetes is postulated to play a role in retinal basement thickening, and capillary cell apoptosis. Thus, mitochondrial oxidative stress is one of the major contributors in the development of diabetic retinopathy (Madsen-Bouterse and Kowluru, 2008). The oxidative stress in the pathogenesis of diabetic nephropathy was ameliorated by supplementation with vitamin E, taurine or lipoic acid (Lee *et al.*, 2010).

Neurodegenerative disorders

The brain is particularly vulnerable to oxidative damage because it is rich in unsaturated fatty acids, but relatively poor in antioxidant defenses, with a low content of antioxidant enzymes. In addition, iron, which promotes cytotoxic radical formation, is accumulated in specific brain regions. Excessive production of hydroxyl radicals can cause neuronal apoptotic nerve death, glutathione depletion, and release of excitatory amino acids (Kim *et al.*, 2008; Yang *et al.*, 2010).

Alzheimer's disease

Alzheimer's disease (AD) is a gradual and irreversible progressive neurodegenerative disorder that results in dementia and death and appears in old age. The products of oxidative and nitrosative stress accumulate with ageing and alteration in the expression of antioxidant systems lends support to a role for free radical damage in AD pathology (Zawia *et al.*, 2009).

The brain of Alzheimer's disease patients is affected by inordinate oxidative stress. This may be due to an increased superoxide dismutase and/or decreased glutathione peroxidase and catalase activities, leading to elevation of H_2O_2 concentration in AD. The generated H_2O_2 is used for hydroxyl radical production via Fenton and Haber-Weiss reactions (Vaisi-Raygani *et al.*, 2007).

Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative disorder, caused by reduced levels of catecholamines. Oxidative stress is one of the contributors in the loss of dopaminergic neurons in PD. In addition, oxidative stress leads to accumulation of unwanted proteins. Reduced glutathione (GSH) and

glutathione peroxidase were found to be decreased in PD. Antioxidant-containing therapies have been shown to be effective in the animal models with PD (Rajasankar *et al.*, 2009; Surendran and Rajasankar, 2010).

Rheumatoid arthritis

Rheumatoid arthritis (RA) is the most common chronic inflammatory arthropathy and it afflicts people of all ages and races. Its prevalence among adults is approximately 1%, with women being at least twice more likely to develop the disease than men. RA is an autoimmune disease involving numerous cells of the immune system with overexpression of inflammatory cytokines like tumor necrosis factor alpha (TNF-α), certain interleukins, proteinases and multiple other chemokines (Tayar and Suarez-Almazor, 2010).

Reactive oxygen species like superoxide and hydrogen peroxide have been implicated as mediators of tissue damage in rheumatoid arthritis. Free radical oxidation products have been identified in the synovial fluid of rheumatoid arthritis patients, which leads to impaired joint function, severe pain and reduced life expectancy. The inflammatory infiltrate that develops is the result of aberrancy in both T cell and macrophage function. In RA, inflammation and bone destruction may occur uncoupled. Therefore, therapeutic agents developed for anti-inflammatory and immunosuppressant activity will be useful and indispensable for RA therapy (Chang *et al.*, 2007; Murphy *et al.*, 2008).

Ageing

Ageing is thought to be a stochastic process combining predictable and random effects that lead to the accumulation of unrepaired cellular damage,

weakening cellular repair and compensatory mechanisms. Much of the individual variation in ageing is accounted for by lifestyle and the effects of the environment, with genes accounting for only 25% of variability (Hindle, 2010). The imbalance between pro-oxidants and antioxidants leads to an accumulation of oxidative damage in a variety of macromolecules with age, resulting in a progressive loss in functional cellular processes, leading to the ageing phenotype (Pérez *et al.*, 2009).

ANTIOXIDANTS

In recent years, there is a tremendous interest in the possible role of nutrition in the prevention of diseases. In this context, antioxidants derived from natural sources such as medicinal plants and herbs require special attention. Antioxidants have many potential applications, especially in relation to human health, both in terms of prevention of disease and therapy (Vaidya and Devasagayam, 2007).

The antioxidative system includes antioxidant compounds such as carotenoids, ascorbate, glutathione and α-tocopherol, and several enzymes involved in the detoxification of ROS. These enzymes include superoxide dismutase, peroxidase, catalase, ascorbate peroxidase and gluthatione reductase (Hernandez *et al.*, 2010).

Superoxide dismutase

Superoxide dismutases (SODs) constitute a primary source of cellular defenses against ROS damages by detoxifying superoxide ions into molecular oxygen and hydrogen peroxide (Xie *et al.*, 2010). SODs are classified according to their metal cofactor and their subcellular localization. The predominant forms

of SOD are the mitochondrial Mn-SOD, the cytosolic Cu/Zn-SOD and the chloroplastic Cu/Zn- and Fe-SODs (Wang *et al.*, 2010b).

Mn-SODs are ubiquitous metalloenzymes responsible for converting superoxide to hydrogen peroxide in the mitochondrial matrix (McAtee and Yager, 2010). Cu/Zn-SOD (SOD1) is an abundant and highly conserved enzyme found in the cytoplasm, nucleus and mitochondrial intermembrane space (Fischer and Glass, 2010).

Literature provides evidence that SOD can protect cells against the harmful effects of oxidative stress, and has clinical application in the treatment of several diseases in which the superoxide radical is involved, such as treatment of rheumatoid arthritis, ageing, cancer and respiratory distress syndrome (Chen *et al.*, 2010b).

Catalase

Catalase is a heme-protein belonging to the class of oxidoreductases with ferriprotoporphyrin-IX at the redox center. It catalyses the disproportionation of hydrogen peroxide into oxygen and water without the formation of free radicals. In addition, catalase is a redox enzyme that is present in all aerobic organisms (Salimi *et al.*, 2007).

Exposure to oxidative stress results in the upregulation of the enzymatic activity of the antioxidant enzymes such as glutathione peroxidase, catalase and the superoxide scavenger superoxide dismutase. Catalase is responsible for the degradation of H_2O_2 encountered from exogenous sources or produced intracellularly as a result of cellular metabolism. The two enzymes, glutathione

peroxidase and catalase are the two principal scavengers of H_2O_2 (Sen *et al.*, 2005).

Peroxidase

Peroxidases are a very large group of enzymes that reduce peroxide and oxidize a wide variety of substrates, such as lignin subunits, lipid membranes, and some amino acid side chains (Passardi *et al.*, 2007). The main function of peroxidase is to reduce H_2O_2 to water, with ascorbate as the electron donor (Karuppanapandian *et al.*, 2006). Glutathione peroxidase catalyses the reduction of hydroperoxides at the expense of GSH. In this process, hydrogen peroxide is reduced to water, whereas organic hydroperoxides are reduced to alcohols (Seufi *et al.*, 2009).

Glutathione reductase

Glutathione peroxidase acts as a second line of defense against ROS toxicity. In the GPx reaction, glutathione is oxidized to glutathione disulfide, which can be converted back to the reduced form of glutathione (GSH) by glutathione reductase (GR) via an NADPH consuming process. Glutathione depletion results in a reduced GPx reaction and an increased cell vulnerability to ROS (Bausenwein *et al.*, 2010).

Glutathione S-transferase

Glutathione S-transferases (GST) are a superfamily consisting of a large multigenic group of detoxifying enzymes (isoenzymes α, θ, μ and π), catalyzing the conjugation of a broad variety of electrophilic and hydrophobic (toxic and mutagenic) compounds with glutathione. The resulting GSH conjugates are

usually more water soluble and often less toxic than the parent compounds (Michałowicz *et al.*, 2010; Özcan *et al.*, 2010; Saadat *et al.*, 2010).

The glutathione S-transferase M1 (GST M1) is found in detoxifying smoke-derived carcinogens such as polycyclic aromatic hydrocarbons and aromatic amines. GST T1 has a higher tendency to detoxify minor substrates such as methyl chloride and methyliodide and GST P1 is involved in conjugation and detoxification of a wide range of xenobiotics (Zeng *et al.*, 2010).

Vitamin C

Ascorbic acid (vitamin C) is a water-soluble vitamin present in vegetables and fruits. It acts as an important biological antioxidant. L-ascorbic acid is present in body fluids and in cerebral fluids. Vitamin C has various physiological and pharmacological functions such as collagen synthesis, intestinal absorption of iron, drug metabolism, immune response, wound healing process and has been used for the prevention and treatment of common cold, mental illness, infertility, cancer and AIDS (Li and Lin, 2006; Thangamuthu *et al.*, 2007; Zare and Nasirizadeh, 2010).

Vitamin A and carotenoids

Vitamin A, a fat-soluble vitamin, has three active forms: retinol, retinal and retinoic acid; these are collectively called retinoids. Dietary sources of vitamin A are provided either by retinol esters, which are present in foods of animal origin or by plant carotenoids. The most important of these is the vitamin A precursor, β-carotene. Vitamin A and carotenoids share some protective mechanisms, such as scavenging of genotoxic ROS, modulation of

signal transduction pathways, inhibition of cell transformation induced by physical and chemical agents and facilitation of intracellular communication inhibited by genotoxic compounds, and have a high antioxidant activity. The carotenoids exert their antioxidant effect by quenching free radical reactions, particularly those involving singlet oxygen; this prevents damaging chain reactions that cause lipid peroxidation (Sethi *et al.*, 2009; Roehrs *et al.*, 2010).

Lycopene is a lipophilic carotenoid pigment with eleven conjugated double bonds and two unconjugated double bonds. The most potent antioxidant activity of lycopene is due to this high degree of conjugated double bonds in the molecule. The singlet oxygen quenching ability of lycopene is two fold higher than β-carotene and ten times higher than that of α-tocopherol (Kavanaugh *et al.*, 2007; Lavecchia and Zuorro, 2008).

Overwhelming evidence from epidemiological studies have indicated that lycopene exerts an antiproliferative effect on cancer cells *in vitro* and xenograft mice. In addition to its antioxidant activities, lycopene also induces apoptosis in cancer cells and significantly inhibits DNA synthesis in primary human epithelial cells. Both epidemiological and case-control studies have suggested that lycopene reduces the risk of prostate cancer (Liu *et al.*, 2008).

Vitamin E

Vitamin E (α-tocopherol) is a group of lipid-soluble, chain-breaking vitamin known to be one of the most potent endogenous antioxidants that prevents the propagation of free radical reaction (Roginsky and Lissi, 2005). Vitamin E deficiency leads to severe degenerative diseases such as ataxia, infertility, and Duchenne-like muscle degeneration. Among all vitamin E derivatives, α-tocopherol is known to be the most abundant and the most

powerful active form of vitamin E *in vivo* (Tsuzuki *et al.*, 2007). Vitamin E functions biologically as a scavenger of different free radicals by working as an antioxidant. As a consequence of its fat-solubility, vitamin E exerts its antioxidant activity in cell membranes, which predominately contain lipids (Isaac *et al.*, 2008). Vitamin C allows α-tocopherol regeneration from α-tocopheroxy radical, thus preventing vitamin E prooxidant activity and tocopherol-mediated peroxidation (Rodríguez *et al.*, 2005). To maintain vitamin E in its active form, it is rapidly regenerated by vitamin C (Blokhina *et al.*, 2003).

Reduced glutathione

Enzymes of the glutathione-dependent antioxidant system, glutathione peroxidase, glutathione reductase and glutathione transferase, play an important role in cell protection from overproduction of reactive oxygen species, including superoxide radical and its derivatives (Goncharova *et al.*, 2007). Reduced glutathione (GSH), the most abundant low molecular weight thiol compound, has unique structural properties and a broad redox potential. GSH protects against a range of peroxides, xenobiotics and heavy metals. Among intracellular antioxidant molecules, GSH is the most important, abundant low molecular weight thiol compound in the cells, thus protecting them from toxic oxygen products. The cellular GSH status has been found to be important in modulating apoptosis in many cell types. Also, depleting GSH by blocking its synthesis induces apoptosis directly in some cell types and sensitizes other cell types to apoptotic stimuli (Ranawat and Bansal 2008).

Alkaloids

Alkaloids are naturally occurring secondary metabolites, low molecular weight, and nitrogen containing compounds that are found in more than 20 per cent of plant species. They show pharmacological effects and are being commonly used as medicines. Alkaloids are biologically significant and can act as stimulators, inhibitors and growth terminators. They also have anti-microbial and anti-parasitic and anticancer properties (Singla *et al.*, 2010; Inui *et al.*, 2007).

Some reports indicate that β-carbolines, harmane, harmaline and harmalol alkaloids have effective antioxidant properties by inhibiting lipid peroxidation. β-carboline alkaloids have a significant antioxidative effect in yeast. The antimutagenic and antigenotoxic effects of alkaloids are suggested to be due to their hydroxyl radical scavenging property (Henriques and Saffi, 2007).

Phenolics

Phenolic compounds can suppress free radical-induced oxidative stress and reduce the onset of chronic illnesses. The consumption of polyphenol-rich foods and beverages is associated with reduced risk of cardiovascular diseases, stroke and certain forms of cancer. Phenolic compounds are important plant constituents because of their scavenging ability, primarily due to their hydroxyl groups. They are found to have excellent antioxidant activity in the inhibition of low-density lipoprotein (LDL) oxidation (Reddivari *et al.*, 2007; Vidović *et al.*, 2010).

Flavonoids

Dietary flavonoids, polyphenolic phytochemicals, are commonly found in a wide variety of plants, fruits, vegetables and medicinal herbs (Auyeung and Ko, 2010). Flavonoids consist of two benzene rings, with a three-carbon connecting group, and are derived from flavone. The subdivision is based on additional oxygen-containing heterocyclic rings and oxygenated functional groups, into chalcones, flavones, flavonols, flavanones, anthocyanins and isoflavonoids (Cassidy and Setzer, 2010).

Several polyphenols have been demonstrated to have clear antioxidant properties *in vitro*, as they can act as chain breakers or radical scavengers depending on their chemical structure. They are also associated with a reduced incidence of age-related illnesses, neurodegenerative diseases (Faria *et al.*, 2010), cardiovascular diseases, anti-inflammatory and antimicrobial activities, and they also possesses anticancer and antitumirogenic properties and proapoptotic activities (Wesołowska *et al.*, 2009).

Anthocyanins

Anthocyanins comprise aglycones and their glycosides. They form a highly differentiated group of compounds. Anthocyanins differ with regard to the number of hydroxyl groups in a molecule, the degree of methylation of these groups, the type, number and place of attachment of sugar molecules, and the type and number of aliphatic or aromatic acids attached to sugars in an anthocyanin molecule (Szajdek and Borowsk, 2008). Anthocyanins are a source of naturally occurring colourants of foods. Studies suggest that anthocyanins have antioxidant activity to scavenge active oxygen radical. In addition to

antioxidant activity, they have been reported to prevent carcinogenesis, improve visual functions and inhibit platelet aggregation (Kano *et al.*, 2005).

Silymarin

Silymarin is a standardized extract obtained from the seeds of *Silybum marianum* (L.) containing silymarin flavonolignans and polymeric and oxidized polyphenolic fractions. The main component of the silymarin complex is silybin. Silymarin/silybin is known to provide a wide range of hepatoprotective effects, especially against diseases like hepatitis, cirrhosis and jaundice. They also act as chemopreventive and cancer protective agents. Silymarin/silybin inhibits the carcinogenic action of many chemicals (Křen and Walterova, 2005; Angeli *et al.*, 2009).

PROGRAMMED CELL DEATH

In animals, as in plants, cell death is an essential process during development and in response to many stresses. The term programmed cell death (PCD) defines any form of cell death involving a series of orderly processes mediated by intracellular death programs, regardless of the triggers or the hallmarks it exhibits. PCD is a tightly regulated process for ensuring the proper development. It can also occur in response to environmental stresses and is often a part of the defense mechanism against pathogen attack via the hypersensitivity response (Zhang and Xing, 2008; Doyle *et al.*, 2010).

APOPTOSIS

Apoptosis is an evolutionarily conserved, multi-step cell death process that occurs in a variety of physiological situations. An apoptotic stimulus induces an initiation, commitment and a degradation phase. The type I

'extrinsic' or death receptor pathway and type II 'intrinsic' pathway, are the two pathways that mediate apoptosis (Smith *et al.*, 2010). Both these pathways induce the activation of caspase 3, which ends up with apoptosis. The events associated with apoptosis include the cleavage of cell proteins, plasma membrane blebbing, cell rounding and shrinkage, chromatin condensation, nuclear fragmentation and apoptotic body formation (Yamanouchi *et al.*, 2010).

Accordingly, caspases are responsible for many of the biochemical and morphological hallmarks of apoptotic cell death by cleaving a range of substrates in the cytoplasm and nucleus (Fulda, 2010). During apoptosis, the integrity of the cell membrane is maintained, thereby preventing the release of histotoxic cell contents (Shaw *et al.*, 2009).

ALTERNATIVES TO *in vivo* MODELS

Alternative models for biomedical research seek to address refinement, reduction and/or replacement of existing animal models. In this focus, recent years have seen the advent of non-mammalian models for drug discovery and development. The most commonly used models are yeast, cell line, primary culture, *Drosophila*, *Caenorhabditis elegans* and Zebrafish (Bjornsti, 2003; Tweats *et al.*, 2007). In addition to that, several *in vitro* human liver models such as perfused liver, liver slices, primary hepatocytes, cytosol, S9 fractions, supersomes, cell lines, transgenic cell lines and microsomes have been developed during the past few decades. These *in vitro* model systems represent an effective approach to estimate the human drug metabolic fates *in vivo* (Brandon *et al.*, 2003; Li, 2004).

Precision-cut liver slices

Liver is the most important and large organ of the human body, rich in heme-containing enzymes, cytochromes P450, which play a major role in phase I oxidation reactions (Asha and Vidyavathi, 2010). Precision-cut liver slice is one of the powerful *in vitro* tools to study the metabolic processes and events related to oxidative stress. This is due to the fact that the precision cut-liver slice model preserves the normal lobular architecture, allows the maintenance of cell–cell interactions within their original extracellular matrix as well as being rapid and simple in preparation (Guyot *et al.*, 2007). Thus, this *in vitro* tool functions in a relatively similar way to the situation *in vivo* (Glöckner *et al.*, 2008).

Saccharomyces cereviciae

The yeast *Saccharomyces cerevisiae* represents a eukaryotic system highly suitable for testing the activity of antioxidants. It is a standard object of genetic manipulations and expression of proteins and, unlike with animals, work with it does not involve ethical issues (Krasowska and Sigler, 2007). Studies in yeast have revealed the fundamental cellular processes, including cell cycle control, neurodegenerative disorders, apoptosis, longevity and ageing (Kohlwein and Petschnigg, 2007). The yeast genome codes for many proteins of the basic molecular machinery executing cell death, including orthologues of caspases, apoptosis inducing factor, HtrA2/Omi and inhibitor of apoptosis proteins (Eisenberg *et al.*, 2007).

Chorioallantoic membrane of chick embryo

Now-a-days fertilized chicken eggs have been used as an alternative to animal experiments. Chorioallantoic membrane surrounds the embryo during

embryo development. This membrane forms an excellent substrate for the seeding of cultured cells or the transplantation of xenogeneic tissue. Many investigations have shown that the results obtained with this model correlated both with *in vitro* and *in vivo* results. Since the model is inexpensive and can be handled relatively easy, it is well suited for screening procedures (http://www.medcare-techarea.com/magazin/umfeld/archiv_2009/index.html?lang=en&artikelid=artikel/04218/index.html).

Zebrafish

In the past decade, the zebrafish (*Danio rerio*) has also become a popular model system for the study of vertebrate development. The embryos and larvae of this species are small, transparent and undergo rapid development ex-utero which allows *in vitro* analysis of embryogenesis and organogenesis. Zebrafish is used as a model system to study human diseases and as a tool to study disease modeling and drug discovery (Fleming, 2007).

MEDICINAL PLANTS

The free radicals are known to be scavenged by synthetic antioxidants, but due to their adverse side effects leading to carcinogenicity, search for effective and natural antioxidants has become crucial (Choi *et al.*, 2007; Adeolu *et al.*, 2009). Several epidemiological studies suggest that plants rich in antioxidants play a protective role in health and against diseases, and their consumption lowered the risk of cancer, heart disease, hypertension and stroke (Muanda *et al.*, 2009).

Utilization of plants for medicinal purposes has been documented long back in ancient literature because they are essential to human survival. The

DiTommaso, 2002). *Artemisia vulgaris* has not only been used as an edible plant (spice) but also as a folk medicine resource (Judzentiene and Buzelyte, 2006).

In spite of such studies being reported, there are no studies that have concentrated on the type of antioxidant responses evoked by the leaf extracts of *Artemisia vulgaris* on oxidant-stress imposed events at a molecular level. The present study is an elaborate probe into the antioxidant, anti-apoptotic and anticancer activities of *Artemisia vulgaris* leaves and their effects on cellular biomolecules.

The layout of the study, the materials used and the methodology adopted are explained, with appropriate references quoted, in the next chapter.