ANTIOXIDANTS: THE NEED OF HOUR!!!

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Summary

Oxygen is rightly called as “breath of life and death”. A paradox is that while the vast majority of complex life on Earth requires oxygen for its existence, oxygen is a highly reactive molecule that damages living organisms. The answer to its toxicity lies in the paramagnetic behavior of oxygen (O_2). It is increasingly being realized that many of today's diseases are due to the "oxidative stress" that results from an imbalance between formation and neutralization of prooxidants. Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation. These changes in the level of free radicals produces deleterious effects of free radicals in the human body contribute to cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases. All human cells protect themselves against free radical damage by enzymes such as superoxide dismutase (SOD) and catalase, or compounds such as ascorbic acid, tocopherol and glutathione. Sometimes these protective mechanisms are disrupted by various pathological processes, and antioxidant supplements are vital to combat oxidative damage. Thus this article draws much attention on the need for development of ethnomedicines with strong antioxidant properties but low cytotoxicities.

Key words: Oxygen, Free radicals, Antioxidants

Introduction

The 21% Oxygen diradical atmosphere in which we now live has presented the living systems with an oxygen paradox i.e. that while the vast majority of complex life on Earth requires oxygen for its existence, oxygen is a highly reactive molecule that damages living organisms.

Oxygen forms the basis of aerobic life ever since its existence; it forms an integral part in the regulation of cellular processes in the body. It is ironic that oxygen an element indispensable for life, can under certain situations, have severely deleterious effects on human body. An elusive molecule, oxygen plays contradictory roles, one essential for life and other as a toxic substance.

Oxygen is required by the cells for energy production via the electron transport chain in the mitochondria. The electron transport chain accounts for 90% of our total oxygen consumption and all the other oxygen requiring reactions in the body account for only 5-10%. However about 5% or more of the inhaled oxygen is converted to Reactive Oxygen Species (ROS) or free radicals by univalent reduction of oxygen (1, 2).

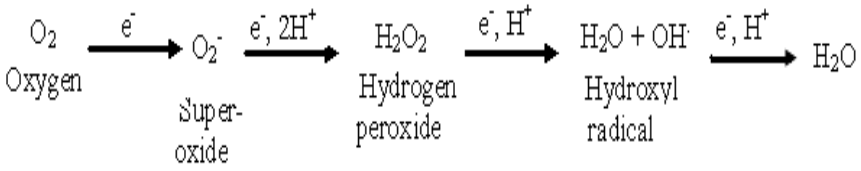
Free Radicals:

Free radicals are known since the beginning of 20^{th} century. A free radical may be defined as any species that has one or more unpaired electrons in their outermost orbital. This broad definition includes the hydrogen atom (one unpaired electron), most transition metals and the oxygen molecule itself (3, 4)

The answer to the toxicity lies in the paramagnetic behavior of oxygen (O_2), which implies to the two unpaired electrons having the same spin state. This electronic structure constitutes a barrier to the insertion of pair of electron by preventing oxidation by 2 electron transfers.

Oxidation by molecular oxygen can only occur by the transfer of single electrons. As a result, the simplistic route of oxygen reduction is by a series of univalent electron transfers.

The reduction of O_2 to $2H_2O$ requires four electrons. Hence, intermediates will be encountered on this univalent pathway and these are superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^\cdot$) collectively called as Reactive Oxygen Species (ROS). It is these intermediates that are responsible for the toxicity of O_2 also called as oxidative stress[17].Generation of these ROS can be shown as below:



Free radicals also include Reactive Nitrogen Species these are radical nitrogen-based molecules that can act to facilitate nitrosylation reactions. Reactive Nitrogen Species (RNS) include Nitrous oxide, Peroxynitrite, Peroxynitrous acid, Nitroxyl anion, Nitryl chloride, Nitrosyl cation, Nitrogen dioxide, Dinitrogen trioxide and Nitrous acid (5)

Reactive Oxygen Species (ROS)

Some of the major oxygen metabolites which are formed by one-electron reduction of oxygen are discussed below (2, 3, 4, 6, 7)

I. Superoxide anion $\{O_2^{-\cdot}\}$

The superoxide free radical anion is formed when oxygen is reduced by the transfer of a single electron to its outer shells i.e. it is formed when oxygen requires additional electron. Estimated 1-3% of all consumed oxygen ends up as superoxide anion. Due to its pKa of 4.8, superoxide can exist in the form of $O_2^{-\cdot}$ Or at low pH hydroperoxyl ($HO_2^\cdot$) which can penetrate membranes more easily than charged form.

Superoxide anion can be formed by:

- Mitochondrial and microsomal electron transport chain i.e. passing electrons directly on to oxygen.
- Xanthine oxidase and other flavoprotein oxidases.
- Auto-oxidation of hydroquinone, catecholamine, thiols
- Neutrophils
- Oxygen interaction with haemoglobin:
  $Haem\text{-}Fe^{+2}\text{-}O_2 \rightarrow Haem\text{-}Fe^{+3}\text{-}O_2^{-\cdot}$ (Methaemoglobin).
- Ionizing radiation.

Superoxide anion is a negatively charged ion with short life of few milliseconds in physiological pH. The Physiological concentrations are about $10^{-10\text{-}11}$ M. It cannot permeate membrane due to its charged state except erythrocyte membrane which has an anion channel that helps its crossing

Mechanism of action of superoxide radical in oxidation are:

1. Hydrogen peroxide formation
2. Reduction of catalytic metals
3. Release of iron from ferritin
4. Peroxynitrite formation.

On its own it isn't particularly damaging. Its main significance lies in its being a main source for the generation of hydrogen peroxide and as a reductant of transition metals, which are precursors to the formation of the lethal hydroxyl radical.

Major reactions involving superoxide anion:

- Dismutation:

$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$ (At physiological pH: k~ 10^5 $M^{-1}s^{1}$)

Hydrogen peroxide

- Haber-Weiss reaction:

$O_2^- + H_2O \rightarrow O_2 + OH^- + OH^{\cdot}$

Hydroxyl radical

- Reaction with nitric oxide:

$O_2^- + NO \rightarrow ONOO^-$

Peroxynitrite

- Reduction of haem bound, haem free iron, ascorbate.

$Fe^{+3} + O_2^- \rightarrow Fe^{+2} + O_2$

II. Hydrogen peroxide (H_2O_2)

Hydrogen peroxide can be generated from the two electron reduction of oxygen. Hydrogen peroxide is not a free radical but falls in the category of reactive oxygen species. It is an oxidising agent that is not particularly reactive but its main significance lies in that it is the main source of hydroxyl radicals in the presence of transition metal ions. It is also involved in the production of HOCl by neutrophils.(19)

Hydrogen peroxide is formed by:

- Dismutation of O_2^- (spontaneously or SOD catalysed)

  $2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2$
  (Radical reactants produce non-radical products)
- Xanthine oxidase, glucose oxidase and other oxidases viz. peroxisomal oxidases and flavoproteins, D-amino acid oxidase, L-hydroxy acid oxidase and fatty acyl oxidase

Hydrogen peroxide is an uncharged, non radical, stable, and is estimated to have a half life of 10^{-9} seconds. Physiological concentrations are about 10^{-8} M. It is not highly reactive by itself but easily penetrate membranes (Aquaporins) causing toxic to most cells at > 100μM.

Major reactions involving hydrogen peroxide:

- Fenton reaction:
  $Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^- + OH^{\cdot}$
- Haber-Weiss reaction:
  $O_2^- + H_2O_2 \rightarrow O_2 + OH^- + OH^{\cdot}$
  $2\ H_2O_2 \rightarrow 2\ H_2O + O_2$ (Catalase)
  $H_2O_2 + AH_2 \rightarrow 2\ H_2O + A$ (Peroxidases)

III. Hydroxyl radical ($OH^{\cdot}$)

The hydroxyl radical is an extremely reactive oxidising radical that will react to most biomolecules at diffusion controlled rates which means that reactions will occur immediately with biomolecules. The hydroxyl free radical is important in radiobiological damage and is several orders of magnitude more reactive towards cellular constituents than

superoxide radicals (and many orders more reactive than hydrogen peroxide)

Hydroxyl radical is formed by:

- UV irradiation of peroxide (possibly during sunlight exposure)
- Ionizing radiation
  $HOCl + O_2^- \rightarrow OH^\cdot + O_2 + Cl^-$
- Fenton reaction:
  $Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^- + OH^\cdot$
- Haber-Weiss reaction:
  $O_2^- + H_2O_2 \rightarrow O_2 + OH^- + OH^\cdot$

Hydroxyl radical is uncharged, radical, Very short lived. Its Physiological concentrations are about 10^{-14} M. It is highly reactive by itself and will not penetrate membranes because will react with every molecule in its vicinity.

Major reactions involving hydroxyl radical:

- Hydrogen atom extraction:
  $RH + OH^\cdot \rightarrow R^\cdot + H_2O$

The resulting radicals are less destructive.

- Electron transfer
  $Cl^- + OH^\cdot \rightarrow Cl^\cdot + OH^-$
- Addition to double bond

$$>C=C< + OH^\cdot \rightarrow HO\text{-}C\text{-}C^\cdot<$$

IV. Singlet Oxygen {$^1O_2$}

It is a nonradical (does not have an unpaired electron) reactive oxygen species often associated with oxygen free radicals that has strong oxidising activity. Singlet oxygen ($^1O_2$) is an electronically excited and mutagenic form of oxygen. In this case the two electrons are formally paired in one of the π orbitals leaving the other vacant.

It is formed by :

- Input of energy, example radiation
- By the action of peroxidases or lipoxygenases (enzymatically)

- By the reaction of hydrogen peroxide with hypochlorite or peroxynitrite
- Thermo-decomposition of dioxetanes
- During the respiratory burst of phagocytes.
- Also generated in biological systems in a number of pigment reactions including chlorophylls, retinal, porphyrins and flavins when they are illuminated in the presence of oxygen.
- Formation of singlet oxygen occurs *in vivo* in illuminated chloroplasts and in both the lenses and retina of the mammalian eye.

  Major properties of single oxygen are:
- It has limited life-time (3 μsec.) since it is in excited state.
- Responsible for photo bleaching and fading with sun exposure.

VI. Nitric Oxide {$NO^{\cdot}$}

It is a common gaseous free radical and is now recognised to play a role in vascular physiology. It is also known as endothelium derived relaxing factor.

Nitric oxide is formed by:
Nitric Oxide Synthetase (Arginine→ NO + Citrulline)
Vascular endothelium produces nitric oxide, as do neutrophils and macrophages from arginine using the enzyme nitric oxide synthetase. This event can be stimulated by cytokines, tumour necrosis factor, or interleukins. Inhibition of production is known to reduce microbicidal and tumoricidal activities of macrophages.

Major properties of nitric oxide are Uncharged, Radical. It has relatively long- lived in the absence of oxygen (seconds). Its physiological concentrations are about 10^{-8} M. Reactive by itself it penetrate membranes thus acting as an endogenous vasodilator. It also binds to haem iron or thiol groups of Hb.

Mechanism of action:
Activation of guanyl cyclase due to haem binding.

Major reactions involving nitric oxide:

- $4NO^{\cdot} + O_2 + H_2O \rightarrow 4H^{+} + 4\ Fe^{+2}$ in aqueous solutions
- $O_2^{-} + NO^{\cdot} \rightarrow ONOO^{-}$
  (Peroxynitrite)
- Degradation of iron containing prosthetic groups
- $NO + Fe^{+3} \rightarrow Fe^{+3}\text{-}NO \leftrightarrow Fe^{+2} - NO^{+}$
  $Fe^{+2} - NO^{+} + H_2O \rightarrow Fe^{+2} + NO_2^{-} + 2H^{+}$
- $NO + H_2O_2 \rightarrow NO_2^{-} + OH^{\cdot} + H^{+}$
  (Nitrite)
- $NO + OH^{\cdot} \rightarrow NO_2^{-} + H^{+}$

VII. Peroxynitrite {$ONOO^{\cdot}$}

Peroxynitrite is formed by:

- Reaction of nitric oxide with superoxide.

$O_2^{-\cdot} + NO^{\cdot} \rightarrow ONOO^{-}$ (At physiological pH: k~ 10^{10} $M^{-1}s^{-1}$)
It is a radical-radical reaction.

Major properties:

- It is very reactive when protonated (ONOOH) and causes rapid damage.
- It is stable at alkaline pH
- At physiological pH it also rapidly decomposes to form nitric acid.
  $O{=}N\text{-}O\text{-}OH \rightarrow NO_3^{-} + H^{+}$

VIII. Hypochlorous acid {HOCl}

Hypochlorous acid is formed by:

- The action of myeloperoxidase on chloride ions in the presence of H_2O_2. $H_2O_2 + Cl^{-} \rightarrow HOCl + OH^{-}$. This reaction occurs in the neutrophils phagocytic vacuole after fusion with the myeloperoxidase containing lysosomal vesicles.
- Activated polymorphonuclear cells produce HOCl as a major bactericidal agent.

Major properties of hypochlorous acid are:

- Hypochlorous acid can cross cell membranes and, in the presence of transitional metal ions, generate hydroxyl radicals.
- Highly reactive hydroxyl radicals can be formed from $HOCl/OCl^-$ on reaction with reductants that are one-electron donors. Important examples include superoxide radicals and ferrous iron:

$$HOCl + O_2\cdot^- \rightarrow \cdot OH + Cl^- + O_2$$
$$HOCl + Fe^{2+} \rightarrow \cdot OH + Cl^- + Fe^{3+}$$

- HOCl has been shown to be capable of initiating lipid peroxidation, combining with H_2O_2 to damage DNA and DNA repair processes and altering intracellular free Ca^{2+} and pH
- It may contribute to tissue damage during the inflammatory process. This latter event may result from the activation of collagenases or the inactivation of alpha-1 antiproteinase.

IX. Ozone {O_3}

It is a triatomic gas. It is not a free radical but a powerful oxidising agent. It acts as an important shield of solar radiation.

Major reactions involving ozone:

- Photo dissociation of chlorofluorocarbons (contained in aerosol sprays)can lead to chlorine radicals ($Cl^\cdot$) and then $O_3 + Cl^\cdot \rightarrow ClO + O_2$

Sources of Free Radicals:

The cell is exposed to large variety of ROS and RNS from the following sources: (3, 6)

- Endogenous sources
- Exogenous sources

Endogenous sources:

1. Autoxidation:

Autoxidation is a by-product of the aerobic internal milieu. Some of the molecules that undergo autoxidation are catecholamines, haemoglobin, myoglobin, reduced cytochrome C and thiol. Autoxidation of any of the above molecules in a reaction results in the reduction of the oxygen diradical and the formation of reactive oxygen species. Superoxide is the primary radical formed. Ferrous ion (Fe^{+2}) also, can have its electron stolen from it by oxygen to produce superoxide and ferric ion (Fe^{+3}), by the process of autoxidation.

2. Enzymatic oxidation:

A variety of enzyme systems is capable of generating significant amounts of free radicals, including xanthine oxidase (activated in ischemia-reperfusion), prostaglandin synthase, lipoxygenase, aldehyde oxidase and amino acid oxidase. The enzyme myeloperoxidase produced in activated neutrophils, utilizes hydrogen peroxide to oxidize chloride ions into the powerful oxidant hypochlorous acid (HOCl).

3. Respiratory burst:

It is a term used to describe the process by which phagocytic cells consume large amounts of oxygen during phagocytosis. It is characterized by 20 fold increase in oxygen consumption. Between 70 and 90% of this oxygen consumption can be accounted for in terms of superoxide production. These phagocytic cells possess a membrane bound flavoprotein cytochrome-b-245 NADPH oxidase (Nicotinamide Adenine Dinucleotide Phosphate-oxidase) system. Cell membrane enzymes such as the NADPH-oxidase exist in an inactive form. It is the exposures to immunoglobulin-coated bacteria, immune complexes, complement 5a, or leukotriene, however, which activates the

enzyme NADPH-oxidase. NADPH serves as a donor of electrons to an activated enzymatic complex in the plasma membrane. This NADPH- oxidase complex utilizes electrons to produce superoxide radicals from oxygen molecule. H_2O_2 is then formed from superoxide by dismutation with subsequent generation of ·OH and HOCl by bacteria.

4. Subcellular organelles:

Organelles such as mitochondria, chloroplasts, microsomes, peroxisomes and nuclei have been shown to generate O_2^- and this is easily demonstrated after the endogenous superoxide dismutase has been washed away. Mitochondria are the main cellular organelle for cellular oxidation reactions and the main source of reduced oxygen species in the cell. The leaks in mitochondrial electron transport system allow O_2 to accept a single electron forming O_2^-. It has been shown that superoxide production by the mitochondria increases in two conditions; either when the oxygen concentration is greatly increased or when the respiratory chain becomes fully reduced (as happens during ischemia).
Microsomes are responsible for 80% of the H_2O_2 produced *in vivo* at 100% hyperoxia sites. Peroxisomes are known to produce H_2O_2, but not $O_2^{\cdot -}$, under physiologic conditions. Although the liver is the primary organ where peroxisomal contribution to the overall H_2O_2 production is significant, other organs that contain peroxisomes are also exposed to these H_2O_2 -generating mechanisms. Peroxisomal oxidation of fatty acids has recently been recognized as a potentially important source of H_2O_2 production with prolonged starvation.

5. Transition metals ions:

Iron and copper play a major role in the generation of free radicals injury and the facilitation of lipid peroxidation. Transition metal ions participate in the Haber-Weiss reaction that generates ·OH from $O_2{\cdot}^-$ and H_2O_2.

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

The Haber-Weiss reaction accelerates the non-enzymatic oxidation of molecules such as epinephrine and glutathione that generates O_2^- and H_2O_2 and subsequently $\cdot OH$.

6. Diseases:

Diseases such as ischemia reperfusion injury confers a number of effects all contributing to the production of free radicals. Normally xanthine oxidase is known to catalyse the reaction of hypoxanthine to xanthine and subsequently xanthine to uric acid. This reaction requires an electron acceptor as a cofactor. During ischemia two factors occur, first the production of xanthine and xanthine oxidase are greatly enhanced. Second, there is a loss of both antioxidants - superoxide dismutase and glutathione peroxidase. The molecular oxygen supplied on reperfusion serves as an electron acceptor and cofactor for xanthine oxidase causing the generation of the $O_2\cdot^-$and H_2O_2. Strenuous exercise has been proposed to activate xanthine oxidase-catalysed reactions and generate free radicals in skeletal muscle and myocardium.

Exogenous sources:

1. Drugs:

A number of drugs can increase the production of free radicals in the presence of increased oxygen tensions. The agents appear to act additively with hyperoxia to accelerate the rate of damage. These drugs include antibiotics that depend on quinoid groups or bound metals for activity (nitrofurantoin), antineoplastic agents as bleomycin, anthracyclines (Adriamycin) and methotrexate, which possess pro-oxidant activity. In addition radicals derived from penicillamine, phenylbutazone, some fenamic acids and the aminosalicylate component of sulphasalazine might inactivate protease and deplete ascorbic acid accelerating lipid peroxidation. Drugs like nitroglycerine acts as $NO\cdot$ donors that can produce ROS indirectly.

2. Radiation:

Radiotherapy may cause tissue injury that is caused by free radicals. Electromagnetic radiation (X rays, gamma rays) and particulate radiation (electrons, photons, neutrons, alpha and beta particles) generate primary radicals by transferring their energy to cellular components such as water. These primary radicals can undergo secondary reactions with dissolved oxygen or with cellular solutes.

Exposure of the cell to γ- irradiation results in the production of radical and non radical species from ionization of intracellular water (e.g. aqueous electron, $OH^{\bullet}$, H_2O_2). Exposure to the cell to non ionizing radiation such as UV-C (< 290 nm), UV-B (290-320 nm), UV-A (320-400 nm) can indirectly produce a variety of ROS including $^1O_2$, $O_2^{\bullet -}$ and H_2O_2 radicals; hemolytic cleavage of H_2O_2 by UV radiation yields $OH^{\cdot}$ radicals.

3. Tobacco smoking:

Oxidants in tobacco exist in sufficient amounts to suggest that they play a major role in injuring the respiratory tract. It has been shown that tobacco smoke oxidants severely deplete intracellular antioxidants in the lung cells *in vivo* by a mechanism that is related to oxidant stress. It has been estimated that each puff of smoke has an enormous amount of oxidant materials. These include aldehydes, epoxides, peroxides, and other free radicals that may be sufficiently long lived as to survive till they cause damage to the alveoli. In addition nitric oxide, peroxyl radicals and carbon centred radicals are present in the gas phase. In addition it also contains other relatively stable radicals in the tar phase. Examples of radicals in the tar phase include the semiquinone moieties derived from various quinones and hydroquinones. Again micro-haemorrhages are most probably the cause of iron deposition found in smokers' lung tissue. Iron in this form leads to the formation of the lethal hydroxyl radical from hydrogen peroxide. It was also found that smokers have elevated amounts of neutrophils in the lower respiratory tract that could contribute to a further elevation of the concentration of free radicals.

4. Inorganic particles:

Inhalation of inorganic particles also known as mineral dust (e.g. asbestos, quartz, silica) can lead to lung injury that seems at least in part to be mediated by free radical production. Asbestos inhalation has been linked to an increased risk of developing pulmonary fibrosis (asbestosis), mesothelioma and bronchogenic carcinoma. Silica particles as well as asbestos are phagocytosed by pulmonary macrophages. These cells then rupture, releasing proteolytic enzymes and chemotactic mediators causing infiltration by other cells such as neutrophils, thus initiating an inflammatory process that leads to increased production of free radicals and other reactive oxygen species. Furthermore, asbestos fibres contain iron, which may have been derived form haemoglobin liberated from micro-haemorrhages. This iron can stimulate the formation of hydroxyl radicals.

5. Gases:

Ozone is not a free radical but a very powerful oxidising agent. Ozone (O_3) contains two unpaired electrons and degrades under physiological conditions to ·OH, suggesting that free radicals are formed when ozone reacts with biological substrates. In support of this hypothesis, ozone can generate lipid peroxidation *in vitro*, although similar findings *in vivo* have not been demonstrated.

6. Food:

A large portion of food we consume is oxidized to a large degree and contains different kinds of oxidants such as peroxides, aldehydes, oxidized fatty acids and transition metals. Food debris that reaches the intestinal tract places an enormous oxidative pressure on the intestinal tract mucosa.

7. Others:

Fever, excess glucocorticoid therapy and hyperthyroidism decrease oxygen tolerance in experimental animals. The decrease is attributable to the increased generation of oxygen-derived radicals that accompanies increased metabolism. In addition, a wide variety of environmental

agents including photochemical air pollutants as solvents, anaesthetics, exhaust fumes and the general class of aromatic hydrocarbons, also cause free radical damage to cells. Xenobiotics (e.g. toxins, pesticides and herbicides such as Paraquat) and chemicals (e.g. alcohol) produce ROS as a by product of their metabolism *in vivo*.

Damaging Reactions of Free Radicals

"We need free radicals to live, but they're also the bane of our existence,"

Lester Packer- A biochemist who is studying free radicals since 1970.

Reactive oxygen species can attack vital cell components like polyunsaturated fatty acids, proteins, lipids and nucleic acids. Lipids are probably the most susceptible biomolecules to free radical attack. To a lesser extent, carbohydrates are also the targets of ROS. These reactions can alter intrinsic membrane properties like fluidity, ion transport, loss of enzyme activity, protein cross-linking, inhibition of protein synthesis, DNA damage which ultimately results in cell death. Some of the well-known consequences of generation of the free radicals *in vivo* are: DNA strand scission, nucleic acid base modification, and protein oxidation and lipid peroxidation. (1, 3, 6, 8)

Lipid peroxidation:

Oxygen radicals catalyze the oxidative modification of lipids. Cell membranes are a rich resource of Poly Unsaturated Fatty Acids (PUFA), which are readily attacked by oxidising agents; a process that is called lipid peroxidation. Consequently it causes changes in membrane fluidity, permeability, and cellular metabolic functions.

Lipid peroxidation is not initiated by O_2^{-} and H_2O_2 but $OH^{\cdot}$, alkoxy radicals ($RO^{\cdot}$), and peroxy radicals ($ROO^{\cdot}$) result in initiating the lipid peroxidation. This can lead to a self perpetuating process since peroxy radicals are both reaction initiators as well as the products of lipid peroxidation.

Hence it is particularly damaging. The breakdown of lipid hydroperoxides often involves transition metal ion catalysis.

Lipid Peroxidation occurs in three stages:

Step 1: Initiation:

$$LH + R^{\cdot} \rightarrow L^{\cdot} + RH$$

Lipid peroxidation is initiated by a free radical compound, such as hydroxyl radical, which extracts a hydrogen from polyunsaturated lipids (LH), resulting in the formation of lipid radical ($L^{\cdot}$)

Step 2: Propagation:

$$L^{\cdot} + O_2 \rightarrow LOO^{\cdot}$$

$$LOO^{\cdot} + LH \rightarrow LOOH + L^{\cdot}$$

The free radical chain reaction is propagated by addition of oxygen, which forms lipid peroxy radical ($LOO^{\cdot}$) and lipid peroxide (LOOH).

Step 3: Degradation:

Malondialdehyde + Degraded lipid peroxides.

Rearrangements of single electron results in degradation of lipid to form various compounds of which one is malondialdehyde which is soluble and appears in blood.

Step 4: Termination:

$$LOO^{\cdot} + L^{\cdot} \rightarrow LOOH + LH$$

Or

$$L^{\cdot} + \text{Vit E} \rightarrow LH + \text{Vit E}^{\cdot}$$

$$\text{Vit E}^{\cdot} + L^{\cdot} \rightarrow LH + \text{Vit E}_{ox}$$

The chain reaction can be terminated by using antioxidants like vitamin E, which donates single electrons in two subsequent steps to form a stable oxidized compound.

Protein damage:

During mitochondrial electron transport chain, free radicals are produced which can stimulate protein degradation.
Proteins and nucleic acids are less susceptible to free radicals than Poly Unsaturated Fatty Acids (PUFAs), in that there seems to be less possibility in the formation of rapidly progressing chain reactions.

Random attack of radicals on proteins is unlikely unless extensive. This happens only if radicals are allowed to accumulate (which is not likely in normal cells), or if the damage is focussed on a particular site of the protein which happens when the protein binds a transition metal ion.

Proteins can undergo direct and indirect damage following interaction with ROS, including peroxidation, damage to specific amino acid residues, changes in their tertiary structure, degradation and fragmentation. It can also cause altered electrical charge, aggregation of cross-linked reaction products and increased susceptibility to proteolysis. The consequences of protein damage as a response mechanism to stress are loss of enzymatic activity, altered cellular functions such as energy production, interference with the creation of membrane potentials, and changes in the type and level of cellular proteins. Protein oxidation products are usually aldehydes, keto compounds, and carbonyls.

Oxidative protein damage may be brought about by metabolic processes which degrade a damaged protein to promote synthesis of a new protein. e.g. In the process of cataractogenesis, oxidative modification plays a significant role in cross-linking of crystalline lens protein, leading to high molecular weight aggregates, loss of solubility, and lens opacity. Lipofuscin- an aggregate of peroxidized lipid and proteins accumulates in lysosomes of aged cells, Alzheimer's disease brain cells, and iron overloaded hepatocytes.

DNA damage:

Oxidising radicals readily attack DNA if they are formed in its vicinity. 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. This leads to formation of abnormal components of the electron transport chain. It further results in the generation of more ROS through increased leakage of electrons, and therefore further cell damage. Oxidative damage to mitochondrial DNA may promote cancer and aging.

ROS and Human Diseases:

Oxidative stress has been implicated in the pathogenesis of various diseases viz. Cardiovascular dysfunction, neurodegenerative diseases, gastroduodenal pathogenesis, metabolic dysfunction of almost all vital organs, cancer as well as premature aging. Thus there is enough evidence that mechanisms that involve free radicals are implicated at some stage of the development of human diseases and the maintenance of well being depends on the supply through the diet of antioxidant nutrients.

Halliwell has defined antioxidant as a substance that when present in low concentrations relative to the oxidizable substrate significantly delays or reduces oxidation of substrate. [3] Antioxidants get their name because they combat oxidation. They are substances that protect other chemicals of the body from damaging oxidation reactions by reacting with free radicals and other reactive oxygen species within the body, hence hindering the process of oxidation. [9]

Mode of action of antioxidants

There are four routes: (9)

1. Chain breaking reactions, e.g. alpha-tocopherol which acts in lipid phase to trap radical.
2. Reducing the concentration of reactive oxygen species e.g. glutathione.
3. Scavenging initiating radicals e.g. superoxide dismutase which acts in aqueous phase to trap superoxide free radicals.
4. Chelating the transition metal catalysts: A group of compounds serves an antioxidant function by sequestration of transition metals that are well-established pro-oxidants. In this way, transferrin, lactoferrin, and ferritin function to keep iron induced oxidant stress in check and ceruloplasmin and albumin as copper sequestrants.

Various antioxidant defenses in human body include:

Enzymatic antioxidants- Superoxide dismutase, Catalase and Glutathione peroxidase.

Non enzymatic antioxidants- Lipid soluble vitamins (Vitamin E and Provitamin A) and water soluble vitamin (Ascorbic acid). [10]

Enzymatic Antioxidants

These antioxidants are the primary defense against ROS in human body. (1, 2, 9, 11)

Superoxide dismutase (SOD):

It is the primary defense against oxidative stress because superoxide is a strong initiator of chain reactions.

Source and Nature: SOD is an endogenously produced intracellular enzyme present in essentially every cell in the body. Cellular SOD is actually represented by a group of metalloenzymes with various prosthetic groups. The prevalent enzyme is cupro-zinc (CuZn) SOD, which is a stable dimeric protein (32,000 D).

SOD appears in three forms:

- Cu-Zn SOD in the cytoplasm with two subunits
- Mn-SOD in the mitochondrion
- extracellular SOD- CuSOD

Mechanism of action: SOD is considered fundamental in the process of eliminating ROS by reducing superoxide to form H_2O_2. Catalase and the selenium-dependent glutathione peroxidase are responsible for reducing H_2O_2 to H_2O.

$$2O_2\cdot^- + 2H^+ + SOD \rightarrow H_2O_2 + O_2$$

The activity of SOD is increased through enzyme induction by chemicals or conditions which increase the production of superoxide.

Glutathione peroxidase enzyme:

It is one of the body's principal means of protecting against oxidative damage.

Source and Nature: It is a tetrameric protein 85,000-D. It has 4 atoms of selenium (Se) bound as seleno-cysteine moieties that confer the catalytic activity. One of the essential requirements is glutathione as a co-substrate. It is present in cytoplasm.

Mechanism of action: Glutathione peroxidase reduces H_2O_2 to H_2O by oxidizing glutathione (GSH/ γ-glutamylcysteinylglycine)

$$H_2O_2 + 2GSH \rightarrow GSSG + 2H_2O$$

Re reduction of the oxidized form of glutathione (GSSG) is then catalysed by glutathione reductase.

$$GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+$$

Catalase:

Source and Nature: Catalase is a protein enzyme present in most aerobic cells in animal tissues. Catalase is present in all body organs being especially concentrated in the liver & erythrocytes. The brain, heart, skeletal muscle contains only low amounts.

Mechanism of action: Catalase and glutathione peroxidase seek out hydrogen peroxide and convert it to water and diatomic oxygen. An increase in the production of SOD without a subsequent elevation of catalase or glutathione peroxidase leads to the accumulation of hydrogen peroxide which gets converted into the hydroxyl radical. 2

$$H_2O_2 \rightarrow 2\ H_2O + O_2$$

Non-Enzymatic Antioxidants: [1, 2, 9, 11]

In addition to primary defense against ROS by antioxidant enzymes, secondary defense is also offered by small molecules called as 'scavengers' which react with radicals to produce another radical compound. When these scavengers

produce a lesser harmful radical species they are called antioxidants.

α-Tocopherol (Vitamin E):

It is the major lipid soluble antioxidant found in cells. In nature, eight substances have been found to have vitamin E activity: d-α, β, γ, δ- tocopherol (which differ in methylation site and side-chain saturation) and d-α, β, γ and δ-tocotrienol. Of all these d- α tocopherol has the highest biopotency and its activity is the standard against which all the others must be compared. It is the predominant isomer in plasma.

Source and Nature: Tocopherols are present in oils, nuts, seeds, wheat germ and grains.

Mechanisms of Action: It prevents the peroxidation of membrane phospholipids and avoids cell membrane damage through its antioxidant action. Tocopherol-OH can transfer a hydrogen atom with a single electron to a free radical, thus removing the radical before it can interact with cell membrane proteins or generate lipid peroxidation. When tocopherol-OH combines with the free radical, it becomes tocopherol-O·, itself a radical. When ascorbic acid is available, tocopherol-O· plus ascorbate (with its available hydrogen) yields semidehydroascorbate (a weak radical) plus tocopherol-OH. By this process, an aggressive ROS is eliminated and a weak ROS (dehydroascorbate) is formed, and tocopherol-OH is regenerated.

α Tocopherol + $LOO^{\cdot}$ → α $Tocopherol^{\cdot}$ + LOOH

α $Tocopherol^{\cdot}$ + $LOO^{\cdot}$ → LOO-α Tocopherol

β Carotene (Provitamin A):

Carotenoids are precursors of vitamin A and have antioxidant effects. It is composed of two molecules of vitamin A (retinol) joined together.

Source and Nature: Carotenoids are pigmented micronutrients present in fruits and vegetables.

Mechanisms of Action: The antioxidant function of β carotene is due to its ability to quench singlet oxygen, scavenge free radicals and protect the cell membrane lipids from the harmful effects of oxidative degradation. The quenching involves a physical reaction in which the energy of the excited oxygen is transferred to the carotenoid, forming an excited state molecule.

In addition to singlet oxygen, carotenoids are also thought to quench other oxygen free radicals. It is also suggested that β carotene might react directly with the peroxyl radical at low oxygen tensions; this may provide some synergism to vitamin E which reacts with peroxyl radicals at higher oxygen tensions.

$$\beta\text{-carotene (CAR)} + LOO^{\bullet} \rightarrow LOO\text{-}CAR^{\bullet}$$

$$LOO\text{-}CAR^{\cdot} + LOO^{\cdot} \rightarrow LOO\text{-}CAR\text{-}OOL$$

Ascorbic acid (Vitamin C):
It is a water-soluble antioxidant.

Source and Nature: It is present in citrus fruits, potatoes, tomatoes and green leafy vegetables. Humans are unable to synthesize l-ascorbic acid from d-glucose due to absence of the enzyme L-gulacolactone oxidase. Hence humans must therefore obtain ascorbic acid from dietary sources.

Mechanism of Action: The chemopreventive action of vitamin C is attributed to two of its functions. It is a water-soluble chain breaking antioxidant. As an antioxidant, it scavenges free radicals and reactive oxygen molecules, which are produced during metabolic pathways of detoxification. It also prevents formation of carcinogens from precursor compounds. Ascorbic acid is a reducing agent with a hydrogen potential of +0.08V, making it capable of reducing such compounds as a molecular oxygen, nitrate and cytochrome a and c. Donation of one electron by ascorbate gives the semi-dehydroascorbate radical (DHA). Ascorbate reacts rapidly with $O_2^{\cdot -}$ and even more rapidly with ·OH to give DHA. DHA itself can act as a source of vitamin C.

$$\text{Ascorbic acid} + 2O_2{\cdot}^- + 2H^+ \rightarrow H_2O_2 + \text{DHA}$$

It has also been shown that ascorbate is more potent than α-tocopherol in inhibiting the oxidation of LDL in a cell free system. Vitamin C also contributes to the regeneration of membrane bound oxidized vitamin E. It will react with the α -tocopheroxyl radical, resulting in the generation of tocopherol in this process itself being oxidized to dehydroascorbic acid.

Other Antioxidants: (1, 9, 11-14, 18)

Glutathione (GSH):

GSH is synthesized intracellularly from cysteine, glycine and glutamate. Hence it is a tripeptide. Intracellular concentration is 5mM (millimolar).

In addition to its role as a substrate in GSH redox cycle, GSH is also a scavenger of hydroxyl radicals and singlet oxygen. It is capable of either directly scavenging ROS or enzymatically via glutathione peroxidase. In addition, GSH is crucial to the maintenance of enzymes and other cellular components in a reduced state.

Coenzyme Q10:

CoQ10 (Coenzyme Q10) is also known as ubiquinone. It is found in almost every living cell (hence the name "ubiquitous") and is essential to energy production by the mitochondria. Far beyond producing energy, CoQ10 can protect the body from destructive free radicals and enhance immune defences.

Uric acid:

It acts as an endogenous radical scavenger and antioxidant. It is present in about 0.5 mmol/L in body's fluids and is the end product of purine metabolism. Uric acid is a powerful scavenger of singlet oxygen, peroxyl radical (ROO·) and ·OH radical.

Albumin:

Depending on the fact that albumin has one sulfhydryl group per molecule; it itself scavenges several free radicals and thus can be considered as one of the primary extracellular defense systems.

Albumin is an additional sacrificial antioxidant that can bind copper tightly and iron weakly to its surface. The bound metals would still be on its surface. The bound metals would still be available for participation in Haber-Weiss reaction, but any generated ·OH would immediately react with and be scavenged by albumin. The resultant protein damage is biologically insignificant because of the large amount of available albumin and free radicals would be inactivated before reacting with other more vital protein structures.

Other plasma proteins namely ceruloplasmin and transferrin have also shown antioxidant activity.

Flavonoids:

Source and nature: Flavonoids are phenolic substances isolated from a wide range of plants. They exhibit biologic activities including antiallergenic, antiviral, anti-inflammatory and antioxidant activities.

Flavonoids are formed in plants from the aromatic amino acids phenylalanine and tyrosine and malonate. Among the many classes of flavonoids, those of particular interest to this review are flavones, flavanones, isoflavones, flavonols, flavanonols, flavan-3-ols and anthocyanidins. Other flavonoid classes include biflavones, chalcones, aurones, and coumarins. Hydrolysable tannins, proanthocyanidins (flavan-3-ol oligomers), caffeates, and lignans are all plant phenols and they are usually classified separately.
Mechanism of action: Flavonoids inhibit the enzymes responsible for superoxide anion production, such as xanthine oxidase and protein kinase C. Flavonoids have been

also shown to inhibit cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase and NADH oxidase, all involved in ROS generation. A number of flavonoids efficiently chelate trace metals, which play an important role in oxygen metabolism.

Naturally there is a dynamic balance between the amount of free radicals produced in the body and antioxidants to scavenge or quench them to protect body against deleterious effects. However the amount of antioxidant principles present under normal physiological conditions may be insufficient to neutralize free radicals generated. Therefore it is necessary to enrich our diet with antioxidants to protect human body against harmful diseases. Thus antioxidant therapy has gained an important status in the prevention and treatment of various pathological conditions.

While fruits and vegetables are best sources, now herbs can be added to help boost the antioxidant arsenal. Herbs act as excellent natural sources of antioxidants. Herbs also have nutrients that are vital for the healthy maintenance of tissues. Many of the antioxidant herbs are promising candidates in the treatment of cancer, atherosclerosis, diabetes, antimicrobial and anti-inflammatory agents and those with neuroprotective abilities. Hence herbs are the answer for free radical rebellion! [15, 16] there are large no. of plants reported with antioxidant activity: *Allium sativum* (Garlic) *Holarrhena antidysenterica* (Kurchi bark) *Aloe vera* (Aloe, Burn plant), *Picrorhiza kurroa* (Kutki), *Piper longum* (Long pepper), *Curcuma longa* (Turmeric), *Emblica officinalis* (Amla) and *Withania somnifera* (Ashwagandha). Thus the usefulness of herbals in protecting against adverse effects of oxidative injury warrants further study.

Conclusions

Oxidative stress is the usual phrase to identify the association of toxic free radicals with damage to cells and tissues. Build up of free radicals may heighten the damages inflicted by these ROS. It can cause debilitating consequences to our body. Free radicals can damage all important biomolecules which leads to the death of the cell or inability to divide and to make new cells. Antioxidants get a new lease on life as supportive elements of a regimen with least toxic side effects.

However, there are conflicting studies also which not only suggest antioxidant supplementation to be ineffective but in certain conditions detrimental to health also. Moreover, irrational and non-judicial use of antioxidants can also increase the risk of potential toxicity as many antioxidants can also act as pro oxidants under a range of circumstances inspite these concerns they have gained very important status presently. However, under the shadow of these concerns associated with the use of synthetic/pharmacological antioxidants, the best recommended action is to increase the intake of natural dietary antioxidant vitamins by consuming cereals, pulses, nuts, fruits, vegetables, which seems to be a safe and effective approach currently.

Improvements in our diet to increase our intake of antioxidant vitamins or supplementation with antioxidant vitamins may prove to be effective in decreasing the incidence of these diseases. Hence ameliorating oxidative stress can be considered as a potential target for therapeutic treatment of diseases. Suitable antioxidant therapies have already attracted world-wide attraction in the recent years. However isolation of an antioxidant factor which is specific in its actions is non toxic and is obtained from the natural sources (plants) and the therapeutic application of such an antioxidant factor would be one of the best approaches to control ROS-mediated pathogenesis.

Future prospects in this field can be taken forward to understand the molecular mechanism of ROS-mediated pathogenesis and its control by suitable antioxidant therapies. Thus taking into consideration the wide plethora of herbal options available to us in the form of rich traditional heritage, we must take an opportunity to explore the antioxidant potentials of these "green medicines" and utilize them for our health benefits.

References:

1. Marks, D.B., Marks, A.D. and Smith C.M.; Oxygen metabolism and Oxygen toxicity, In: *Basic Medical Biochemistry: A Clinical Approach*; William and Wilkins; 1996: 327-340.
2. Bandyopadhyay, U., Das, D. and Banerjee, R. K.; Reactive oxygen species: Oxidative damage and pathogenesis; *Curr Sci.* September 1999; 77(5): 658-666
3. Kohen, R. and Nyska, A.; Oxidation of biological systems: Oxidative stress phenomena, Antioxidants, Redox reactions and Methods for their quantification; *Toxicol Pathol.* 2002; 30(6): 620–650
4. Halliwell, B. and Gutteridge J.M.C.; Oxygen toxicity, oxygen radicals, transition metals and disease; *Biochem. J.*1984; 219: 1-14
5. www.oxisresearch.com/library/oxidative_stress
6. www.thedoctorslounge.net/medlounge/articles/freeradicals/index
7. www.ttuhsc.edu/SOM/physiology/courses/6311Cellular/PathophysiologyfR0S2002.pdf
8. www.cropsoil.psu.edu/Courses/AGRO518/Oxygen
9. www.thedoctorslounge.net/medlounge/articles/antioxidants
10. Naik, S.R.; Antioxidants and Their Role in Biological Functions: An Overview; *Indian Drugs*; September 2003; 40(9): 501-516
11. www.thenutritionreporter.com/Free-rads.htm
12. Nadendla, R.R.; Role of antioxidants in human diseases-An overview; *Pharma Times*; May 2002; 34: 13-18

13. Pietta, P.G.; Flavonoids as antioxidants; *J.Nat.Prod*. 2000; 63: 1035-1042
14. Tiwari, A.K.; Imbalance in antioxidant defense and human diseases: Multiple approach of antioxidants therapy; *Curr Sci.* November 2001; 81(9): 1179-1187
15. Kolhapure, S.A.; Herbal answer to free radical rebellion; *Express Pharma Pulse*; April 2005: 7-8
16. Sivarajan, V.V., and Balachandran, I.; *Ayurvedic drugs and their plant sources*; Oxford and IBH publishing Co. Pvt. Limited; 1994: 91-94
17. Irwin Fridovich; Oxygen toxicity: A radical explanation; *Journal of Experimental Biology*; 201, 1203–1209 (1998).
18. Michael Antolovich, Paul D. Prenzler, Emilios Patsalides, Suzanne McDonald and Kevin Robards; Methods for testing antioxidant activity; *Analyst*, 2002, 127, 183–198.
19. Chance B, Sies H & Boveris A (1979).Hydroperoxide metabolism in mammalian organs. *Physiological Reviews*, 59: 527-605.