Review of Literature

2. REVIEW OF LITERATURE

Medicinal plants are moving from fringe to main stream, seeking remedies and health approaches free from side effects caused by synthetic chemicals. Considerable attention is being paid to utilize eco-friendly and bio-friendly plant based products for the prevention and cure of different diseases (Dubey *et al.*, 2004).

The advantages of traditional systems of medicine with respect to their safety and efficacy could result in a better utilization of our herbal resources with the application of the scientific methods (Seth and Sharma, 2004). Over the past two decades, an expanding body of evidence from epidemiological and laboratory studies have demonstrated that some edible plants as a whole, or their identified ingredients with antioxidant properties have substantial protective effects on human carcinogenesis (Tsao *et al.*, 2004).

Spices and herbs are recognized as potent resources of natural antioxidants that can protect from oxidative stress and thus play an important role in the chemo prevention of diseases that have their etiology and pathophysiology in reactive oxygen species (Repetto and Llesuy, 2002).

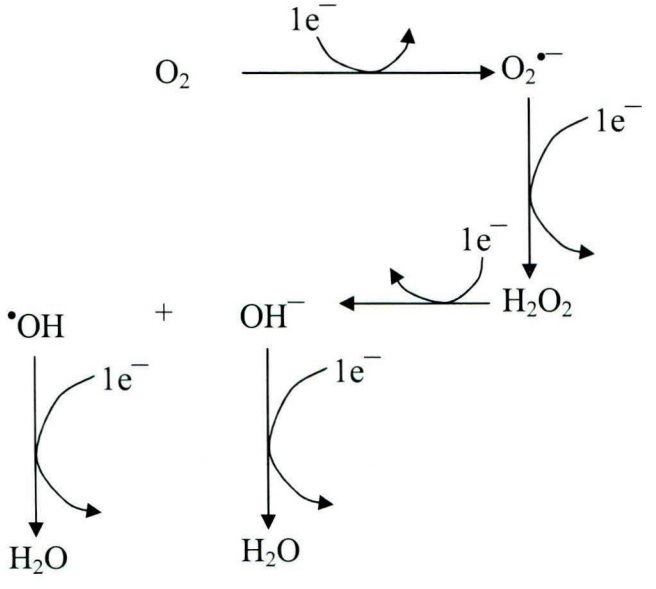
FREE RADICALS

Free radicals can be defined as a chemical species, an atom or a molecule that has one or more unpaired electrons in its valence shell and is capable of existing independently. A free radical contains an odd number of electrons which makes it unstable, short lived and highly reactive. Therefore, it reacts quickly with other compounds in order to capture the needed

electron to gain stability. Generally, free radicals attack the nearest stable molecule, stealing its electron. When the attacked molecule loses its electron, it becomes a free radical itself, beginning a chain reaction cascade resulting in the disruption of a living cell (Kumar *et al.*, 2003).

Many active intermediaries such as electrophiles (tend to accept electrons) and free radicals (with the ability to damage cellular components) are produced during physiological and pathological process, as shown in figure below.

Electron leakage within the mitochondrial respiratory chain



Free radical production in organisms with ascorbic metabolism is a continuous and unavoidable process, since the reduction of molecular oxygen (O_2) to water (H_2O) within the mitochondrial respiratory chain is not 100% efficient. In this way, the mitochondria is the main source of free radicals, due to electron leakage in the respiratory chain, with the resulting formation of reactive species, such as superoxide radical ($O_2^{\bullet -}$), hydrogen

peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$). These free radicals are called reactive oxygen species (ROS), which are prone to oxidize intracellular molecules (Arora *et al.*, 2002).

TYPES OF ROS

ROS represent a broad category of molecules that indicate a collection of radicals and non-radical oxygen derivatives. These intermediates may participate in reactions that give rise to free radicals that damage organic substrate. The terms Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are collective terms that include not only the radicls but also the non-radicals. Free radicals and reactive oxygen / nitrogen species of importance in living organisms include hydroxyl ($OH^{\bullet}$), superoxide ($O_2^{\bullet -}$), nitric oxide (NO), nitrogen dioxide (NO_2) and peroxyl ($ROO^{\bullet}$). Peroxynitrate ($OONO^{-}$), hypochlorous acid (HOCl), hydrogen peroxide (H_2O_2), singlet oxygen ($O_2^{\bullet -}$), ozone (O_3), nitrous acid (HNO_2) and dinitrogen trioxide (N_2O_3) are not free radicals but can easily lead to free radical reactions in living organisms (Mavi *et al.*, 2003).

OXIDATIVE STRESS

The consequences of the damage initiated by these metabolic by products affect a large range of biological reactions, like increases in the mutation rate and alteration of cellular membranes composition, structural proteins, metabolic and detoxifying enzymes and cellular signaling proteins. In many cases, the reactive intermediaries produced are able to convert cellular constituents in second generation reactive intermediaries, which are able to induce more damage (Marnett *et al.*, 2003). Oxidative stress is the

term referring to the imbalance between the generation of reactive oxygen species and the activity of the antioxidant defenses (Saito *et al.*, 2005). Oxidative stress can affect the individual molecules, and thus the entire organism. This is believed to be one of the major causes of many human diseases including Alzheimer's diseases, autoimmune disease, cancer, cardiovascular diseases, diabetes, Parkinson's disease, rheumatoid arthritis, multiple sclerosis, myocardial infarction and reperfusion injury (Mohammed, 2002).

CELLULAR DAMAGE AS A CONSEQUENCE OF OXIDATIVE STRESS

Free radicals are prone to oxidize intracellular molecules, such as lipids DNA and proteins, giving rise to alterations in the cell structure.

DAMAGE TO LIPIDS

Tissue lipid peroxidation is a degradative phenomenon as a consequence of free radical chain production and propagation, which affects mainly the polyunsaturated fatty acids and this event is strongly implied in the pathogenesis of several diseases such as arterio sclerosis, diabetes, cancer and rheumatoid arthritis, as well as toxicity associated to drugs and aging, LPO is the oxidation of polyunsaturated fatty acids (PUFA), the main biological membranes component. Such acids contain methylene groups between double bonds that are prone to react with oxidants. These compounds subtract hydrogen atoms to form free radicals in the central carbons. These radicals react with O_2 giving $ROO^{\bullet}$, which are initial PUFA oxidation products. The $ROO^{\bullet}$ are able to oxidize the PUFAs, spreading the lipid peroxidation (LPO) process, or they can be implied in other kinds of

reactions giving rise to malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) formation, which can diffuse and even escape from the cell acting far from the place of its production (Chen *et al.*, 2000).

DAMAGE TO DNA

The exposure to endogenous oxidants and electrophils gives rise to an increase in the damage to cellular macromolecules as important as DNA. DNA damage can be a result of a series of reactions with the nitrogen bases of nucleic acids, the deoxyribose residues or the phosphodiester backbone. DNA lesions that are not properly repaired, accumulate through time and can contribute to the development of diseases associated with aging. $OH^{\bullet}$ radical is able to add double bonds to nitrogen bases or to subtract hydrogen atoms fro m methyl groups and deoxyribose residues (Chatgilialoglu and Neill, 2001).

Activated oxygen and agents that generate oxygen free radicals such as ionizing radiation, induce numerous lesions in DNA that lead to deletions mutations and other lethal genetic effects (Tominaga *et al.*, 2004).

Pyrimidine bases are most susceptible to ROS attack as are purines and deoxyribose sugar. Oxidation of the sugar by the hydroxyl radical is the main cause for DNA strand breaks (Agarwal and Saleh, 2001). Oxidative damage can cause base degradation, DNA fragmentation and cross-linking to protein (Aitken and Krausz, 2001).

DAMAGE TO PROTEINS

Sulphur containing amino acids having thiol groups specifically are vary susceptible to oxidative stress. Activated oxygen can abstract H atom

from cysteine residues to form a thiyl radical ($RS^{\bullet}$) that will cross-link to a second thiyl radical to form disulphide bridges. Alternatively, oxygen can add to methionine residues to form methionine sulphoxide derivatives. Many amino acids undergo specific irreversible modifications when a protein is oxidized. Oxidative damage can also lead to cleavage of the polypeptide chain and formation of cross linked protein aggregates, histidine, lysine, proline, arginine and serine form carbonyl groups on oxidation (Stadtman and Levine, 2003).

DISEASES CAUSED DUE TO FREE RADICAL DAMAGE

RHEUMATOID ARTHRITIS (RA)

Rheumatoid arthritis is a systemic diseases characterized by progressive, erosize and chronic polyarthritis. Increased oxidative stress and/or defective antioxidant status are known to contribute to the pathology of rheumatoid arthritis (Karatas *et al.*, 2003). Raised levels of malondia ledhyde and low levels of endogenous antioxidants are observed in patients of rheumatoid arthritis. Plasma catalase is lower in patients with RA (Kamanli *et al.*, 2004). These patients have impaired glutathione reductase activity in synovial fluid (Bazzichi *et al.*, 2002).

HEART DISEASE

In vivo and *ex vivo* studies have provided precious evidence supporting the role of oxidative stress in a number of conditions (atherosclerosis, catecholamine-induced cardiomyopathy, diabetic cardiomyopathy, cardiac hypertrophy and congestive heart failure) leading to severe cardiovascular dysfunctions (Dhalla *et al.*, 2000).

DIABETES

The free radicals or oxidative injury now appears to be the fundamental mechanism underlying a number of human disorders. In diabetes, increased oxidative stress, which co-exists with reduction in the antioxidant status, has been postulated. Oxygen free radicals can initiate the peroxidation of lipids, which in turn stimulates the glycation of proteins, inactivation of enzymes and alteration in the structure and function of collagen in basement and other membranes and play a long term complication of diabetes (Sabu and Kuttan, 2002).

CANCER

In carcinogenesis, reactive oxygen species are responsible for initiating the multistage carcinogenesis process, starting with DNA damage and the accumulation of genetic events in one or few cell lines, which leads to progressively dysplastic cellular appearances, deregulated cell growth and finally carcinoma (Tsao *et al.*, 2004).

ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is actually the main cause of dementia. It is characterized by the deposition of the amyloid β-peptide (Aβ) outside of nerve cells forming extracellular plagues. Furthermore, intraneuronal accumulation of tau proteins also occurs, due to abnormal tau phosphorilation (Vickers *et al.*, 2000). It is known that Aβ is neurotoxic and there are evidences suggesting a role for oxidative stress, since Aβ aggregation process is accelerated by Aβ peptide metal catalyzed oxidation, giving rise to H_2O_2 production which is able to react further with transition

metals. Thus an increase in H_2O_2 levels is observed in AD. Free radical production by Aβ aggregation has important consequences to brain physiology since it implies the necessity of a protection system such as antioxidants (Tamagno *et al.*, 2006).

PARKINSON'S DISEASE

Another very well known neurodegenerative disease, Parkinson's disease, is characterized by akinesia, muscular rigidity, tremor at rest, and pastural abnormalities. In early stages of Parkinsonism, there appears to be a compensatory increase in the number of dopamine receptors to accommodate the initial loss of dopamine neurons. As the disease progress, the number of dopamine receptors diseases, apparently due to the concominant degeneration of dopamine target sites on striatal neurons. The loss of dopaminergic neurons in Parkinson's disease results in enhanced metabolism of dopamine augmentating the formation of H_2O_2, thus leading to generation of highly neurotoxic hydroxyl radicals (OH^{β}). The generation of free radicals can also be produced by 6-hydroxydopamine or MPTP which destroys strialal dopaminergic neurons causing Parkensonism (Chiuch *et al.*, 2000).

SCURVY

Deficiency of vitamin C causes the lethal disease scurvy, in which collagen disintegrates within the body, leads to widespread internal bleeding and finally death. Scientists believe that degenerative diseases like atherosclerosis and cancer are manifestations of mild scurvy (http://www.tandurust.com/antioxidants/vitamin_c.html).

ANTIOXIDANT DEFENSES

A number of important physiological antioxidant systems serve to oppose the effects of free radicals and oxidants on target substances. Those capable of sequestering transition metal ions, which in a free form catalase the formation of hydroxyl radicals, can be considered as preventive antioxidants.

An antioxidant has been defined as any substance that when present at low concentration compared with those of an oxidizable substrate significantly delays or prevents the oxidation of that substrate. When ROS/RNS are generated *in vivo* their actions are opposed by intricate and coordinated antioxidant lines of defense systems (Waring, 2001).

These include enzymic and non-enzymic antioxidants that present or alleviate injuries from ROS. The enzymic antioxidants include superoxide dismutase (SOD), catalase (CAT), peroxidase (POx), ascorbate peroxidase (APx), glutathione reductase (GR) and polyphenol oxidase. The nonenzymic antioxidants include compounds like ascorbates (Vitamin C), glutathione, tocopherol, flavonoids and carotenoids (Agarwal and Pandey, 2004). Cooperation among these components is essential for effective protection from ROS.

ENZYMIC ANTIOXIDANTS

The major enzymes, constituting the first line of defence, directly involved in the neutralization of ROS/RNS are : superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (Foyer and Noctor, 2000).

Superoxide dismutase (SOD) can be divided into three different classes according to the catalytic metal present at the active site. SOD (CuZnSOD) is found in the cytosol and contains copper (Cu) and Zn as metal cofactors SOD2 (MnSOD) is present in mitochondria and contains Mn, SOD3 (ECSOD) is present extracellularly. Of these Cu/ZnSOD and MnSOD are the main forms.

SOD catalyses the dismutation of superoxide into hydrogen peroxide and oxygen.

$$2O_2^{\bullet} + 2H^{+} \xrightarrow{SOD} H_2O_2 + O_2$$

SOD scavenges both intracellular and extracellular superoxide radical and prevents the lipid peroxidation of plasma membrane. However, it should be conjugated with catalase or GPx to prevent the action of H_2O_2, which promotes the formation of hydroxyl radicals (Sozmen *et al.*, 2001).

Catalase, an exclusively peroxisomal enzyme in most tissues, converts H_2O_2 to water and O_2. However, the most important H_2O_2-removing enzymes are selenoprotein GPx enzymes. GPx enzymes remove H_2O_2 by using it to oxidize reduced glutathione (GSH) to oxidized glutathione (GSSG).

Glutathione reductase a flavoprotein enzyme, regenerates GSH from GSSG with NADPH as a source of reducing power. Glutathione peroxidase also catalyses the reduction of unstable hydroperoxides at the expense of GSH (Apel and Hirt, 2004).

NON-ENZYMIC ANTIOXIDANTS

Vitamin E is a major chain breaking antioxidant since this interrupts the lipid peroxidation process. It reacts directly with free radicals such as peroxy radical ($ROO^{\bullet}$), yielding lipid hydroperoxides, which can be removed by phospholipase - GSH - Px systems. It scavenges all the three important types of ROS, namely, superoxide, H_2O_2 and hydroxyl radicals (Agarwal *et al.*, 2004).

Ascorbate is like vitamin E, also a chain-breaking antioxidant and is found both intracelluarly and extracellularly. It prevents lipid peroxidation due to peroxy radicals.

It also recycles vitamin E. It protects against DNA damage induced by H_2O_2. Vitamin C has a paradoxical effect, as it can also produce ROs by its action on transition metal ions.

$$AH + Fe^{3+} / Cu^{2+} \rightarrow A^{-} + Fe^{2+} / Cu^{+}$$

$$Fe^{3+} / Cu^{+} + O_2 \rightarrow O_2 + Fe^{3+} / Cu^{2+}$$

$$Fe^{3+} / Cu^{+} + H_2O_2 \rightarrow OH + OH + Fe^{3+} / Cu^{2+}$$

(Lutsenko *et al.*, 2002).

Glutathione may contribute to antioxidant defense by networking with other major antioxidants such as vitamins E and C. It plays a vital role in annihilating oxygen toxicity by interrupting the reaction leading to O_2^{-} formation. In its reduced form, it metabolizes H_2O_2 and $OH^{\bullet}$, it is a peptide composed of glutamate, cysteine and glycine that exists in thiol-reduced glutathione (GSH) and oxidized glutathione. It can restore the physiological

constitution of polyunsaturated fatty acids in the cell membrane (Lenzi *et al.*, 2000).

Flavonoids have become widely accepted as physiological antioxidants with the potential to protect against the many degenerative diseases linked to ROS-related tissue damage (Dreosti, 2000).

Plant phenolic compounds such as flavonoids and legnin precursors are important constituents of the human diet. These dietary phytophenolics have been recognized largely as beneficial antioxidants that can scavenge harmful active oxygen species. In plant systems, phytophenolics can act as antioxidants by donating electrons to guaiacol-type peroxidase (GuPxs) for the detoxification of H_2O_2 produced under stress conditions (Sakihama *et al.*, 2002).

Carotenoids are a group of red, orange and yellow pigments found in plant foods, particularly fruits and vegetables. Some carotenoids, like β-carotene, act as a precursor of vitamin A : others do not. β-carotene is an effective antioxidant as it is one of the most powerful singlet thus preventing this active molecules from generating free radicals. Its other antioxidant properties include the scavenging of free radicals. Besides β-carotene, other important dietary carotenoids include α-carotene, lycopene lutein, zeaxanthin and β-cryptoxanthin. Studies have confirmed that lycopene has a powerful role as an antioxidant (Pillai and Pillai, 2002).

Drosophila melanogaster

Drosophila melanogaster is a two-winged insect that belongs to *Diptera*, the order of the flies. The species is commonly known as the fruit

fly and is one of the most commonly used model organisms in biology-including studies in genetics, physiology and life history evolution. The genome of *D. melanogaster* (sequenced in 2000) contains four pairs of chromosomes : an X/Y pair and three autosomes labeled 2, 3 and 4. The fourth chromosome is so tiny that it is often ignored aside from its important eyeless gene.

Its sequenced genome of 120 million base pairs has been annotated (Adams *et al.*, 2000) and contains approximately 13,767 protein-coding genes which comprise ~20% of the genome. More than 60% of the genome appears to be functional non-protein coding DNA (Halligan and Keightley, 2006) involved in gene expression control.

About 75% of known human disease genes have a recognizable match in the genetic code of fruit flies and 50% of the fly protein sequences have mammalian analogues. *Drosophila* is being used as a genetic model for several human diseases including the neurodegenerative disorders, Parkinson's, Huntington's, spinocerebellar ataxia and Alzheimer's disease. The fly is also being used to study mechanisms underlying aging and oxidative stress, immunity, diabetes and cancer, as well as drug abuse (Reiter *et al.*, 2001).

Drosophila melanogaster is the most studied organism in biological research particularly in genetics and developmental biology. There are several reasons for this.

1) It is small and easy to grow in the laboratory

2) It has a short generation time (about two weeks) and high fecundity (females can lay more than 800 eggs in one day)

3) The mature larvae show giant chromosomes in the salivary glands called polytene chromosomes-puffs indicate regions of transcription and hence gene activity.

4) It has only four pairs of chrosomes three autosomes and one sex chromosomes

5) Males do not show meiotic recombination facilitating genetic studies

6) Its compact genome was sequenced and first published in 2000 (Ashburner *et al.*, 2005).

Triticum aestivum

Triticum aestivum commonly called as wheat grass is consumed for healthy growth of human body. It is also taken in the form of tablets available commercially as green food. Tender leaves and its juice are consumed. A very few publications are available in the literature on the 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).

The 4^{th} day leaves (Plate 1) of wheat grass proved to possess better antioxidant activity compared to 8^{th} and 12^{th} day in an earlier study conducted in our laboratory (Vidya, 2007). The wheat grass was also

reported to have antimutagenic property and is helpful in curing certain diseases such as thalassemia (Marawaha *et al.*, 2004) and distal ulcerative colitis (Ben-Arye *et al.*, 2002).

The present study aimed to analyze the antioxidant effect of *Triticum aestivum* using Drosophila as the model organism. The methodology adopted and the layout of the study are presented in the next chapter with bibliographical and appendix support.

Plate 1

Triticum aestivum **PLANTLETS**
AT FOUR DAYS OF GROWTH