Review of Literature

2. REVIEW OF LITERATURE

Oxygen is a prerequisite to life, but at concentrations beyond the physiological limits, it may be hazardous to the cells. Living creatures therefore have a tightly regulated system to deliver the necessary amount of oxygen to specific tissues at the right time (Hamasaki, 2006). Oxygen also forms potentially harmful reactive oxygen species.

The presence of the free radicals in biological systems represents an evolutionary pleiotropic effect. In the process of producing required energy in mitochondria (ATP), toxic ROS/RNS are also produced through the oxygen's being utilized (Wei and Lee, 2002).

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons. The presence of unpaired electrons usually confers a considerable degree of reactivity upon a free radical. The radicals derived from oxygen represent the most important class of such species generated in living systems (Valko *et al.*, 2004). The harmful effect of free radicals causing potential damage is termed oxidative stress and nitrosative stress (Ridnour *et al.*, 2005).

Oxidative stress results from the metabolic reactions that use oxygen and represents a disturbance in the equilibrium status of pro-oxidant/antioxidant reactions in living organisms. The delicate balance between beneficial and harmful effects of free radicals is a very important aspect of living organisms and is achieved by mechanisms called "redox regulation". The process of "redox regulation" protects living organisms from various oxidative stresses and maintains "redox homeostasis" by controlling the redox status *in vivo* (Droge, 2002).

TYPES OF FREE RADICALS

Reactive species can be classified into two groups namely radical and non-radical reactive species. The oxygen free radicals include superoxide, hydroxyl, peroxyl ($RO_2^{\bullet}$), alkoxyl ($RO^{\bullet}$) and hydroperoxyl ($HO_2^{\bullet}$) radicals. Nitric oxide and nitrogen dioxide ($^{\bullet}NO_2$) are two nitrogen free radicals. Oxygen and nitrogen free radicals can be converted to other non-radical reactive species, such as hydrogen peroxide, hypochlorous acid (HOCl), hypobromous acid (HOBr) and peroxynitrite ($ONOO^-$). ROS, RNS and reactive chlorine species are produced in animals and humans under physiologic and pathologic conditions (Evans and Halliwell, 2001).

SUPEROXIDE RADICAL ($O_2^{\bullet-}$)

Superoxide anion arising either through metabolic processes or following oxygen "activation" by physical irradiation, is considered the "primary" ROS and can further interact with other molecules to generate "secondary" ROS, either directly or prevalently through enzyme- or metal-catalysed processes (Valko *et al.*, 2005).

The mitochondrial electron transport chain is the main source of ATP in the mammalian cell and thus is essential for life. During energy transduction, a small number of electrons "leak" to oxygen prematurely, forming the oxygen free radical superoxide, which has been implicated in the pathophysiology of a variety of diseases (Kovacic *et al.*, 2005).

Under stress conditions $O_2^{\bullet-}$ acts as an oxidant of [4Fe-4S] cluster-containing enzymes and facilitates $^{\bullet}OH$ production from H_2O_2 by making Fe^{2+} available for the Fenton reaction (Leonard *et al.*, 2004).

$$O_2^{\bullet-} + H_2O_2 \longrightarrow O_2 + {}^{\bullet}OH + OH^-$$

HYDROXYL RADICAL ($^{\bullet}OH$)

The hydroxyl radical, $^{\bullet}OH$ is the neutral form of the hydroxide ion. The hydroxyl radical has a high reactivity, making it a very dangerous radical with a very short *in vivo* half-life of approximately 10^{-9}S (Pastor *et al.*, 2000).

The majority of the hydroxyl radicals generated *in vivo* comes from the metal catalyzed breakdown of hydrogen peroxide, according to the Fenton reaction

$$M^{n+}(=Cu^{+}, Fe^{2+}, Ti^{3+}, Co^{2+}) + H_2O_2 \longrightarrow M^{(n+1)+}(=Cu^{+}, Fe^{3+}, Ti^{4+}, Co^{3+}) + {}^{\bullet}OH + OH^{-}$$

where m^{n+} is a transition metal ion (Liochev and Fridovich, 2002).

PEROXYL RADICAL

Typical of additional radicals derived from oxygen that can be formed in living systems are peroxyl radicals ($ROO^{\bullet}$). The simplest peroxyl radical is $HOO^{\bullet}$, which is the protonated form and is usually termed either hydroperoxyl radical or perhydroxyl radical. They are high-energy species and ~0.3% of any superoxide present in the cytosol of a typical cell is in the protonated form (De Grey, 2002).

SINGLET OXYGEN

Singlet oxygen can be generated by a range of enzymatic and non-enzymatic reactions including processes mediated by heme proteins, lipoxygenases and activated leukocytes, as well as radical termination reactions. Singlet oxygen mediates protein oxidation by bringing about changes to both the side chains and backbone of amino acids, peptides and proteins (Davies, 2003).

HYPOCHLOROUS ACID (HClO)

Hypochlorous acid is an effective biological oxidant produced by activated neutrophils. Hypochlorous acid plays a role of the major inflammation mediator in mammalian tissues (Lapshina *et al.*, 2006).

HClO is a powerful natural oxidant that damages bacteria, endothelial cells, tumor cells and erythrocytes. In blood red cells it oxidizes the protein SH^- groups, lysine residues, cholesterol and fatty acids, causes K^+ leaks, membrane deformability, cross-linking of membrane proteins and lysis (Zavodnik *et al.*, 2001).

HYDROGEN PEROXIDE

Peroxisomes are known to produce H_2O_2 under physiologic conditions. Oxygen consumption in the peroxisome leads to H_2O_2 production. When peroxisomes are damaged and their H_2O_2 consuming enzymes downregulated, H_2O_2 releases in the cytosol significantly contributes to oxidative stress (Valko *et al.*, 2004).

H_2O_2 is also produced through two-electron reduction of O_2 by cytochrome P450, D-amino acid oxidase, acetyl coenzyme A oxidase or uric acid oxidase (Evans and Halliwell, 2001; Ignarro *et al.*, 1999).

NITRIC OXIDE

NO is a small molecule that contains one unpaired electron on the antibonding $2\pi_y^*$ orbital and is, therefore a radical. NO is generated in biological tissues by specific nitric oxide synthases (NOSs), which metabolise arginine to citrulline with the formation of NO via a five electron oxidative reaction (Ghafourifar and Cadenas, 2005).

NO has a half-life of only a few seconds in an aqueous environment. NO has greater stability in an environment with a lower oxygen concentration. Since it is soluble in both aqueous and lipid media, it readily diffuses through the cytoplasm and plasma membranes (Chiueh, 1999).

Overproduction of reactive nitrogen species is called nitrosative stress. Nitrosative stress may lead to nitrosylation reactions that can alter the structure of proteins and so inhibit their normal function (Klatt and Lamas, 2000).

PEROXYNITRITE

Cells of the immune system produce both the superoxide anion and nitric oxide during the oxidative burst triggered during inflammatory processes. Under these conditions, nitric oxide and the superoxide anion may react together to produce significant amounts of a much more oxidatively active molecule, peroxynitrite anion ($ONOO^-$) which is a potent oxidizing agent that can cause DNA fragmentation and lipid oxidation (Carr *et al.*, 2000).

$$NO^{\bullet} + O_2^{\bullet -} \rightarrow ONOO^-$$

SOURCES OF FREE RADICALS

Oxygen and its reactive species are very important in oxidative metabolism. They are produced in living organisms by normal metabolism and by exogenous sources (Vertuani *et al.*, 2004). Exogenous sources of free radicals include ozone, exposure to ultraviolet radiation in sunlight, electromagnetic radiation and cigarette smoke. Potential endogenous sources include mitochondria, cytochrome P450 metabolism, peroxisomes and inflammatory cell activation (Inoue *et al.*, 2003).

ETHANOL

The ability of ethanol to increase the production of reactive oxygen species (ROS) and enhance peroxidation of lipids, proteins and DNA has been demonstrated in a variety of systems, cells and species including humans (Cederbaum, 2001). The mechanism of ethanol-induced oxidative stress and cell injury appear to involve redox state changes (decrease in the NAD^+/NADH redox ratio) produced as a result of ethanol oxidation by alcohol and acetaldehyde dehydrogenases, production of the reactive metabolite acetaldehyde, one electron oxidation of ethanol of the 1-hydroxy ethyl radical (Tsukamoto and Lu, 2001).

Several mechanisms contribute to alcohol-induced liver injury, the linkage between CYP2E1-dependent oxidative stress, mitochondrial injury, stellate cell activation and GSH homeostasis (Wu and Cederbaum, 2005). Alcohol-inducible cytochrome P450 (CYP2E1) exposes the liver and peripheral tissues to a range of pathogenic and potentially carcinogenic substances owing to the low substrate specificity of this enzyme (Lieber, 2004a).

CCl_4

Carbon tetrachloride is an extensively used industrial solvent and is the best characterized animal model of xenobiotic induced free radical mediated hepatotoxicity and is also nephrotoxic. It may reduce antioxidant enzyme and antioxidant substrates to induce oxidative stress. CCl_4 requires bioactivation by cytochrome P450 system of phase I in liver and yields the reactive metabolic trichloromethyl radical ($CCl_3^{\bullet}$) and peroxy trichloromethyl radical ($^{\bullet}OOCCl_3$). These free radicals can bind with polyunsaturated fatty acid (PUFA), alkoxy ($R^{\bullet}$) and peroxy radicals ($ROO^{\bullet}$), that can generate lipid peroxide, cause damage in cell membrane, change enzyme activity and finally induce hepatic injury or necrosis (Weber *et al.*, 2003).

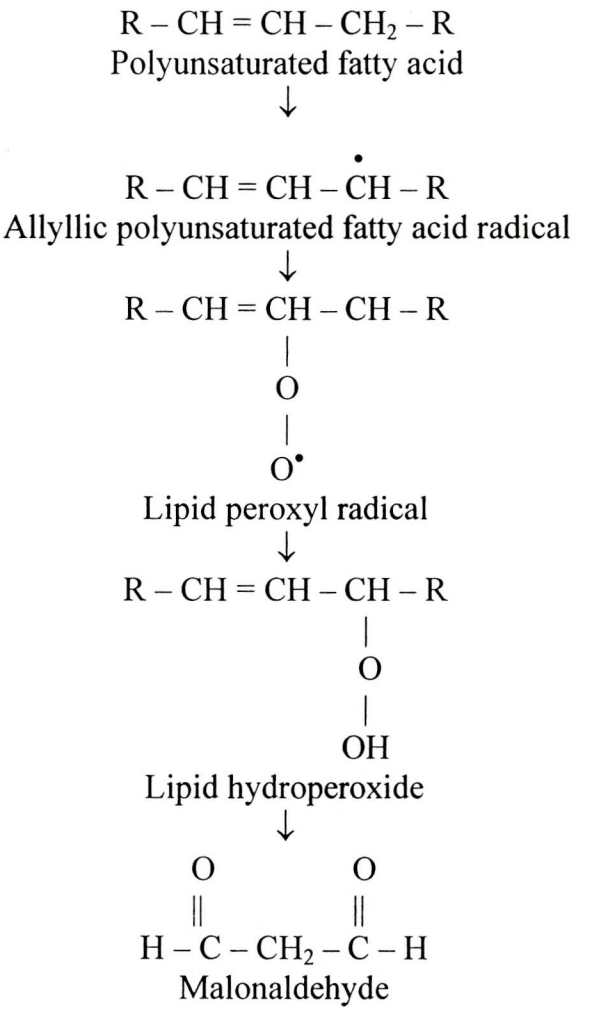
OXIDATIVE DAMAGE TO BIOMOLECULES

Oxidation and production of free radicals are an integral part of human metabolism. Reactive oxygen and nitrogen species can attack various substrates in the body including lipids, nucleic acids and proteins. Oxidation of any of these substrates can theoretically contribute to chronic diseases such as cancer, cardiovascular disease and age-related macular degeneration and to aging (Seitz and Stickel, 2006).

LIPID PEROXIDATION

Lipid peroxidation has been shown to be of great importance in mammalian physiology and pathophysiology. Once generated, ROS initiate the peroxidation of membrane lipids as well as a wide range of biological molecules through a process that is believed to be implicated in the etiology of several disease conditions (Lefer and Granger, 2000). Lipid peroxidation of fats and fatty acids in foods results not only in their spoilage but is also a source of peroxyl and hydroxyl radicals that are associated with carcinogenesis, mutagenesis and aging (Finkel and Holbrook, 2000).

The overall process of lipid peroxidation consists of three stages namely initiation, propagation and termination (Kohen and Nyska, 2002). Once formed, peroxyl radicals ($ROO^{\bullet}$) can be rearranged via a cyclisation reaction to endoperoxides (precursors of malondialdehyde) with the final product of the peroxidation process being malondialdehyde (Marnett, 1999). Peroxidation of lipids is an autocatalytic process which is terminated for example by the recombination of radicals ($R^{\bullet} + R^{\bullet}$) $\rightarrow$ non-radical product or depletion of the substrate.



Steps of lipid peroxidation (Alessio, 2000)

MDA can react with DNA bases G, A and C to form adducts M_1G, M_1A and M_1C respectively (Marnett, 1999).

PROTEINS

Oxidation of proteins is associated with a number of age-related diseases and ageing (Stadtman, 2001). It has been reported that two mitochondrial proteins namely aconitase and adenine nucleotide translocase may be important targets to long-term oxidative damage. The side chains of all amino acid residues of proteins are susceptible to oxidation by ionizing radiation and by the action of ROS/RNS (Stadtman, 2004).

The hydroxyl radical represents the major species responsible for the oxidation of proteins. Welch *et al.* (2001) demonstrated the site-specific modification of ferritin by iron which involved the oxidation of cysteine, tyrosine and also some other residues.

Protein oxidation by ROS is associated with the formation of many different kinds of inter- and intra-protein cross-linkages, including those formed, (i) by addition of lysine amino groups to the carbonyl group of an oxidized protein, (ii) by interaction of two carbon-centered radicals obtained by the hydroxyl radical-driven abstraction of hydrogens from the polypeptide backbone, (iii) by oxidation of sulphydryl groups of cysteine residues to form -S-S-cross-links, (iv) the oxidation of tyrosine residues to form -tyr-tyr-cross-links. Cysteine and methionine residues of proteins are particularly susceptible to oxidation by ROS (Levine *et al.*, 1996).

DNA

Reactive oxygen species (ROS)-induced oxidative DNA damage producing a variety of modifications at DNA level including base and sugar lesions, strand breaks, DNA-protein cross-links and base free sites. However, DNA of all mammalian cells contains trace amounts of modified bases that are indicative of attack by oxidizing species and its are removed by excision repairing enzymes, they are known to accumulate with age being associated with disease processes (Dizdaroglu *et al.*, 2002).

DNA damage can result either in arrest or induction of transcription, induction of signal transduction pathways, replication errors and genomic instability, all of which are associated with carcinogenesis (Cooke *et al.,* 2003). The hydroxyl radical is known to react with all components of DNA molecule, damaging both the purine and pyrimidine bases and also the deoxyribose backbone. Permanent modification of genetic material resulting from these

"oxidative damage" incidents represents the first step involved in mutagenesis, carcinogenesis and ageing (Dizdaroglu *et al.*, 2002).

In addition to ROS, reactive nitrogen species (RNS), such as peroxynitrites and nitrogen oxides, have also been implicated in DNA damage. Upon reaction with guanine, peroxynitrite has been shown to form 8-nitroguanine. Due to its structure, this adduct has the potential to induce G : C → T : A transversions (Brown and Borutaite, 2001).

OXIDATIVE STRESS AND HUMAN DISEASES

Oxidative stress has been implicated in various pathological conditions involving cardiovascular disease, cancer, neurological disorders, diabetes, other diseases and aging (Dhalla *et al.*, 2000; Jenner, 2003; Dalle-Donne *et al.*, 2006). These diseases fall into two groups : (i) the first group involves diseases characterized by pro-oxidants shifting the thiol/disulphide redox state and impairing glucose tolerance - the so-called "mitochondrial oxidative stress" conditions (ii) the second group involves disease characterized by the "inflammatory oxidative conditions" and enhanced activity of either NAD(P)H oxidase or xanthine oxidase-induced formation of ROS.

CANCER

Oxidative stress induces a cellular redox imbalance which has been found to be present in various cancer cells compared with normal cells; the redox imbalance may be related to oncogenic stimulation (Marnett, 2000). DNA damage, mutations and altered gene expression are key players in the process of carcinogenesis. The involvement of oxidants appears to be the common denominator of all these events (Valko *et al.*, 2004).

In addition to ROS, various redox metals due to their ability to generate free radicals, or non-redox metals, due to their ability to bind to critical thiols, have been implicated in the mechanisms of carcinogenesis and aging (Santos *et al.*, 2005).

ROS, SIGNAL TRANSDUCTION AND CANCER

Cell signaling refers to the process by which extracellular substances produce an intracellular response. Aberrant signaling mechanisms are related to various disease states (Brown and Borutaite, 2001).

Since one of the most fundamental processes regulated through signal transduction mechanisms is cell growth, alterations in the normal regulatory processes of cells may lead to cancer. The abnormal behaviour of neoplastic cells can often be traced to an alteration in cell signaling mechanisms, such as receptor or cytoplasmic tyrosine kinases, altered levels of specific growth factors, intracellular processes for conveying membrane signals to the nucleus and the regulation of DNA replication. It has been clearly demonstrated that ROS interfere with the expression of a number of genes and signal transduction pathways and are instrumental in the process of carcinogenesis (Poli *et al.*, 2004).

CARDIOVASCULAR DISEASES

The ROS-induced oxidative stress in cardiac and vascular myocytes has been linked with cardiovascular tissue injury (Dhalla *et al.*, 2000). ROS-induced oxidative stress plays a role in various cardiovascular diseases such as atherosclerosis, ischemic heart disease, hypertension, cardiomyopathies, cardiac hypertrophy and congestive heart failure (Kukreja and Hess, 1992).

The major sources of oxidative stress in cardiovascular system involve (i) the enzymes xanthine oxidoreductase (XOR) (ii) NAD(P)H oxidase (multisubunit

membrane complexes) and (iii) NOS as well as (iv) the mitochondrial cytochromes and (v) hemoglobin (Berry and Hare, 2004; Hare and Stamler, 2005).

One of the first events in atherogenesis is the oxidative modification of LDL induced by ROS and/or RNS. Oxidized LDL stimulates CD36 and scavenger receptor-A expression in monocytes, macrophages and smooth muscle cells induces hypertrophy and hyperplasia of smooth muscle cells and enhances many factors that contribute to the formation of an atherosclerotic lesion (Hamilton *et al.*, 2004).

ROS-induced oxidative stress in hypertensive patients is accompanied by decreased levels of antioxidants such as vitamin E, GSH and SOD, all good scavengers of free radicals. The interaction of $O_2^{\bullet-}$ and NO (leading to peroxynitrite formation) seems to be also involved in the process of hypertension (Li and Forstermann, 2000).

There is growing evidence that the altered production, spatiotemporal distribution of ROS/RNS induces oxidative/nitrosative stresses in the failing heart and vascular tree, which contribute to the abnormal cardiac and vascular phenotypes (Ignarro *et al.*, 2002).

DIABETES

Under normal conditions, the key sites of superoxide formation in the mitochondrial membrane are complex I and ubiquinone-complex III but diabetes alters the primary sites of superoxide generation so that complex II becomes the primary source of electrons that contribute to superoxide formation under diabetic conditions (Nishikawa *et al.*, 2000).

Another source of ROS in diabetes is NAD(P)H. Several lines of evidence support that NAD(P)H oxidases are a major source of glucose-induced ROS

production in the vasculature and kidney cells, confirming thus NAD(P)H as a mediator of diabetic complications (Li and Shah, 2003).

In addition to ROS, RNS have been implicated as one of the sources of nitrosative stress in diabetes. NO can react with superoxide forming peroxynitrite, a highly reactive oxidant linked with many disease states including diabetes (Zou *et al.*, 2002).

Glucose can react directly with free amine groups on protein and lipids, finally yielding a diverse group of modifications referred as advanced glycation end products (AGE) (Ling *et al.*, 2001).

NEUROLOGICAL DISORDERS

The brain and nervous system are particularly vulnerable to oxidative stress due to limited antioxidant capacity. The brain makes up about 2% of a person's mass but consumes 20% of their metabolic oxygen. The vast majority of this energy is used by the neurons (Shulman *et al.*, 2004). The brain has limited access to the bulk of antioxidants produced by the body, neurons are the first cells to be affected by a shortage of antioxidants, and are most susceptible to oxidative stress (Perry *et al.*, 2004).

The brain produces high levels of superoxide and nitric oxide, and these molecules can react to form peroxynitrite, one of the most reactive oxygen species (Porasuphatana *et al.*, 2003). For these reasons, oxidative stress plays a pivotal role in neurodegenerative disorders. High levels of both Cu and Fe, with relatively low levels of Zn and Mn, play a crucial role in brain cancer and in degenerative diseases of the brain (Parkinson's and Alzheimer's diseases, multiple sclerosis, etc.) (Johnson, 2001).

FEMALE INFERTILITY

Post operative adhesions and endometriosis are among the most common causative factors of female infertility. These two conditions have a progressive and recurrent course, which is generally unpreventable (Alpay *et al.*, 2006).

Reactive oxygen species are involved in adhesion development following surgery. Free radicals are likely to be produced locally in peritoneal tissue during laparoscopy as a result of ischemia/reperfusion process; in part, their production may be related to exposure of hyperoxic environment during laparatomy. The predisposition to adhesion development is due to either an increase in free radical activity or a decrease in scavenger molecules (Binda *et al.*, 2003).

Endometriosis is a chronic inflammatory disease, which is characterized by implantation and growth of endometrial tissue outside the uterine cavity. Increased oxidative stress in endometriosis is strongly associated with decreased fertility (Agarwal *et al.*, 2005). Elevated levels of iNOS and NO have been demonstrated in both eutopic and ectopic endometrium in women with endometriosis (Wu *et al.*, 2003). Since NO can induce endometrial cell apoptosis, high NO levels in endometrium may also impair embryo implantation and development (Khorram, 2002).

AUTOIMMUNE DISEASES

ROS, RNS and released enzymes such as proteases play some role in tissue damage in the autoimmune diseases. Hence therapy against them might prove beneficial. The levels of nitrate plus nitrite tend to be higher in the patients suffering from autoimmune diseases, indicating more NO generation (Halliwell and Gutteridge, 1999).

AGING

According to the free radical theory, aging can be considered as a progressive, inevitable process partially related to the accumulation of oxidative damage into biomolecules - nucleic acids, lipids, proteins or carbohydrates due to an imbalance between prooxidants and antioxidants in favour of the former (Mariani *et al.*, 2005).

The genesis of aging starts with oxygen, occupying final position in the electron transport chain. The leaking of electrons interact with oxygen to produce superoxide radicals, so that under physiological conditions, about 1-3% of the oxygen molecules in the mitochondria are converted into superoxide. The primary site of radical oxygen damage from superoxide radical is mitochondrial DNA. The cell repairs much of the damage done to nuclear DNA (nDNA), but mtDNA cannot be readily fixed. Excessive mtDNA damage accumulates over time and shuts down mitochondria, causing cells to die and organism to age (Cadenas and Davies, 2000).

ANTIOXIDANTS

Oxygen is vital for all the living system, except for anaerobes. However, the paradox of aerobic life is that oxidative damage occurs at the key biological sites, threatening their structure and function. Oxygenic threat is met by an array of antioxidants that evolved in parallel with our oxygenic atmosphere (Benzie, 2003).

The human body is equipped with an antioxidant defense system that deactivates these highly reactive free radicals. Antioxidant enzymes (made in the body) and antioxidant nutrients (found in foods) soak up all the excess energy that these free radicals have, turning them into harmless particles that can be

metabolized, so these antioxidant nutrients are functional components of foods that have extra health benefits in the body (Oboh, 2005).

ENZYMIC ANTIOXIDANTS

Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), etc. Antioxidant enzymes like SOD and CAT are not consumed and have high affinity and rate of reaction with ROS. They afford more effective protection against acute massive oxidative insults (Christofidou-Solomidou and Muzykantov, 2006).

SUPEROXIDE DISMUTASE

Superoxide dismutases (SOD), a group of metal-containing enzymes, have a vital antioxidant role in human health, conferred by their scavenging of one of the reactive oxygen species, superoxide anion. SODs are among the first line of defense in the detoxification of products resulting from oxidative stress (Johnson and Giulivi, 2005).

SODs can be categorized into one of three types : copper/zinc (Cu/Zn) SOD found primarily in the cytosols of eukaryotes, in chloroplasts. Manganese (Mn) SOD in the mitochondria of eukaryotes and prokaryotes and iron (Fe) SOD in prokaryotes. Mitochondria are particularly susceptible to damage induced by ROS, which are generated continuously by the mitochondrial respiratory chain and is the reason for the existence of a specific SOD in this organelle (Raha and Robinson, 2000).

The SODs catalyze the reaction:

$$2O_2^{\bullet-} + 2H^+ \longrightarrow H_2O_2 + O_2$$

Experimental knockout models have also shown that MnSOD plays an essential role in protecting lung tissue against exogenous oxidants (Kinnula *et al.*, 2004).

CATALASE

The decomposition of hydrogen peroxide to form water and oxygen is accomplished in the cell by catalase. This antioxidative enzyme is widely distributed in the cell, with the majority of the activity occurring in the mitochondria and peroxisomes (Powers and Lennon, 1999).

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$

Over expression of catalase targeted to mitochondria showed extension of murine life span (Schriner *et al.*, 2005). Catalase is the only antioxidant enzyme increased both at the mRNA and at the activity levels during human lung morphogenesis (Asikainen *et al.*, 1998).

GLUTATHIONE PEROXIDASE

Glutathione peroxidases are ubiquitously distributed. In the gastrointestinal tract the isoenzyme provides a barrier against hydroperoxides derived from the diet or from the metabolism of ingested xenobiotics. These isoenzymes may have a role in the regulation of the regional redox balance, to evoke several cellular responses, for example, programmed cell death, proliferation, cytokine production, and so on (Nakashima *et al.*, 2005).

Extracellular glutathione peroxidase has been shown to protect alveolar epithelial cells against hyperoxia-induced injury in rat (Roum *et al.*, 2001).

$$H_2O_2 + 2GSH \xrightarrow{GPx} GSSG + 2H_2O$$
$$GSSH + NADPH + H^+ \longrightarrow 2GSH + NADP^+$$

GLUTATHIONE REDUCTASE

Glutathione reductase a flavoenzyme, catalyses the reduction of oxidized GSH back into GSH, the latter being the co-substrate of GSH-Px (Gul *et al.*, 2000).

$$GSSH + NADPH + H^{+} \xrightarrow{GR} 2GSH + NADP^{+}$$

In the presence of oxidative stress, GSH concentration rapidly decreases while GSSG-potentially highly cytotoxic, increases because of the reduction of peroxides or as a result of free radical scavenging. Glutathione disulphide is reduced to GSH by the action of GR utilizing NADPH as a reductant (Argyrou and Blanchard, 2004).

GLUTATHIONE S-TRANSFERASE

Glutathione S-transferases are three enzyme families cytosolic, mitochondrial and microsomal-that detoxify noxious electrophilic xenobiotics, such as chemical carcinogens, environmental pollutants and antitumor agents. They protect against reactive compounds produced *in vivo* during oxidative stress by inactivating endogenous unsaturated aldehydes, quinones, epoxides and hydroperoxides, all of which are produced intracellularly after the exposure to pollutants (Hayes *et al.*, 2005).

GLUCOSE-6-PHOSPHATE DEHYDROGENASE

Glucose-6-phosphate dehydrogenase is essential to control intracellular reductive potential by increasing glutathione intracellular levels, which in turn decrease the amount of reactive oxygen species (Bugdayci *et al.*, 2006).

Adipogenic glucose-6-phosphate dehydrogenase overexpression promotes the expression of pro-oxidative enzymes, including nitric oxide synthase, and

NADPH oxidase, and the activation of nuclear factor-kappa B (NF-kappa B) signaling, which eventually leads to the dysregulation of adipocytokines and inflammatory signals (Park *et al.*, 2006a).

NON-ENZYMIC ANTIOXIDANTS

Human antioxidant defense system is incomplete without dietary antioxidants. The diet derived antioxidants vitamin C, vitamin E, carotenoids and along with GSH play an important role in antioxidant defense.

VITAMIN C

The physiological role of vitamin C stems from its very strong reducing power and its ability to be regenerated using intracellular reductants such as glutathione, nicotinamide adenine dinucleotide and nicotinamide, adenine dinucleotide phosphate (Chaudiere and Ferrari-Iltiou, 1999).

Emerging *in vitro* data show that extracellular ascorbic acid selectively kills some cancer but not normal cells by generating hydrogen peroxide (Chen *et al.*, 2005a). According to Vatassery *et al.* (2004) vitamins C and E may be very efficient mitochondrial protectors when there is an excessive formation of superoxide and nitric oxide in normal or pathological conditions.

VITAMIN E

Vitamin E belongs to a group of lipid-soluble antioxidants. The biological activity of vitamin E exhibit tocopherols and tocotrienols, especially α-tocopherol. The predominant reaction responsible for tocopherol antioxidant activity is hydrogen atom donation, where a tocopheroxyl radical is formed (Lampi *et al.*, 2002). Vitamin E provides a first line of defense against DNA oxidative damage and lipid peroxidation of unsaturated fatty acids in the cell membrane (Griffiths *et al.*, 2002).

Vitamin E is particularly effective in preventing lipid peroxidation. Lipid peroxidation is a series of chemical reactions involving the oxidative deterioration of polyunsaturated fatty acids (PUFA) that may disrupt the structure and function of cells. Using PUFA as an example, the peroxidative cascade is likely to be terminated by vitamin E as follows.

$$\underset{\text{Non-radical PUFA}}{PUFA:H} + \underset{\text{FR}}{R^{\bullet}} \longrightarrow \underset{\text{PUFA radical}}{PUFA^{\bullet}} + \underset{\text{non-radical product}}{RH} \quad (1)$$

$$\underset{\text{PUFA radical}}{PUFA^{\bullet}} + \underset{O_2}{O_2} \longrightarrow \underset{\text{PeroxyPUFA radical}}{PUFAOO^{\bullet}} \quad (2)$$

$$\underset{\text{Vitamin E}}{\text{Tocopherol} - OH} + \underset{\text{PeroxyPUFA radical}}{PUFAOO^{\bullet}} \longrightarrow \underset{\text{tocopheroxy radical}}{\text{tocopherol-}O^{\bullet}} + \underset{\text{Lipid hydroperoxide}}{PUFAOOH}$$

When vitamin E donates a hydrogen it becomes a free radical, but it is relatively unreactive. The PUFAOOH can be released from the phospholipid structure in membrane by phospholipase A_2 and then degraded by selenium dependent glutathione peroxidase (Duthie, 2000).

To maintain vitamin E in its active form it is rapidly regenerated by vitamin C (Blokhina *et al.*, 2003).

VITAMIN A

Carotenoids (terpenoid molecules synthesized by plants, among which vitamin A precursors such as β-carotene) are free radical scavengers, and most importantly, singlet oxygen quenchers. This is particularly important for the retina, where singlet oxygen is produced following interactions between visible light, oxygen (triplet) and various photosensitizers (Handelman, 2001).

Retinoic acid plays an important role in controlling cell growth, cell differentiation, and apoptosis, as well as carcinogenesis and is of potential clinical

interest in cancer chemoprevention and treatment (Altucci and Gronemeyer, 2001). In the blood, β-carotene and the other carotenoids are carried mainly in the lipoproteins. The widespread interest in the role of lipoprotein oxidation in the etiology of CVD stimulated much interest in the abilities of β-carotene to scavenge free radicals and thus perhaps to prevent CVD by inhibiting LDL oxidation (Tavani and La Vecchia, 1999).

REDUCED GLUTATHIONE

The major thiol antioxidant and redox buffer of the cell is the tripeptide, glutathione (GSH) (Masella *et al.*, 2005). GSH serves as a significant first line of defense against the oxidative stress and it plays an important role in maintaining the integrity of cells (Das and Vasudevan, 2005).

GSH in the nucleus maintains the redox state of critical protein sulphydryls that are necessary for DNA repair and expression. Oxidized glutathione is accumulated inside the cells and the ratio of GSH/GSSG is a good measure of oxidative stress of an organism (Nogueira *et al.,* 2004).

The main protective roles of glutathione against oxidative stress are : (i) glutathione is a factor of several detoxifying enzymes against oxidative stress, e.g. GPx, GST and others (ii) GSH participates in amino acid transport through the plasma membrane (iii) GSH scavenges hydroxyl radical, singlet oxygen directly, detoxifying hydrogen peroxide and lipid peroxides by the catalytic action of glutathione peroxidase (iv) glutathione is able to regenerate the most important antioxidants, vitamins C and E, back to their active forms (Masella *et al.*, 2005).

PROTEIN THIOLS

Protein-S-thiolation, protein-S-nitrosation and protein-SH plays an important role in redox signaling during normal physiology and under oxidative

stress in controlling the cellular processes (Biswas *et al.*, 2006). Protein thiols is a water soluble antioxidant, S-thiolation of cellular proteins may represent a mechanism for protection of vulnerable thiols against irreversible damage by prooxidant agents (Dominici *et al.*, 1999).

HERBAL MEDICINE

Herbs have recently attracted attention as health beneficial foods (physiologically functional foods) and as source materials for drug development. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases. Recently, interest in complementary and alternative medicine has grown rapidly in industrialized countries and the demand for herbal remedies has currently increased (De Smet, 2002).

Medicinal plants have always had an important place in the therapeutic armoury of mankind. Up to 80% of populations in developing countries are totally dependent on plants for their primary health care. And despite the remarkable progress in synthetic organic chemistry of the twentieth century, over 25% of prescribed medicines in industrialized countries derive directly or indirectly from plants (Colson and De Broe, 2005).

Dietary components, which are capable of acting as antioxidants are likely to be beneficial by augmenting cellular defenses and protecting the cell against damage caused by free radicals, by acting as radical scavengers, reducing agents, potential complexes of prooxidant metals and quenchers of singlet oxygen (Doblado *et al.*, 2005; Oboh, 2006).

The consumption of natural antioxidant phytochemicals was reported to have potential health benefits (Sumino *et al.*, 2002). Many antioxidant compounds, naturally occurring in plant sources have been identified as free radical or active oxygen scavengers. Natural antioxidants can protect the human body from free

radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods. Hence, the studies on natural antioxidants have gained increasingly greater importance (Gulcin *et al.,* 2004a).

Natural antioxidants are presumed to be safe, since they occur in plants and they are more desirable than their synthetic counterparts. Moreover, plants with known antioxidant property usually present some antimutagenic, anticarcinogenic and anti-inflammatory action (Arnao *et al.*, 1999).

One such medicinal plant is *Triticum aestivum* commonly called as wheat grass. Tender wheat grass and its juice are consumed for healthy growth of human body. It is also taken in the form of tablets available commercially as 'green' food. Although antioxidant activity of wheat grass is well believed, the exact reasons are not well established. A very few publications are available in literature on nutritive and antioxidant properties of wheat sprout extracts where it is reported that these extracts inhibit the DNA oxidative damage and are effective in suppressing the superoxide radical that can further lead to various diseases (Falcioni *et al.*, 2002). It has also shown antimutagenicity property (Peryt *et al.*, 1992).

Kulkarni *et al.* (2006a) has reported that wheat grass grown indoors using only tap water could be adequate to provide bare minimum health support. It is also advantageous to consume wheat grass grown using only tap water since there is no extraneous addition of elements/compounds either during growth period or processing. Fresh wheat grass is preferred over wheat grass tablets.

In spite of such reports being reported, not many studies have concentrated on the type of antioxidant responses evoked by the *Triticum aestivum* leaves on oxidative stress-induced events at the molecular level. The present study is an extensive search into the antioxidant and hepatorenalprotective activities of *Triticum aestivum* 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.