Chapter 2: Review of literature
2.1 Mushrooms

Mushrooms are macro fungi with distinctive fruiting bodies which are either epigenous or hypogenous and sufficiently conspicuous to the naked eye to be hand-picked (Chang and Miles, 1982). The appraisal of mushrooms as highly nutritive foodstuff is well founded. Many kinds of mushrooms are edible, and at the same time possess tonic and medical attributes (Chang, 1999). Human use of mushrooms extent as early to 5000 BC. About 2000 species of edible mushrooms are known all over the world. One of the most delicious and excellently edible mushrooms is the European button mushroom. The total production of edible mushrooms is about 3.75 million tones. Extensive clinical studies, conducted primarily in China and Japan, have explicitly illustrated that a number of mushroom species have medicinal and therapeutic value in the prevention /treatment of cancer, viral disease, hypercholesterolemia, blood platelet aggregation, and hypertension (Jong et al., 1991).

Mushrooms have been important in human history as food, as medicine, as legends, and in folk lore and religion. Mushrooms are lively in folklore as “witches egg and fairy egg” (Molitoris, 2001). Mushrooms are basically consumed for their texture and flavor. They have recently become attractive as health - beneficent food and as sources for the development of drugs. Many higher Basidomycetes mushrooms are known to contain a number of biologically active components that show promising antitumour and immunomodulating, cardiovascular, hepatoprotective, hypocholesterolemic, antiviral, antibacterial, antiparasitic and antidiabetic effects (Didukh, 2001).

2.1.1. Nutritional value of mushrooms

Nutritional value of mushrooms lies between that of meat and vegetables. The rich source of proteins, vitamins and minerals and low in fat content (2-8%) unique chemicals constitution of mushrooms makes them low calorie food
and choice diet for those suffering from hypertension, artherosclerosis, diabetes, obesity etc. (Subramanian, 1995). Mushrooms normally contain 19-35% protein. Mushroom proteins contain all the essential amino acids and are especially rich in lysine and leucine, which are lacking in most staple cereal food. The low total fat content, and high proportion of polyunsaturated fatty acids (72-85%) relative to total fatty acids, is considered a significant contribution to the health value to mushrooms. Fresh mushrooms contain relatively large amount of carbohydrate and further range from 51-88% and 4-20% mushroom appear to be a good source of vitamin including thiamine, riboflavin, niacin, biotin and ascorbic acid (Andrae et al., 1999).

Most mushroom derived preparation and substances find their use not as pharmaceutical but as a novel class of dietary supplements (DS) or “nutraceutical”. A mushroom nutraceuticals is a refined or partially refined extract or dried biomass from either the mycelium or the fruiting body of the mushroom, which is consumed in the form of capsule or tablets as a dietary supplement and which may enhance the immune response of human body, thereby increasing resistance to disease and in some cases causing regression of a disease state (Wasser et al., 2000). Many pharmaceutical substances with potent and unique properties were isolated from mushroom and distributed worldwide (Wasser and Weis, 1999).

2.1.2. Medicinal properties of mushrooms

Fungi of various types normally inhabit the different parts of a plant body such as roots, stem and leaves. Some of these may be harmless while others may be weak or dangerous pathogens. A higher basidiomycetes mushrooms (HBM) are historically and economically highly praised for their nutritional value and acceptability, as well as their pharmacological properties. They include species from the Basidiomycetes class that have macroscopic fruit
bodies large enough to be seen by the naked eye, and usually picked by hand. HBM contain approximately 10000 species from 55 genera and 80 families (Wasser and Weis, 1999).

The characteristic features of higher Basidiomycetes could be divided into terrestrial or hypogenous, lignicolous or saprobic, mycorrhizal or pathogenic and edible, medicinal, hallucinogenic and poisonous mushrooms. Among the medicinal mushrooms, poisonous and some inedible had a very prominent place in the folklore; represent a major and as yet largely untapped source of powerful new pharmaceutical products. Edible ones are known to possess all essential amino acids, minerals, vitamins etc in adequate quantities and low sugars (Chang and Miles, 1982). Among the 14,000 species known, 2000 are safe and about 600 have significant pharmacological properties (Wasser et al., 2000). Most of the traditional knowledge about medicinal properties comes from the Far East (China, Japan, Korea, and Russia). Anticancer drugs isolated from mushrooms such as *Lentinus edodus*, *Coriolus versicolor* and *Schizophyllum commune* are sold in Japan (Table 2.1) (Jong and Birmingham, 1992).

Medicines from poisonous mushrooms have been considered in China as ‘poison as an antidote for poison’ (Yang and Jong, 1989). Various preparations are still used as medicine e.g. *Amanita muscaria* used therapeutically as a powder, tincture for swollen glands, nervous troubles and epilepsy etc. A lotion made out of this can be used externally and internally for the ailments of heart and inflammation of eye. *Amanita phalloids* is used against cholera and intermittent fever. Psilocybin and psilocin are two other drugs extracted from *Psilocybe mexicana* used to treat mental disorders (Bahl, 1987). Significant pharmacological effects or physiological properties of mushrooms are bioregulation (immunological enhancement), maintenance of homeostasis, and regulation of biorhythm, cure of various diseases and prevention and
improvement of life threatening diseases such as cancer, cerebral stroke and heart diseases. It is also confirmed that mushrooms have effective substances for antifungal, anti-inflammatory, antitumor, antiviral, antibacterial, hepatoprotective, antidiabetic, hypolipidemic, antithrombotic, hypotensive and other applications (Wasser and Weis, 1999). Medicinal properties of some of the commercially/non-commercially cultivated mushrooms are given in table 2.3. Recently extracts from fruiting bodies and mycelia of *Ganoderma* species and *Volvariella* found to possess *in vitro* antioxidant activity (Jones and Janardhanan, 2000; Lakshmi *et al.*, 2003; Mau *et al.*, 2002; Mathew *et al.*, 2008) and antimutagenic activities (Lakshmi *et al.*, 2003). Oyster mushrooms (species of genus *Pleurotus*) are highly edible and nutritious, rank second among the commercially cultivated mushrooms in the world (Chang, 1999) and are demonstrated to possess antioxidant, anti-inflammatory and antitumor activities (Jose and Janardhanan, 2000).

### 2.1.3. *Volvariella volvacea*

*Volvariella volvacea* (also known as straw mushroom or paddy straw mushroom) is a species of edible mushroom cultivated throughout East and South East Asia and used extensively in Asian countries. In Chinese they are called caoga. They are often available fresh in Asia, but are more frequently found in canned or dried from outside their nations of cultivation. Straw mushrooms are grown on rice straw beds and picked immature, before the caps open. They are adaptable and take 4 – 5 days to mature, and are most successfully grown in subtropical climates with high annual rainfall. There is no record of their cultivation before 19th century. They look similar to poisonous death caps, but can be distinguished by their pink spore print, which is white for death caps. Many sources list *Volvariella* as a member of the *Pluteaceae* family, but recent DNA studies have revealed that *Pluteus* and *Volvariella* evolved separately and have very different DNA. These studies
show that *Volvariella* is very closely related to *Schizophyllumoid* mushrooms like *Schizophyllum commune*. The genus *Volvariella* comprised a group of several species which can be found growing on a variety of substrates in tropical and subtropical regions.

List of species:
- *Volvariella bombycina*
- *Volvariella gloiocephala*
- *Volvariella hypopithys*
- *Volvariella jamaicensis*
- *Volvariella lepiotospora*
- *Volvariella peckii*
- *Volvariella specioisa*
- *Volvariella surrecta*
- *Volvariella volvacea*

At present time *V. volvacea* is the third most important cultivated mushroom reaching the total production of 287 metric tons (Chang and Miles, 1993). In India Su and Seth (1940) have first cultivated straw mushroom but the scientific cultivation using spawn was successfully demonstrated by Thomas *et al.* (1943). It is commonly known as Chinese mushroom, the most favorite mushroom in South Asian countries because of its excellent delicacy, high protein, amino acid, vitamins and minerals contents (Thakur and Yadav, 2006).

### 2.1.4. Importance of *Volvariella volvacea*

- *Volvariella* sp. contains moderate quantities of good quality protein and is good sources of dietary fiber, vitamin C, vitamin B and minerals.
- Lipids levels are low but unsaturated to saturated fatty acid ratio are high (about 2.0-4.5: 1).
- *V. volvacea* contain toxic substance such as the heat labile cardio toxic proteins volva toxin.

- Extensive clinical studies, have clearly demonstrated that this has medicinal and therapeutic value by injection or oral administration for the prevention or treatment of cancer, viral diseases (influenza, polio), hypercholesterolemia, blood platelet aggregation and hypertension.

- Many of the active substances which include polysaccharide (e.g.: β-glucans), nucleic acid derivatives (the hypercholesterolemia eritadenine) lipids, peptides and proteins and glycoprotein have been isolated and identified.

- Some of the mechanism of activity have been elucidated e.g.: antiviral activity via stimulation of interferon production in the host. Additional medicinal claims less well documented may nonetheless have some validity and merit.

- They lower serum cholesterol. They have strong antitumor and antiviral properties.

- They are very low in fat content, no starch, more vitamin B12 than meat and fish.

- Several proteins from edible fungi such as *V. volvacea* share similar amino acid sequence and immunomodulatory functions. These proteins were named as fungal immunomodulatory proteins.

*V. volvacea* lectin was isolated and purified from the fruiting body by extraction with 5% cold acetic acid in the presence of 0.1% 2-mercaptoethanol, followed by ammonium sulphate fractionation, DEAE-C-52 column chromatography (Lin and Chou, 1984). The molecular weight is estimated to be 26 K Da, and the lectin comprises two dissimilar subunits as evidenced by gel filtration and SDS-PAGE. The lectin is lacking in half-cystine, methionine and histidine but the amino acids aspartic acid, glutamic
acid, tyrosine and leucine occur frequently. Its haemagglutinating activity is preserved after incubation at 80°C for 2h, but 25% of the activity is destroyed when the lectin is subjected to 90°C for 30 min. The haemagglutinating activity of the lectin is not thwarted by any of the common simple sugars. It is likely that the lectin has an affinity for complex carbohydrate structures on the cell surface. The *V. volvacea* lectin is toxic its LD₅₀ in mice is 17.5 mg kg⁻¹ bodyweight. It has a mild retarding effect on the growth of tumour cells. The life span of the control group, after injection of 0.2ml of ascetic fluid containing 2x10⁷ sarcoma 180 cells/mouse, was 12.5 ± 5 days. The tested group, which was given in addition 85 mg of the lectin intraperitoneally, had their life span lengthened by 63%. The group which received 175 mg of the lectin experienced about 100% prolongation of life span (Lin and Chou, 1984).

Recently some groups of workers reported the purification of fungal immunomodulatory protein designated FIP-*vvo* from *V. volvacea* (Hsu et al., 1997). Its haemagglutinating activity, which is not affected by monosaccharides and disaccharides, and its molecular weight as estimated by gel filtration (26 KDa) and SDS-PAGE, suggest that it is similar to the previously isolated lectin at least in some respects (Lin and Chou, 1984). The protein is unglycosylated and blocked at the N-terminus. It maximally stimulates proliferation of human peripheral blood lymphocytes *in vitro* at 5mg ml⁻¹. The generation of BSA- induced Arthus reaction in mice is drastically attenuated after treatment with the protein *in vitro*; but systemic anaphylaxis reactions can hardly be prevented. Transcriptional expression of interleukin – 2, interleukin – 4, interferon-γ, tumour necrosis factor –α, lymphotoxin and interleukin – 2 receptor is selectively augmented, indicating that FIP-*vvo* exerts its immunomodulatory effects via cytokine regulation. The complete aminoacid sequence of FIP-*vvo* has been elucidated (Hsu et al., 1997).
Homology was detected with those of the immunomodulatory proteins from *Flammulina velutipes* and *Ganoderma lucidum*.

### 2.2. Free radicals

#### 2.2.1. Introduction and significance of free radicals

‘Free radical’ can be defined as an atom or a group of atoms having an unpaired electron. Because of the presence of unpaired electron, free radicals are highly reactive. In popular scientific/biomedical literature the term 'free radical' is used in a broad sense and also includes related reactive species such as 'excited states' that lead to free radical generation or those that results from free radical reactions. In general, free radicals are extremely short lived, with half-lives in milli-, micro- or nanoseconds. The area of research dealing with this frontier area of biomedical sciences is termed ‘free radical biology’. In the human body, most of the free radicals capable of influencing cellular functions are derived from oxygen. Because of its paramagnetic nature, oxygen has spin restriction that forbids it from reacting freely with other molecules. In biological systems incomplete reduction of oxygen gives rise to a large number of free radicals and related reactive species collectively known as 'reactive oxygen species' (ROS).

During cellular respiration molecular oxygen undergoes incomplete reduction in the mitochondria producing superoxide (\(O_2^-\)), hydrogen peroxide (\(H_2O_2\)), hydroxyl radical (\(^{•}OH\)), and finally water. Superoxide is formed by the addition of one electron to ground state dioxygen. It is unstable due to its spontaneous reaction with another \(O_2^-\) forming \(H_2O_2\) and \(O_2\) (dismutation reaction) (Fridovich, 1989). \(H_2O_2\) is very harmful to cells since it may cross biological membranes and also can form highly reactive \(^{•}OH\) radicals. Another group of reactive species, the reactive nitrogen species (RNS), have both nitrogen and oxygen and include physiologically important nitric oxide
(NO\(^*\)) and toxic peroxynitrite (ONOO\(^-\)). All reactive species have several biological implications (Table 2.4 and 2.5) (Thomas and Kalyanaraman, 1997; Halliwell and Gutteridge, 2000; Yoshikawa et al., 2000). Apart from ROS and RNS, there are also other reactive species such as carbon-centered radicals, triplet carbonyls, thiyl radicals and thiyl peroxyl radicals that are formed in biological systems. Under certain conditions these are also important.

2.2.2. Generation of free radicals in biological systems

Free radicals are being constantly generated in our body by a large number of reactions involving either endogenous systems, exogenous xenobiotics or during exposure to physicochemical agents and pathological conditions (Kelly et al., 1998).

2.2.2.1. Endogenous sources of free radicals

In our body, normal metabolism generates a number of free radicals that are either required for carrying out biochemical reactions, being produced as signaling molecules or as by-products of metabolism. The endogenous systems that generate free radicals include:

(i) cellular respiration in mitochondria that involves the reduction of molecular oxygen (O\(_2\)) to water in the electron transport chain.

(ii) functioning of the microsomal electron transport involving cytochrome P-450 during drug metabolism generating O\(_2^\cdot\).

(iii) during release of free metal ions and haemoproteins, iron and copper salts promote generation of oxidizing radicals like \(^*\)OH from peroxides, by a process known as Fenton reaction,

\[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{•OH}. \]

(iv) under certain conditions, peroxisomes, the organelles responsible for degrading fatty acids and other molecules, produce H\(_2\)O\(_2\) as a by-product and this ROS along with O\(_2^\cdot\) produces more potent \(^*\)OH.
(v) auto-oxidation of natural compounds such as catecholamines, coenzyme Q\(_{10}\) and epinephrine generates O\(_2^•\) (in blood vessels catecholamine autooxidation is an important source of ROS generation which is released under certain pathophysiological conditions).

(vi) oxidizing enzymes like diamine oxidase, tryptophan dioxygenase, xanthine oxidase, lipoxygenase, cyclooxygenase, nitric oxide synthase (under low arginine conditions) guanyl cyclase, glucose oxidase, myeloperoxidase, lactoperoxidase and chloroperoxidase produce ROS (Sies, 1997; Halliwell and Gutteridge, 2000; Yoshikawa et al., 2000).

Recent studies by Wentworth et al. (2003) showed that antibodies, regardless of origin or antigenic specificity, could convert \(^1\)O\(_2\) into H\(_2\)O\(_2\) via a process that they have postulated to involve dihydrogen trioxide (H\(_2\)O\(_3\)). Regardless of antibody specificity, this process can be used to kill bacteria, thus linking recognition and killing within the same molecule of immune system. They suggested that the hydrolating species might be the hydrotrioxy radical (HO\(_3^•\)), and point to the remarkable potential of either H\(_2\)O\(_3^–\) or O\(_3^–\)-derivable species to act as a masked \(^•\)OH in a biological environment.

### 2.2.2.2. Exogenous sources of free radicals

There are many exogenous sources that generate ROS or RNS. These include redox cycling of xenobiotics, exposure to ionizing radiations such as X-rays and \(\gamma\)-rays, besides visible light or UV in the presence of oxygen and a photosensitizer. This latter can be in the form of cytochromes, porphyrins, riboflavins, tetracycline, phenothiazines, chemical toxicants or air pollutants (Devasagayam and Kesavan, 1996). Cigarette smoke contains a large amount of reactive species including several oxides of nitrogen and stable free radicals such as semiquinone radicals. Smokes from other sources are also potent generators of these free radicals (Devasagayam and Kamat, 2002).
2.2.2.3. Radiation as a source of free radicals

To a large extent biological effects are mediated through the action of radiation on water, which represents about 80% of the weight of living organisms. When cells are exposed to radiation, the water molecules undergo dissociation (radiolysis) producing free radicals. The first step in radiolysis of water is the absorption of energy. When a water molecule absorbs radiation energy, it becomes ionized or excited. During ionization, the radiation energy is transferred to an orbital. As a result, that electron is removed, creating a positively charged radical ion of water (Hall and Keriakes, 1998).

\[
\text{H}_2\text{O} \quad \rightarrow \quad \text{H}_2\text{O}^+ + e^-
\]

This reaction needs about 13eV of energy. These ions are short lived, with half-life of about $10^{-10}$ s and will soon decay to produce uncharged free radicals.

\[
\text{H}_2\text{O}^+ \quad \rightarrow \quad \text{OH}^+ + \text{H}^+
\]

At lower energies the water molecules get excited

\[
\text{H}_2\text{O} \quad \rightarrow \quad \text{H}_2\text{O}^*
\]

The excited water molecules are not stable and give rise to H\(^+\) and OH radicals

\[
\text{H}_2\text{O}^* \quad \rightarrow \quad \text{OH} + \text{H}^+
\]

The \(^{\cdot}\)OH radicals have a lifetime of about 10\(^{-9}\)s. They are powerful oxidizing agents. Some of these \(^{\cdot}\)OH and \(^{\cdot}\)H radicals may recombine to form water. But, those with sufficient energy will react with biological molecules causing damage. The electrons acquire kinetic energy and will move away from the site of formation, which reduces their chances of recombination. They lose their energy by collisions and are finally captured by water molecules, forming aqueous electrons

\[
e^- + \text{H}_2\text{O} \quad \rightarrow \quad e_{aq}^-
\]

The aqueous electrons are very strong reducing agents and cause dissociation of water molecules lying in their vicinity forming OH and H radicals.
They may also react with hydrogen ions and get neutralized. Two OH radicals may interact to form hydrogen peroxide.

\[
\cdot \text{OH} + \cdot \text{OH} \rightarrow \text{H}_2\text{O}_2
\]

This last reaction depends upon the radiation quality as described by its linear energy transfer (LET) and is more frequent with high LET radiations.

### 2.2.2.4 Free radical generation during patho-physiological conditions

The following pathophysiological conditions also lead to the generation of ROS/RNS (Sies, 1996; Thomas and Kalyanaraman, 1997). During phagocytosis, phagocytic cells (neutrophils and monocytes) destroy bacteria or virus-infected cells with an oxidative burst, releasing several reactive species such as NO\(^*\), O\(_2\)^*\(\cdot\), ONOO\(^-\), \(\cdot\)OH, H\(_2\)O\(_2\), hypochlorous ion (OCl\(^-\)) and \(\cdot\)O\(_2\).

During chronic infection leading to inflammation, various ROS/RNS are generated. Nitric oxide, an RNS, reacts with O\(_2\)^*\(\cdot\) to form the highly reactive ONOO\(^-\). The free radical NO\(^*\), first described as endothelium derived relaxation factor (EDRF), is produced from arginine by nitric oxide synthase (NOS).

\[
\text{L-Arg} + \text{O}_2 + \text{NADPH} \rightarrow \text{NO}^* + \text{citrulline} + \text{NADP}
\]

An inducible nitric oxide synthase (NOS) is capable of continuously producing large amounts of NO\(^*\). In activated immune cells (phagocytes) it acts as a ‘killer molecule’. Although the direct toxicity of NO\(^*\) is modest, it gets greatly increased when it reacts with O\(_2\)^*\(\cdot\) to form peroxynitrite, a very strong oxidant.

\[
\text{NO}^* + \text{O}_2^\cdot \rightarrow \text{ONOO}^-
\]

While it is clear that cells and tissues containing NOS and extracts from them can make NO\(^*\), this does not necessarily mean that it is the initial RNS formed.
Nitroxyl (NO\textsuperscript{−}) ion or the protonated species (HNO) could in principle be formed first. If nitroxyl is the product of NOS, its reaction with oxygen can form peroxynitrite. This reaction has a much lower rate constant than that of NO\textsuperscript{•} with O\textsuperscript{2−}; but this is offset by the much higher concentration of oxygen available (Alderton \textit{et al.}, 2001).

\[ \text{NO}\textsuperscript{•} + \text{O}_2 \rightarrow \text{ONOO}^{-} \]

In certain clinical conditions there is a stoppage and restoration of blood supply (ischaemia-reperfusion). During this process oxidants like O\textsuperscript{2−}, \textsuperscript{•}OH and H\textsubscript{2}O\textsubscript{2} are produced. Such occurrences are called 'oxygen paradox', since though oxygen is essential; its supply causes damage due to generation of ROS and RNS. This occurs during non-fatal myocardial infarction, surgeries, stroke, transplantation and blockage of arteries under pathological conditions. During ischemia in the heart (in myocyte mitochondria) conversion of ATP to adenosine causes the generation of O\textsuperscript{2−}; while in the blood vessels (endothelium) the pathway involved in the transition from xanthine to uric acid generates superoxide (Liversidge \textit{et al.}, 2002; Cooper, 2002). In cardiovascular diseases involving angiotensin II (Ang II), ROS acts as critical signaling molecules in a wide range of cellular processes.

2.2.3. Free radicals: Friends or Foes?
The events of World War II (1939-1945) led directly to the birth of free radical biochemistry. The two atom bombs (in August 1945, Hiroshima and Nagasaki) led to massive deaths of an entire population, and the survivors had short life spans. After the discovery of free radicals, in 1956, Harman proposed the free radical-induced damage as a cause of ageing. Since then free radicals have gained notoriety. But it has to be emphasized that ROS and RNS are both produced in a well-regulated manner to help maintain
homeostasis at the cellular level in the normal healthy tissues and play an important role as signaling molecules. Most cells can produce superoxide (O$_2^\cdot$), H$_2$O$_2$ and NO$^\cdot$ on demand.

Hence it is worth emphasizing the important beneficial roles of ROS and RNS, as given below:

- Generation of ATP (universal energy currency) from ADP in the mitochondria: oxidative phosphorylation
- Detoxification of xenobiotics by cytochrome P450 (oxidizing enzymes)
- Apoptosis of effective or defective cells
- Killing of micro-organisms and cancer cells by macrophages and cytotoxic lymphocytes
- Oxygenases (e.g. cyclo-oxygenase, lipo-oxygenase) for the generation of prostaglandins and leucotrienes, which have many regulatory functions.
- ROS exert critical actions such as signal transduction, gene transcription and regulation of soluble guanylate cyclase activity in cells

2.2.4. ROS/RNS as second messengers to modulate signaling pathways
In recent years, it has become interestingly evident that ROS can function as second messengers and, at low levels, can activate signaling pathways resulting in a broad array of physiological responses from cell proliferation to gene expression and apoptosis. NO$^\cdot$ produced by endothelial cells is essential for regulating relaxation and proliferation of vascular smooth muscle cells, leukocyte adhesion, platelet aggregation, angiogenesis, thrombosis etc. (Liversidge et al., 2002; Cooper, 2002).
- NO$^\cdot$ produced in neurons acts as a neurotransmitter
- Exogenous H$_2$O$_2$ can mimic the activity of the insulin growth factor
Thus the presence of free radicals can be advantageous for cells. However, damaging effects as a result of overproduction of ROS and RNS are due to their high reactivity. They are also potentially toxic, mutagenic and carcinogenic. The targets for ROS-induced damage include all major groups of biomolecules such as lipids, proteins and DNA. Their alterations can eventually lead to diseased states.

2.3. Oxidative stress

Oxidative stress is the situation of serious imbalance between production of ROS /RNS and antioxidant defense. According to Seis (1991), oxidative stress is a disturbance in the prooxidants-antioxidant balance in favor of the former, leading to potential damage. Oxidative stress can results from diminished antioxidants or increased production of ROS/RNS. In a normal healthy state generation of pro-oxidants in the form of ROS/RNS by various biochemical reactions are effectively neutralized by defense systems present in our body in the form of antioxidants. However, when our body gets exposed to different physicochemical and pathological agents there is an excess generation of pro-oxidants present. Hence the delicately maintained balance is shifted in favor of pro-oxidants resulting in oxidative stress.

Oxidative stress may results in adaptation, cell injury or cell death. Cells can usually tolerate mild oxidative stress, which often results in up-regulation of the synthesis of antioxidant defense systems in an attempt to restore the oxidant /antioxidant balance. This up- regulation of oxidative defenses helps the cells to protect against much more severe oxidative stress applied subsequently leading to adaptation. Moderate oxidative stress may lead to cell injury and cells exposed to severe oxidative stress may die. DNA and lipids are the primary targets of oxidative damage. Cell death can result from multiple mechanisms, such as bleb rupture. Cell death occurs essentially by
two mechanisms, necrosis and apoptosis, based on the severity of oxidative stress. Oxidative stress also has striking effects on cell calcium metabolism, resulting in rises in free intra cellular calcium levels, leading to cell injury and death by apoptosis or necrosis.

Certain environmental agents can also deplete cellular antioxidants thereby reducing the defense that normally counteracts the pro-oxidants, leading to oxidative stress. There are many environmental agents that can induce tissue damage through oxidative stress. These include extreme high and low temperatures, trauma, ultra sound, excess of exercise, ionizing and non-ionizing radiations. Apart from this, infection, pathological conditions, hypoxia and bacterial/chemical toxins, air pollutions, also induce oxidative stress. During this phenomenon, the pro-oxidants are free to act and damage crucial cellular molecules leading to severe alteration of various tissues. This can eventually lead to diseased states.

2.3.1. Ionising radiations and oxidative stress
Whenever a large enough particles, such as iron, passes through a cell, it is capable of physically damaging molecules. The absorption of energy from radiation in biologic material may lead to excitation or to ionization. If the radiation has sufficient energy to eject one or more orbital electrons from the atom or molecule, the process is called ionization, and that radiation is said to be ionizing radiation. The important characteristic of ionizing radiation is the localized release of large amounts of energy. It has long been recognized that the damaging effects of ionizing radiation are brought about by both direct and indirect mechanisms. The direct action produces disruption of sensitive molecules in the cells whereas the indirect actions of ionizing radiation occur when it interacts with water molecules in the cell, resulting in the production of highly reactive free radicals, such as •OH, •H, and e\textsubscript{aq} \cdot. High energy
radiation breaks chemical bonds and this creates free radicals, like those produced by other insults as well as by normal cellular processes in the body. The free radicals can change chemicals in the body. The half life of these free radicals is extremely short, (10–6–10–10 seconds); however, they immediately react with any biomolecules in the vicinity and produce highly site-specific oxidative damage. These changes can disrupt cell function and may kill cells (Shirazi et al., 2007).

Gamma irradiation disrupts water molecules, producing hydroxyl radicals and thus leading to oxidative damage and apoptosis in dividing cells. Biological molecules are susceptible to physical and chemical modifications whenever they are exposed to ionizing radiation. An estimated 60%–70% of tissue damage induced by ionizing radiation is believed to be caused by •OH radicals. The other form of oxidative damage most recognized to result from radiation exposure is oxidative damage to biomolecules, including proteins, lipids, and DNA results from the formation of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radicals. ROS can induce the cellular antioxidant defence enzymes such as superoxide dismutase and glutathione peroxidase. Reactive oxygen species (ROS) and free radicals induced by partial reduction of oxygen (O₂) react with cellular macromolecules (i.e., nucleic acids, lipids, proteins, and carbohydrates) and damage them. This becomes critical in the case of DNA, resulting in mismatch repair and chromosomal rearrangement in which the DNA strands do not reattach to the correct corresponding piece of DNA, or segments of DNA are deleted altogether. In addition to being mutagenic, these ROS are carcinogenic in animals and humans because of their likelihood of forming oxidative adducts with the nucleic acids of DNA. The most prevalent oxidative DNA adducts, 8-oxodeoxyguanosine, causes G-to-T transversions that have been implicated in carcinogenesis. A series of glycosylates are involved in repairing
a variety of oxidative DNA adducts, including 8-oxodeoxyguanosine. Defects in the nucleotide excision repair pathway compromises DNA transcription, which leads to accelerated ageing, loss of bone mineral density, and cancer predisposition. When sufficient levels of ROS are detected by a cell, apoptosis can be triggered, thereby removing the potentially damaged cell from the population and reducing the likelihood it would continue towards tumor formation.

Major biomarkers of oxidative damage to living cells are (i) lipid peroxidation (LPO) products, comprising volatile hydrocarbons measurable in exhaled air, such as ethane and pentane, and isoprostanes and aldehydic products measurable in tissues and body fluids; (ii) DNA hydroxylation products (e.g., 8-hydroxy-2′-deoxyguanosine (8-OHdG)) and microscopic indices of damage such as chromosomal aberrations and micronuclei; and (iii) protein hydroxylation products such as oxidized amino acids.

2.3.2. Cancer therapy and oxidative stress

Cancer is a class of diseases or disorders characterized by uncontrolled division of cells and the ability of these cells to spread, either by direct growth into adjacent tissue through invasion or by implantation in to distant sites by metastasis. The role of ROS in different stages of carcinogenesis such as tumour initiation, promotion and progression is described (Athar, 2002). Cancer may affect people at all ages, but risk tends to increase with age. It is one of the principal causes of death in developed countries. There are many types of cancer. Severity of symptoms depends on the site and character of the malignancy and whether there is metastasis. Most cancers can be treated and some cured, depending on specific type, location, and stages. As research develops, treatments are becoming more specific for the type of cancer pathology. Although substantial progress has been made in the
chemotherapeutic design and implementation, metastatic cancer is a disease for which definite treatment is rarely predictable. If untreated, cancers may eventually cause illness and death, though this is not always the case.

Three established strategies are used to kill cancer cells in the patient’s body. They are physical removal of tumor mass (resection), which is the foundation of surgery, radiotherapy and chemotherapy. Once diagnosed, cancer is usually treated with a combination of surgery, chemotherapy and radiotherapy. Surgery procedures are used to physically remove malignant tissues, and it remains one of the important modality of treatment for malignant tumors. It has a role in cure, prevention, diagnosis, staging and papillation. It may also increase the sensitivity to other therapeutic modalities. Ionic radiation continues to be a curative option for many patients alone or in combination with other modalities. Mammalian cells are most sensitive to radiation-induced damage in the late G2 and M-phase of the cell cycle. Proliferation tissues are reported to be more sensitive to radiation than non-proliferative ones. Curative radiation treatment involves selective destruction of the proliferative capacity of tumor cells while sparing normal tissues within and around the irradiated volume to the degree that they can carry out necessary functions. The purpose of treating cancer with chemotherapeutic agent is to prevent cancer cells from multiplying, invading, metastasizing and ultimately killing the host (patients). The era of modern chemotherapy may be started in 1948 with the introduction of nitrogen mustard. Most agents currently in use appear to exert their effects primarily on cell multiplication and tumor growth. Because cell multiplication is characteristic of many normal cells as cancer cells, most of the chemotherapeutic agents also have toxic effects on normal cells. Inhibition of cell multiplication and tumor growth takes at several levels within the cell.
Chemotherapy involves several classes of drugs: - hormones (prednisone), antimetabolites (5-flurouracil), plant products (camptothecin, etoposide) and antibiotics (doxorubicin, bleomycin). Most of these work inhibiting by some metabolic pathway or DNA synthesis, which ultimately lead to cytotoxicity. Though chemotherapy has limited use for localized tumours, it is often the most effective agent for the management of disseminated or systemic cancer. These include the hematological malignancies (leukemia, lymphoma), metastasis of the primary solid tumor and potential micro metastasis after surgery or radiation (De Vita et al., 2001). The main goal of cancer chemotherapy is to eradicate the malignant disease while minimizing severe toxic effects (Paule et al., 1998). More than hundred drugs are currently used for chemotherapy, either alone or in combination with other drugs or treatments. Drugs that target specific cancers already exist for several cancers. The discovery of drugs for the effective treatment of cancer is an extra ordinary challenge. The drug must kill or disable a variety of subpopulations of a tumor, without any harm to normal tissue of the same origin and also to normal tissue of other types throughout the body.

Ionizing radiations and some chemotherapeutic drugs are capable to inflict deleterious effects to living cells by causing damages to cellular membranes and DNA. Although, radiotherapy and chemotherapy are the common and effective tools for cancer treatment; the chemo-toxicity and radio-sensitivity of normal tissues especially adjacent to the tumor which are unavoidably exposed to radiation and chemicals, limit the therapeutic gains. Protection of normal tissues against cellular damage is of immense importance in radiotherapy and chemotherapy. The interaction of ionising radiations or some chemotherapeutic drugs with biological systems results in generation of free radicals. Radio and chemo protective compounds are highly important because of their relevance in military, space expeditions, nuclear accidents
and industrial applications. A wide variety of naturally occurring and synthetic drugs used for treating several illness exhibit efficient free radical scavenging properties and these could act as radio and chemo protectors. The identification of a novel, nontoxic, effective and convenient compounds to protect human against radiation and chemical induced tissue injuries is of paramount importance for effective chemo and radiotherapy.

2.3.3. Oxidative stress mediated damage to bio molecules

Oxidative stress can cause damage to all types of biomolecules including DNA, proteins, lipids and carbohydrates. In many situations it is unclear that which is the most important target, since injury mechanisms overlap widely. The primary cellular target of oxidative stress can vary depending on the cell, the type of stress imposed and how severe the stress is.

2.3.3.1. Damage to DNA

ROS can cause oxidative damages to DNA, both nuclear and mitochondrial. The nature of damages includes mainly base modification, deoxyribose oxidation, strand breakage, and DNA–protein cross-links. Among the various ROS, OH• generates various products from the DNA bases which mainly include C-8 hydroxylation of guanine to form 8-oxo-7,8 dehydro-2¢-deoxyguanosine, a ring-opened product; 2,6-diamino-4-hydroxy-5-formamimidopyrimidine, 8-OH-adenine, 2-OH-adenine, thymine glycol, cytosine glycol, etc. ROS-induced DNA damages include various mutagenic alterations as well. For example, mutation arising from selective modification of G:C sites specially indicate oxidative attack on DNA by ROS. The action of 8-oxodeoxy- guanosine as a promutagen, as well as in altering the binding of methylase to the oligomer so as to inhibit methylation of adjacent cytosine has been reported in cases of cancer development. ROS have also been shown to activate mutations in human C-Ha-ras-1 protooncogene, and to induce
mutation in the p53 tumour-suppressor gene. Besides, ROS may interfere with normal cell signaling, resulting thereby in alteration of the gene expression, and development of cancer by redox regulation of transcriptional factors/activator and/or by oxidatively modulating the protein kinase cascades. ROS also induce various early response or stress-response genes like c-fos, c-jun, jun-B, jun-D, cmyc, erg-1, and heme oxygenase-1. Activation of the early response proto oncogenes plays a vital role in signal transduction, leading to cell proliferation and transformation.

ROS/RNS can lead to DNA damage by direct chemical attack on DNA, and also by indirect mechanisms. Direct damages occur to the purine/ pyrimidine bases and/or to the deoxyribose sugar. Indirect mechanisms include the activation of Ca²⁺ dependent endonucleases as a consequence of rises in intracellular free Ca²⁺, and interference with enzymes that replicate or repair DNA. Much of the cell damage caused by ionising radiation involves the formation of OH• radicals by homolysis of water. Ionising radiation mediated oxidative stress can produce base and sugar modifications, single and double strand breaks. Double strand breaks are the most important damage caused by ionising radiations. Double strand breaks may be caused by a large amount of radiation energy being deposited in one place, causing multiple attacks by OH• on the same short stretch of DNA. Nuclear proteins can be attacked by radiations and the resulting protein derived radicals can cross link to base derived radicals giving DNA – protein cross links that interfere with chromatin unfolding, DNA repair, replication and transcription. Irradiation or treatment with toxins like theothyline, theobromine and 3-amino benzamide leads to depletion of cellular ATP and NAD⁺ levels with excessive DNA damage, often maintaining cell viability. The depletion of NAD⁺ levels often occurs because a chromatin bound enzyme, poly (ADP-ribose) polymerase (PARP) splits the NAD⁺ molecule and transfers the ADP
ribose portion on to nuclear proteins, including itself (auto modification). ADP ribosylation proteins are thought to facilitate DNA repair. However excessive activation of PARP can deplete the NAD+ pool, interfering with ATP synthesis and perhaps even leading to cell death. This effect has sometimes been called a suicide response. DNA damage plays an important role in a number of disease processes such as in carcinogenesis and neurodegenerative diseases such as Alzheimer’s disease. The oxidative damage of mitochondrial DNA also involves base modification and strand breaks, which leads to formation of abnormal components of the electron transport chain. This result in the generation of more ROS through increased leakage of electrons therefore further cell damage and may promote cancer and ageing.

2.3.3.2. Damage to lipids
Membrane lipids present in subcellular organelles are highly susceptible to attack by free radicals. Lipids, on reaction with free radicals can undergo the highly damaging chain reaction of lipid peroxidation (LP) leading to both direct and indirect effects. Since membranes form the basis of many cellular organelles like mitochondria, plasma membranes, endoplasmic reticulum, lysosomes and peroxisomes, the damage caused by LP is highly detrimental to the functioning of the cell and can even lead to cell death (Devasagayam et al., 2003).

Lipid peroxidation can be a major contributor to the loss of cell function under oxidative stress situations. LP damages cells ‘directly’ by attacking membrane structures and ‘indirectly’ by releasing reactive products. The process of LP gives rise to many ROS such as peroxyl and alkoxyl radicals, which are capable of disrupting cellular components and causing cell death. The major products of LP of toxicological interest are malondialdehyde (MDA), 4-hydroxynonenal (4-HNE), various 2-alkenals and F2-isoprostanes,
which are unique products of lipid peroxidation. Peroxidation in microsomal membranes has been shown to lead to calcium release and uncontrolled activation of calcium – dependent proteases and lipases and peroxidation and permeabilization of mitochondrial membranes can induce disruption of cellular energetics. The overall effects of lipid peroxidation are to decreased membrane fluidity, make it easier for phospholipids to exchange between the two monolayers, increase the ‘leakiness’ of the membrane bilayer to substances that do not normally cross it other than through specific channels and inactivate membrane bound enzymes. Cross linking of membrane proteins decreases their lateral and rotational mobility. Lipid peroxidation is particularly harmful in mitochondria, that contain cardiolipin as a major component of inner mitochondrial membrane, and which required for the activity of cytochrome oxidase. Peroxidation products in mitochondrial membranes were significantly greater than in microsomal membrane.

LP has been implicated in the pathogenesis of a large number of diseases and clinical conditions such as disorders associated with premature birth, diabetes, adult respiratory distress syndrome, aspects of shock, Parkinson’s disease, Alzheimer’s disease, various chronic inflammatory conditions, ischemia-reperfusion mediated injury to organs including heart, brain and intestine, atherosclerosis, organ injury associated with shock and inflammation, fibrosis and cancer, pre-eclampsia and eclampsia, inflammatory liver injury, type-1 diabetes, anthracycline induced cardiotoxicity, silicosis and pneumoconiosis (Halliwell and Gutteridge, 2000; Yoshikawa et al., 2000).

2.3.3. Damage to proteins
Apart from damage to DNA and lipids, protein damage induced by free radicals has significant implications in health and radiation injury. Free
radicals can oxidize proteins increasing their hydrophobicity and sensitivity to proteolysis. Free radical mediated damage to proteins may be initiated by electron leakage, metal-ion-dependent reactions and autoxidation of lipids and sugars. The consequent protein oxidation is oxygen-dependent, and involves several propagating radicals, notably alkoxyl radicals. The chemical reactions resulting from attack of reactive oxygen and nitrogen species (ROS/RNS) on proteins are complex and lead to a variety of products, many as yet uncharacterized. Among the reactive products, protein hydroperoxides can generate further radical fluxes on reaction with transition-metal ions; protein-bound reductants can reduce transition-metal ions and thereby facilitate their reaction with hydroperoxides; and aldehydes may participate in Schiff-base formation and other reactions. The oxidative damage to proteins is reflected by increase in levels of protein carbonyls (PCO) and decrease in levels of protein thiols (P-SH).

Attack by ROS upon proteins can damage several amino acid residues, including histidine, proline, arginine, and lysine. A number of oxidized proteins and amino acid species are found in biological systems. These include 2-oxohistidine, 3-chlorotyrosine, 3-nitrotyrosine, 5-hydroxy-2-aminovaleric acid, aminomalonic acid, dimers of hydroxylated aromatic acids, DOPA, hydro(pero)xy leucine, hydro(pero)xy valines, n-formyl kynurenine, o- and m-tyrosine, p-hydroxy phenyl acetaldehyde, protein carbonyls. Although most oxidized proteins that are functionally inactive and are rapidly removed, while some can gradually accumulate with time and thereby contribute to the damage associated with ageing as well as various diseases including diabetes, atherosclerosis and neurodegenerative diseases. Oxidative damage to several of these amino acid residues and/or to the peptide backbone of proteins can generate PCO products. Oxidative
inactivation of several enzymes has been associated with ageing as well as pathological states (Yohikawa et al., 2000).

2.3.3.4. Damage to carbohydrates

In common with other biological structures, carbohydrates are susceptible to the effects of oxygen free radicals. Their rate of reaction is dependent on structure. Free radicals such as OH\(^{-}\) react with carbohydrates by randomly abstracting a hydrogen atom from one of the carbon atoms, producing a carbon-centered radical. They oxidize monosaccharides and cause depolymerization of polysaccharides. This leads to chain breaks in important molecules like hyaluronic acid in a process involving intermediates such as peroxyl radicals. In the synovial fluid surrounding joints, an accumulation and activation of neutrophils during inflammation produces significant amounts of oxyradicals. This phenomenon apparently accounts for a significant decrease in the synovial fluid of affected joints, and is also being implicated in rheumatoid arthritis (Drinda et al., 2002). Advanced glycation end products (AGEs) is a class of complex products. They are results of reaction between carbohydrates and free amino group of proteins. Most of the AGEs are very unstable, reactive compounds and the end products are difficult to be completely analysed (Valko et al., 2007).

2.3.4. Oxidative stress and human diseases

Despite the existence of endogenous defense mechanisms against ROS, it has been observed that whenever either the level of the cellular antioxidant systems goes down or when the ROS reach abnormally high levels, oxidative damage to the cells occurs, finally leading to several pathological conditions. About 100 disorders, like rheumatoid arthritis, hemorrhagic shock, cardiovascular diseases, cystic fibrosis, metabolic disorders, neurodegenerative disease, gastrointestinal ulcerogenesis and AIDS have
been reported as the ROS-mediated disorders. Some specific examples of the ROS-mediated diseases are Alzheimer’s disease, Parkinson’s disease, oxidative modification of low-density lipoprotein in atherosclerosis, cancer, Down’s syndrome, and ischemic reperfusion injury in different tissues including heart, brain, kidney, liver, and gastro-intestinal tract. Among these, role of ROS in atherosclerosis, and ischemic injury in heart and brain have been studied extensively (Halliwell and Gutteridge, 1990). The major role played by ROS in stress-induced gastric ulcer and inflammatory bowel diseases has been recently established (Das et al., 1997; Murthy et al., 1997). The involvement of ROS in aging has been documented as well (Stadtman, 1991; Ames, 1993; Starke-Reed, 1997). The detailed mechanism of the oxygen-radical-mediated disease process has recently been reviewed (Thomas and Kalyanaraman, 1997). Halliwell (1998) has summarized the mechanism of formation of various oxygen-derived free radicals, and the role of antioxidant defense system in controlling development of pathological conditions.

2.4. Antioxidants

Antioxidants are substances that neutralize free radicals and attenuate free radical-induced damage. They can be enzymatic or non-enzymatic. Another definition of antioxidant is “any substance that when present at low concentrations compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substrate”. The term ‘oxidisable substrate’ includes almost everything found in foods and in living tissues including lipids, proteins, carbohydrates and DNA.

2.4.1. Levels of antioxidant action

Antioxidants act at different levels of action such as prevention, interception and repair. Preventive antioxidants attempt to stop the formation of ROS. These include superoxide dismutase (SOD) that catalyses the dismutation of
superoxide to H₂O₂ and catalase that further breaks it down to water. Other enzymes such as glutathione peroxidase (GPx), glutathione reductase (GR), glutathione transferase (GT) also help in non-radical decomposition of hydroperoxide and H₂O₂. It also involves sequestration of metal by chelation using transferrin, lactoferrin, hemoglobin, ceruloplasmin and albumin (Cadenas and Packer, 1996; Sies, 1996). In hyperthermophilic anaerobes, another enzyme, superoxide reductase (SOR) reduces O₂⁻ to H₂O₂ using electrons from NADPH. Hence it confers selective advantage to anaerobes (Cooper, 2002). Interception of free radicals is mainly by radical scavenging involving removal of primary radicals such as •OH, O₂⁻, ¹O₂ and NO• while at the secondary level scavenging of lipid peroxyl, protein peroxyl and DNA peroxyl radicals is effected. The effectors include various antioxidants like vitamins C and E, glutathione, other thiol compounds, bilirubin, albumin, ubiquinol, carotenoids and flavonoids. Polyphenols in foods, cosmetics and pharmaceutical preparations can readily donate an electron or a hydrogen atom to a peroxyl or alkoxyl radical to terminate a lipid peroxidation chain reaction. At the repair level, mainly lipases, proteases, transferases and DNA repair enzymes such as glutathione peroxidase, DNA glycosylases, DNA ligase and mismatch correction enzymes are involved. The de novo enzymes help in the repair of damage and reconstitution of membranes (Sies, 1996; Cadenas and Packer, 1996; Halliwell and Aruoma, 1993). At the adaptation level, there is generation of antioxidant enzymes and their transfer to the correct site at right time and in required concentration (Cadenas and Packer, 1996).

There are some natural barriers designed to protect the genome from radical attack. These include compartmentalization of the sensitive target molecules and shielding of non-replicating DNA by histones and polyamines. If protection of DNA is not successful, cellular regulatory mechanisms such as
induction of apoptosis and inhibition of cell cycle progression may prevent transfer of damaged DNA to the offspring. Alternatively, DNA repair processes can correct the damage (Halliwell and Aruoma, 1993; Cadenas and Packer, 1996; Sies, 1996). Antioxidants include polyphenols, lipoic acid, carotenoids, and tocotrienols. These 'neutraceuticals' have demonstrated greater antioxidant and anti-cancer activity than what has been achieved previously in nutritional protocols and cosmetics formula. The benefits of tocotrienols reach from decreasing platelet aggregation (clumping of blood) to anti-inflammatory action and anti-cancer activity.

Reactive oxygen and nitrogen species (ROS and RNS) have significant implications in human health. They are generated by many biochemical reactions in our body during metabolism and if left unchecked, they can cause adverse alterations in many biological molecules. This can lead to diseased conditions. Such damage has been implicated in the etiology of large number of human ailments, including cardiovascular diseases, neurological disorders, cancer, diabetes, etc. Hence, antioxidants capable of neutralizing these reactive species have potential health benefits (Yoshikawa et al., 2000; Devasagayam et al., 2004). Many epidemiological studies have revealed an inverse correlation between the levels of conventional antioxidant/phytonutrients present in tissue/blood samples and incidence of many human diseases such as cardiovascular disease, cancer, arteriosclerosis or mortality due to these diseases. Tocotrienols have reputed to have potent therapeutic effects due to their role in the prevention or delaying the onset of many such diseases.

Vitamins are prominent among natural or endogenous compounds that are considered to be beneficial. Vitamin E group of compounds are among the most visible of the vitamins due to their suggested health benefits including
antioxidant and related protective properties. Among these, tocotrienols have gained prominence in recent years due to their potential applications and better protective effects in certain systems. These tocotrienols are vitamin E derivatives that are analogues of the more established forms of vitamin E namely Tocopherol. Vitamin E is an efficient lipid soluble antioxidant that functions as a ‘chain breaker’ during lipid peroxidation in cell membranes and various lipid particles including LDL (Meydani et al., 1992). It has been suggested to help the normal functioning of various organs including heart, brain, lungs, skin and eyes besides in improving immunity (Ong and Packer, 1992; Packer and Ong, 1998; Kagan et al., 2002; Vivekananthan et al., 2003). Vitamin E is considered as the ‘standard antioxidant’ to which other compounds with antioxidant activities are compared, especially in terms of its biological activity and clinical relevance. In the antioxidant activity of vitamin E, a radical (R°/ROO°) abstracts a hydrogen atom from the aromatic hydroxyl group of the chroman head rather than from a poly unsaturated fatty acid, and a chromanoxyl radical is formed that is fairly unstable. In order to give efficient protection, vitamin E has to be regenerated from the vitamin E radical. It has been suggested that both vitamin C and GSH are able to mediate the regeneration of vitamin E. The daily dietary allowance varies between 400 IU to 800 IU (Sies, 1996; Thomas and Kalyanaraman, 1997; Stocker, 1999; Kagan et al., 2002). Vitamin C (ascorbic acid) is a water-soluble free radical scavenger (Frei, 1994) and it also regenerates vitamin E in cell membranes in combination with glutathione or compounds capable of donating reducing equivalents and maintains LDL particle integrity (Niki, 1987; Diaz et al., 1997). This vitamin is important in neutrophil functions and in preventing DNA damage (Wilson, 1983).
2.4.2. Natural antioxidants

Natural compounds with possible health benefits have become attractive targets for research in areas pertaining to human health. For both prevention and therapy of various human ailments, such compounds are preferred over synthetic ones due to their lesser toxicity. They are also easily absorbed and processed by our body. Natural products have long been a fertile source of cure for cancer, which is projected to become the major causes of death in this century. There are at least 250,000 species of plants out of which more than one thousand plants have been found to possess significant anticancer properties. While many molecules obtained from nature have shown wonders, there are a huge number of molecules that still remains to be trapped or studied in details by medicinal chemists.

Potential of herbs and other plant-based formulations have been increasingly recognized in prevention and treatment of human diseases including cancer. There exist enormous prospect for screening and evaluation of herbal/plant products for developing effective radio sensitization and radioprotection relevant to nuclear research program. In recent years, the use of some synthetic antioxidants has been restricted because of their possible toxic and carcinogenic effects. This concern has resulted in an increase interest in the investigation of the effectiveness of naturally occurring compounds with antioxidant properties (Duh et al., 1992), food rich in antioxidants have been shown to play an essential role in the prevention of cardiovascular diseases, cancer, neurodegenerative diseases, hepatotoxicity, inflammation and problems caused by cell and cutaneous ageing (Ames et al., 1993). Thus the natural antioxidants present in foods and other biological materials have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic effects (Ames et al., 1993).
Natural compounds in the diet provide functional antioxidants, such as vitamins, minerals and enzymes. Reduction of oxidation damage by such natural antioxidants provides a degree of protection against ionizing radiation injury. Although thiol synthetic compounds show good radioprotection, their toxicity at optimum protective doses promoted the search for alternatives to synthetic compounds that would be less toxic and highly effective. In general, natural radioprotectors have a lower degree of protection compared to synthetic thiol agents. Most of the antioxidants are also anti-tumor agents. Recent research has revealed the significant role of free radicals in human health and diseases. Free radicals and antioxidants have attracted lot of attention from both health professionals and basic scientists involved in human health care.
Table 2.1. Anticancer drugs from mushrooms sold in Japan

<table>
<thead>
<tr>
<th>Name</th>
<th>Source</th>
<th>Active principle</th>
<th>Route</th>
<th>Type of cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krestin</td>
<td><em>Coriolus versicolor</em></td>
<td>Protein bound polysaccharide</td>
<td>Oral</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>Lentinan</td>
<td><em>Lentinus edodes</em></td>
<td>β-1,3-D-glucans</td>
<td>Injection</td>
<td>Breast, lung</td>
</tr>
<tr>
<td>Schizophyllam</td>
<td><em>Schizophyllum commune</em></td>
<td>β-1,3-D-glucans</td>
<td>Injection</td>
<td>Stomach</td>
</tr>
</tbody>
</table>

(Adopted from Jong and Birmingham, 1992)

Table 2.2. Reported benefits of medicinal mushrooms

<table>
<thead>
<tr>
<th>Benefit</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Analeptic</td>
<td>Diuretic</td>
</tr>
<tr>
<td>Analgesic</td>
<td>Dyspepsia remedy</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>Hemostatic</td>
</tr>
<tr>
<td>Anticarcinogenic</td>
<td>Hemorrhoid remedy</td>
</tr>
<tr>
<td>Antimour</td>
<td>Hepatic function stimulating</td>
</tr>
<tr>
<td>Anticonvulsives</td>
<td>Hypoglycemic</td>
</tr>
<tr>
<td>Antidiarrhetic</td>
<td>Hypocholesterolemic</td>
</tr>
<tr>
<td>Antiphlogistic</td>
<td>Hypotensive</td>
</tr>
<tr>
<td>Antiparasitic</td>
<td>Immuno regulatory</td>
</tr>
<tr>
<td>Antipyretic</td>
<td>Laxative</td>
</tr>
<tr>
<td>Antirheumatic</td>
<td>Metabolic regulating</td>
</tr>
<tr>
<td>Antithrombotic</td>
<td>Mitogenic</td>
</tr>
<tr>
<td>Antitussive</td>
<td>Nephritis remedy</td>
</tr>
<tr>
<td>Antiviral</td>
<td>Radioprotection</td>
</tr>
<tr>
<td>Antistrigent</td>
<td>Respiratory stimulating</td>
</tr>
<tr>
<td>Chronic disease remedy</td>
<td>Sedative</td>
</tr>
<tr>
<td>Cardiotonic</td>
<td>Diaphoretic</td>
</tr>
<tr>
<td>Coronary heart disease remedy</td>
<td>Tonic</td>
</tr>
<tr>
<td>No</td>
<td>Medicinal properties</td>
</tr>
<tr>
<td>----</td>
<td>----------------------</td>
</tr>
<tr>
<td>1</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>2</td>
<td>Antifungal</td>
</tr>
<tr>
<td>3</td>
<td>Antiviral</td>
</tr>
<tr>
<td>4</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>5</td>
<td>Blood pressure regulation</td>
</tr>
<tr>
<td>6</td>
<td>Hypolipidemic</td>
</tr>
<tr>
<td>7</td>
<td>Antidiabetic</td>
</tr>
<tr>
<td>8</td>
<td>Immunomodulating</td>
</tr>
<tr>
<td>9</td>
<td>Kidney tonic</td>
</tr>
<tr>
<td>10</td>
<td>Hepatoprotective</td>
</tr>
<tr>
<td>11</td>
<td>Nerve tonic</td>
</tr>
<tr>
<td>12</td>
<td>Chronic bronchitis</td>
</tr>
</tbody>
</table>
### Table 2.4. Reactive oxygen species of biological interest

<table>
<thead>
<tr>
<th>Reactive oxygen species</th>
<th>Symbol</th>
<th>Half life</th>
<th>Reactivity/ Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide</td>
<td>O₂•</td>
<td>10⁻⁶ s</td>
<td>One-electron reduction state of O₂, formed in many autoxidation reactions and by the electron transport. Rather unreactive but can release Fe²⁺ from iron-sulfur proteins and ferritin. Undergoes dismutation to form H₂O₂ spontaneously or by enzymatic catalysis and is a precursor for metal-catalyzed ⋅OH formation.</td>
</tr>
<tr>
<td>Hydroxyl radical</td>
<td>⋅OH</td>
<td>10⁻⁹ s</td>
<td>Three-electron reduction state, formed by Fenton reaction and decomposition of peroxynitrite. Highly reactive, attacks most cellular components. Two-electron reduction state, formed by dismutation of O₂• or by direct reduction of O₂.</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>H₂O₂</td>
<td>10⁻⁹ s</td>
<td>Lipid soluble and thus able to diffuse across membranes. Yields potent species like ⋅OH.</td>
</tr>
<tr>
<td>Alkoxyl and Peroxyl radical</td>
<td>RO*/ROO</td>
<td>s</td>
<td>Oxygen centred organic radicals produced in the presence of oxygen by radical addition to double bonds or hydrogen abstraction.</td>
</tr>
<tr>
<td>Organic hydroperoxide</td>
<td>ROOH</td>
<td>Stable</td>
<td>Formed by radical reactions with cellular components such as lipids and nucleobases. Reacts with transient metal ions to yield reactive species.</td>
</tr>
<tr>
<td>Reactive Nitrogen species</td>
<td>Symbol</td>
<td>Half life</td>
<td>Reactivity/ Remarks</td>
</tr>
<tr>
<td>---------------------------</td>
<td>--------</td>
<td>----------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>NO⁺</td>
<td>s</td>
<td>Neurotransmitter and blood pressure regulator, can yield potent oxidants during pathological states.</td>
</tr>
<tr>
<td>Peroxynitrite</td>
<td>ONOO⁻</td>
<td>10⁻³ s</td>
<td>Formed from NO⁺ and superoxide, highly reactive.</td>
</tr>
<tr>
<td>Peroxynitrous acid</td>
<td>ONOOH</td>
<td>fairly stable</td>
<td>Protonated form of ONOO.</td>
</tr>
<tr>
<td>Nitrogen dioxide</td>
<td>NO₂</td>
<td>s</td>
<td>Formed during atmospheric pollution.</td>
</tr>
</tbody>
</table>

Table 2.5. Reactive nitrogen species of biological interest
Figure 2.1

Fruiting body of *Volvariella volvacea*
Figure 2.2

a. *Volvariella volvacea* mycelium

b. Submerged culturing of *Volvariella volvacea* mycelium
Figure 2.3
Overview of oxidative stress
Figure 2.4

Ionising radiation mediated oxidative stress
Figure 2.5
Oxidative stress mediated damage to biomolecules
Figure 2.6
Levels of antioxidant action

Antioxidants
Substances capable of inhibiting formation and spread of free radicals, and their damage

Antioxidant

Preventive
Suppress radical formation

Free radicals
Break chain initiation

Target molecules: Lipids, Sugars, proteins, DNA etc.
Break chain propagation

Chain oxidation
Reconstitute membranes

Damage
Repair damage

Disease, cancer, ageing