**Free radicals and antioxidants in health and disease: a review**

Ritesh Kumar Upadhyay1, Alok K Soni2

School of Pharmaceutical studies, Faculty of health science Dr K.N. Modi University, Newai, 304021

Institute of Pharmaceutical sciences, SAGE University Indore,

[**Riteshupadhyay307@gmail.com**](mailto:Riteshupadhyay307@gmail.com)

**Abstract:**

1. **INTRODUCTION**

Since humans or human ancestors first evolved a destructive class of chemical agents has assailed the human body. They are called free radicals though they are also termed reactive oxygen species and abbreviated to ROS. They assailed even our pre-primate ancestors. They assailed the dinosaurs and all other life forms that exist in the fossil record even the simplest single cell organisms that have an oxidative metabolism are and always have been assailed by these same free radicals. The free radicals come from oxygen and highly oxygenated molecules.[1]

Free radicals are atoms or molecules containing unpaired electrons. Electrons normally exist in pairs in specific orbitals in atoms or molecules. Free radicals which contain only a single electron in such any orbital are usually unstable toward losing or picking up an extra electron so that all electrons in the atom or molecule will be paired. Free radicals can be positively charged negatively charged or neutral. The presence of and unpaired electron in an atom or molecule provide great reactivity thus shortening its half Life.[2]

Free radicals are commonly generated via NADPH cytochrome p-450 reductase or other flavin containing reductases, although cytochrome p-450 itself may involve CCl2O2. Many radical can participate in recycling reaction, resulting in a sustained level of free radical in the cell, result in depletion of reduced cofactor and hypoxia.[3]

* 1. **Types of free radicals**

Most free radicals are coming from oxygen atoms and are called reactive oxygen species such as superoxide ion, hydroxyl radical, hydrogen peroxide and singlet oxygen.

**Super oxide ion (or reactive oxygen species)**

It is an oxygen molecule with an extra electron. This free radical can cause damage to mitochondria DNA and other molecules. Our body can neutralize super oxide ions by producing superoxide dismutase.

**Hydroxyl radical**

It is formed by the reduction of an oxygen molecule in the electron transport chain. It is a neutral not charged form of the hydroxide ion. Hydroxyl radical are highly reactive and form an important part of radical biochemistry unlike superoxide the hydroxyl radical cannot be eliminated by an enzymatic reactions. It has a very short half-life and will only react with molecules it's vicinity because of its high reactivity it will damage most organic molecules such as carbohydrate, DNA, lipid and proteins.

**Singlet oxygen**

Singlet oxygen is formed by our immune system. Singlet oxygen causes oxidation of LDL cholesterol.

**Hydrogen peroxide**

It is not a free radical but it is involved in the production of many reactive oxygen species. Hydrogen peroxide is a by-product of oxygen metabolism and is neutralized by peroxidases.

Sometime reactive nitrogen atoms are involved and these free radicals grouped under Reactive Nitrogen Species (RNS). Nitric acid is the most important RNS. Some transitional metals such as iron and copper have many number of unpaired electrons and can also act as free radicals. These metals do not have that strong electron affinity but can easily except and donate electrons.[4]

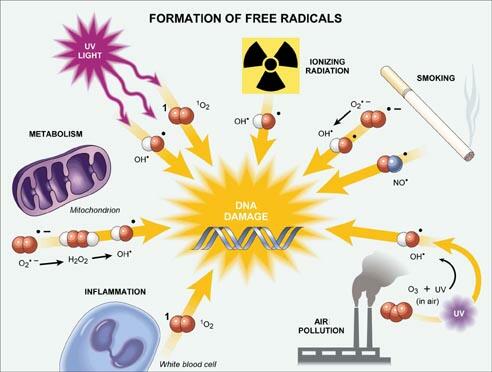
1. **GENERATION OF FREE RADICAL**

Many chemical compounds can we converted into free radical forms. The later spaces are usually quite reactive and short lived because they have a single unpaired electron in there outer or orbital. Recently, evidence has accumulated to indicate that free radical intermediate may be important in the toxicity of a large number of substances. Free radicals can be formed by several mechanisms, including one-electron oxidation, one-electron reduction homolytic cleavage of a C-H Bond and in some cases by two electron oxidation/reduction reactions. Enzyme-catalysed oxidation also provides a mechanism by which free radical in term meditates can be formed.[5]

Oxidative stress maybe defined as an imbalance between pro-oxidant and antioxidant agents, in favour of the former, this imbalance maybe due to excess of pro-oxidant agents, a deficiency of antioxidant agents or both factors simultaneously. The origin of oxidative stress is an alteration of the redox status in cells, leading to a cellular response to counteract the oxidising action.

Pro-oxidant agents are all those that can directly or indirectly oxidise molecules. The most important pro-oxidant agents in biological systems are those derived from oxygen more commonly known as reactive oxygen species (ROS). Although there are also reactive species derived from nitrogen (RNS) or sulphur (RSS). Some of these molecules exhibit great reactivity, such as hydroxyl radicals (OH), and others present mild reactivity the biological importance of the latter release on their capacity to be easily transformed into the hydroxyl radical, especially in the presence of iron, as in the case of super superoxide radical O2 or hydrogen peroxide (H2O2).

The production of these reactive species occurs continuously in the organism; this production may be endogenous or exogenous. Some of these reactive species are generated as “chemical accidents” i.e. undesired secondary reactions between biomolecules or alternatively in the detoxification of xenobiotic. Other reactive species however are generated in vivo for a specific aim such as in the case of activated phagocytes which produce O2 and H2O2. [6]



1. **STEPS INVOLVING FREE RADICAL GENERATION**

In chemistry free radicals take part in radical addition and radical substitution as reactive intermediates chain reaction involving free radicals can usually be divided into three distinct processes: initiation, propagation and termination.

**Initiation** reactions are those, which result in a net increase in the number of free radicals. They may involve the formation of free radicals from stable species or they may involve reactions of free radicals with stable species to form more free radicals.

**Propagation** reactions involve free radicals in which the total number of free radicals remains the same.

**Termination** reactions are those reactions resulting in a net decrease in the number of free radicals. Typically two free radicals combine to form a more stable species, for example: 2Cl\*-----˃ Cl2.

The formation of radicals may involve breaking of covalent bonds homolytically, a process that requires significant amount of energy. The bond energy between two covalently bonded atoms is affected by the structure of the molecule. Homolytic bond cleavage most often happens between two atoms of similar electronegativity. However, propagation is a very exothermic reaction. Radicals may also be formed by single electron oxidation or reduction of an atom or molecule .An example is the production of superoxide by the electron transport chain.[7]

**Reactive Oxygen Species (ROS)**

Reactive oxygen species (ROS) are very small molecules and are highly reactive due to the presence of unpaired valence shell electrons. ROS is formed as a natural by-product of the normal metabolism of Oxygen and have important roles in cell signalling. However, during times of environmental stress ROS levels can increase dramatically, which can result in significant damage to cell structure. Platelets involved in wound repair and blood homeostasis release ROS to recruit additional platelets to sits of injury. Generally harmful effects of reactive oxygen on the cell are most often like damage of DNA; oxidation of poly-DE saturated fatty acid in lipids oxidants of amino acid in proteins, oxidation of amino acid in proteins, oxydatively inactivates specific enzymes by oxidation of co-factors.[7]

1. **SOURCES OF FREE RADICAL**
   1. **Purine metabolism**

The enzyme xanthine dehydrogenase (XDH) mainly located in the vessel walls of most tissue including cardiac and skeletal muscles, catalyses the oxidation of hypoxanthine to xanthine and xanthine to uric acid. Under certain conditions like Ischemia reperfusion and extreme hypotension as in haemorrhagic shock xanthine dehydrogenase, maybe either reversibly or irreversibly transformed to xanthine oxidase (XO). In contrast to xanthine dehydrogenase, xanthine oxidase utilise the oxygen as electron acceptor and produces super oxide as a result while catalysing the oxidation of hypoxanthine to uric acid.[8]

* 1. **Phagocytes**

As weapons for pathogen destruction and immune protection ROS have been put to good use by phagocytes NADPH oxidase located in the plasma membrane of neutrophils produce superoxide’s on purpose following spontaneous dismutation superoxide’s generated as such remarkably contribute to H2O2 formation, cytoplasmic azurophilic granules of neutrophils and monocytes contain heamo proteins peroxidase called Myloperoxidase when activated by immune challenge or such others stimuli, neutrophils release myloperoxidase into the extra cellular medium.[9] The released myloperoxidase complexes with H2O2 to form an enzyme substrate complex with oxidizing potential. The complex oxidises chloride to produce hypochlorous acid HOCl.[10]

* 1. **Drug metabolism**

Microsomal and nuclear membrane electron transport system mainly involved in drug metabolism (via cytochrome p-450 and B5 systems) also host ROS production, NADPH oxidation both in presence and absence of mixed function, oxidase substrate contribute to ROS (O2 and H2O2) formation as well. Mechanism of cytochrome p-450 driven reactions involves the formation of oxy and subsequently peroxy intermediates.[11] Breakdown of these intermediates yield ROS. Cytochrome p-450 has functional multiplicity and also acts as peroxidase in which peroxidases are used as oxygen donor.[12]

* 1. **Nitric oxide synthase**

It is widely believed that endothelium derived relaxing factors (EDRF) produced by vascular endothelial cells is identical with nitric oxide NO (Nitric oxide).[13] Nitric oxide is synthesized in a wide variety of tissues and is known to be implicated in a number of crucial physiological functions eg: control of systemic blood pressure, respiration, digestion, platelet aggregation etc. The enzyme primarily responsible for the synthesis of NO is tissue specific. Nitric oxide synthase in the endothelium and neurones is calmodulin activated enzyme that oxidizes Ariginin to Citrulin in the presence of biopterin, NADPH and oxygen. Cells like macrophages which are capable of producing both NO and superoxide’s are the likely host of a very powerful deleterious ROS the peroxy nitrite anion (ONOO). This formed peroxy nitrate anion is relatively long lived ROS and the nitric oxide may magnify superoxide toxicity remarkably. [14]

* 1. **Transition metals**

Conditions (eg: Plasma pH < 6, haemolysis and ischemia-reperfusion that lead to the release of transition metal ions (that of Iron and Copper) may remarkably amplify ROS toxicity.[15] Iron and Copper ions are capable of converting in the presence of the free transition metal ions ascorbic acid a commonly known antioxidant functions as a pro-oxidant.[16]

* 1. **Other some other possible resources**

There are some other enzymes known to be responsible for the generation of free radicals are listed below with their respective subcellular localization. [17]

* Glycolate oxidase (peroxisome)
* L-Alpha hydroxyl acid oxidase (peroxisome)
* L-gulonlactone oxidase (cytosol)
* Aldehyde oxidase (Cytosol)
* Monoamino oxidase (Mitochondrial outer membrane)
* Urate oxidase (peroxisome core)
* Diamine oxidase (Endoplasmic reticulum)

PGH (Prostaglandin) synthase dependent arachdonic acid metabolism generate superoxide’s in the presence of NADH Once other possible biological source of ROS is myoglobin oxidation of ferrous myoglobin to its hypervalent ferryl form is suggested to contribute to ischemia reperfusion injury in the heart.[18]

1. **TARGETS OF FREE RADICLE IN VIVO**

Free radicles attack three main cellular components.

* 1. **Lipids**

Lipids peroxidation is the introduction of a functional group containing two catenated oxygen atoms, O-O; in to unsaturated fatty acids in a free radicle reaction Polyunsaturated fatty acids susceptible to free radicle attack are initiated by the formation of a carbon cantered radical by the abstraction of hydrogen atom at one of the double bond of the lipid. Lipid peroxidation is also one of the major causes of quality deterioration during the storage of fats, oils or other lipid rich foods. Lipid peroxidation is the most extensively studied manifestation of oxygen activation in biology. It is broadly defined as “Oxidative deterioration of PUFA” which is fatty acids that contain more than two carbon-carbon double bonds. Lipid when reacted with free radicals can undergo the highly damaging chain reaction effects peroxidation of lipids in cell membranes can damage cell membrane by disrupting fluidity and permeability. Lipid peroxidation can also adversely affect the function of membrane bound proteins such as enzymes and receptors.

* 1. **Proteins**

Oxidative damage to proteins can be caused by free radicals. During the mitochondrial electron transport chain, free radicals are produced which can stimulate protein degradation. Oxidative protein damage may be brought about by metabolic processes degrade damaged protein to promote synthesis of a new protein. The mechanism of oxidative damage of proteins by ROS has been studied in vitro by generating these reactive species either in solution or site specifically within the protein. While the former damage is termed as nonspecific (global) and the later damage is termed as site specific (localised damage). Nonspecific damage can be stimulated by generating activated oxygen species in-situ, using a radiation source, CO, or pulse radiolysis techniques which lead to aggregation and fragmentation of the protein and modification of almost all the amino acids.[19]

* 1. **DNA**

Fragmentation of DNA caused by free radical attack causes activation of the poly (ADP-ribose) synthetase enzyme. This splits NAD+ to aid the repair of DNA. However, if the damage is extensive, NAD+ levels may become depleted to the extent that the cell may no longer be able to function and dies. The site of tissue damage by free radical is dependent on the tissue and the reactive species involved. Extensive damage can lead to death of the cell; this may be by necrosis or apoptosis depending on the type of cellular damage. When a cell membrane or an organelle membrane is damaged by free radicals, it loses its protective properties. This puts the health of the entire cell at risk.

1. **FREE RADICAL REACTIONS IN DNA STRAND BREAKAGE**
   1. **Formation of Oxygen Radicals**

Oxygen radicals are usually formed via redox reactions because oxygen is the most efficient biological electron acceptor forms of the superoxide radical O2-.

R-Xˉ+ O2 R-X + O2ˉ

Superoxide radical can undergo dismutation to hydrogen peroxide and oxygen.

2O2ˉ + 2H+ SOD H2O2 + O2

2H2O2  CATALASE 2H2O2 + O2

The peroxide can be removed by catalase or glutathione peroxidase. However, if a transition metal present, commonly Fe or Cu, the metal can be reduced and hydroxyl radical formed.

H2O2 + 2GSH GLUTATHIONE PEROXIDASE  GSSG + H2O

H2O2 + Fe2+ Fe3+ + OH˚ + OHˉ (fentons reaction) Heber Weiss

O2ˉ + Fe3+ Fe2+  + O2

Summarised equation

O2ˉ + H2O2 OH + OHˉ + O2

There is some evidence that free radical damage contributes to the etiology of many chronic health problems such as emphysema, cardiovascular and inflammatory disease, cataracts, and cancer. Defences against free radical damage include Tocopherol (Vitamin E), Escorbic acid (vitamin C), beta-carotene, glutathione, uric acid, bilirubin, and several metallo enzymes including glutathione peroxidase (selenium), catalse (iron), and superoxide dismutase (copper, zinc, manganese) and proteins such as ceruloplasmin (copper). The extent of tissue damage is the result of the balance between the frère radicals generated and the antioxidant protective defence system. Several dietary micronutrients contribute greatly to the protective system.

* 1. **Radical induced DNA damage**

Hydroxyl radical formed by radiolysis or by drug induced mechanism, interacts with DNA bases to form a hydroxyl radical adduct. It appears that Thymidine and to a lesser extent, Adenine may be rather more sensitive bases for hydroxyl radical attack than others, but in any event a neutral hydroxyl radical formed. If an electron is now removed from the neutral hydroxyl base radical to form a positively charged base adduct, the damage is fixed, that is can no longer be repaired. In well oxygenated cell this process is easily accomplished by oxygen which accepts an electron to form superoxide. At this stage hydroxyl base cation can undergo 2 chemical transformations: loss of proton to form hydroxyl base, or a condensation reaction in which a second hydroxyl group is added to the base to form a glycol derivative of the base which is rapidly recognised as aberrant by the cell’s repair enzymes and the base is efficiently removed. The result of the damaged base is strand breakage, the extent of which, if unable to repaired, will result in cytotoxicity, possible mutagenicity and/or cell death.[20]

* 1. **Redox cyclic**

If a drug required reductive activation to produce its damaging effect upon the cell, this reaction may be nullified in the presence of oxygen which is the most electron affinic of all biological molecules. The overall effect is one of redox cyclic, which if it is prevents the drug from being reductively activated, is also called futile cyclic.

E.g. Nitro compound which upon being reduced by a singlet electron forms a nitro radical anion. Oxygen being more electron affinic than the nitro radical, abstract the electron, regenerating the parent drug molecule to form superoxide. The formed hydroxyl radical not only is causing the peroxidation of lipid in membranes or oxidation of amino acids in proteins leading to conformational changes and inactivation of enzymes.

R-NO2 + 1eˉ R-NO2ˉ + O2 R-NO2 + O2ˉ

* 1. **Action of nitro homocyclic and hetrocyclic drugs**

With all nitro compounds their activity is solely dependent upon reduction of the nitro group, the products of which are responsible for DNA damage.[21]

1. **ANTIOXIDANTS**

Antioxidants are an inhibitor of the process of oxidation even at relatively small concentrations and does have diverse physiological role in the body antioxidant constituents of the plant material act as radical scavengers and helps in converting the radicals to less reactive species ability of free radical scavenging antioxidants is found in dietary sources like fruits vegetables and tea etc. antioxidants that have traditionally been used to inhibit oxidation in foods also quench dreaded free radicals and stop oxidation change in vivo so they have become viewed by many as natures answer to environmental and physiological stress aging atherosclerosis and cancer. The nutraceutical trend towards doubling the impact of natural antioxidants that stabilize food and maximize health impact presents distinct challenges in evaluating antioxidant activity of purified individual compounds mixed extract and endogenous food matrices and optimising applications.[22]

Broadly the possible mechanisms by which antioxidants may protect against ROS toxicity are:

1. Prevention ROS formation.

2. Interception of ROS attack by scavenging the reactive metabolites and converting them to less reactive molecules and by enhancing the resistivity of sensitive biological targets to ROS attack.

3. Facilitating the repair of damage caused by ROS.

4. Providing (e.g. as a cofactor by acting to maintain a suitable redox status) a favourable environment for the effective function of other antioxidant.[23]

In human body a complex combination of enzymatic and no enzymatic function to minimise the stress induced by ROS these antioxidants can be classified as:

1. Endogenous antioxidant those which are physiological and origin.

2. Exogenous antioxidant those which cannot be produced by the human body but may protect against pro accident forces when administered as supplement.[24,25]

**9. ENDOGENOUS ANTIOXIDANT**

Although a large number of enzymatic and non-enzymatic physiological substances are known to have antioxidant like functions the primary contributors are;

* 1. **Superoxide dismutase’s (SOD)**

These are the enzymes involved in the cellular defence against uncontrolled oxidative process that catalyse the dismutation of the superoxide radical anion and hence diminishes toxic effect due to this or to other free radical derived from secondary reactions.[26]

In mammalian tissue two types of SOD have been described;

* Cytosolic cuprozinc- SOD(CuZn-SOD)
* Mitochondrial mangano-SOD(Mn-SOD)

The principal function of SOD is to catalyse the conversion of one form of ROS to the other. The principal function of SOD is mainly controlled by its substrate the O2.

2O2 + 2H SOD H2O2 + O2

H2O2 thus produced is detoxified either by catalase or reduced glutathione (GSH) dependent reaction.[27]

* 1. **Catalase**

Catalases are present virtually in all mammalian cells and are suggested to play a dual role;

1. A true catalytic role in the decomposition of H2O2

H2O2 catalase H2O2 + O2

1. A peroxide role in which the peroxide is utilized to oxidize a range of H donors (AH2) such as methanol, ethanol and format.

AH2 + H2O2 A + H2O2

In each case active enzyme-H2O2 complex is formed initially followed by an exceedingly rapid second stage in which a second molecule of H2O2 serves as an H donor for the enzyme-H2O2 complex. The enzyme is most localized in the peroxisomes (mitochondria) of liver & Kidney & in much smaller aggregates (micro peroxisomes) found in other cells. [28]

* 1. **Glutathione (L-Gamma-glutyl-L-cysteinyl glycine)**

It is important in the circumvention of oxidative stress detoxification of electrophile maintenance of intracellular thiol redox status.[29]

* 1. **Glutathione peroxidase (GSHPx) –** glutathioneperoxidase catalase the reaction of hydro peroxide with reduced glutathione (GSH) to form glutathione disulphide (GSSG) and the reduction product of hydro peroxide. This enzyme is specific for its hydrogen donor GSH and non-specific for the hydro peroxide ranging from H2O2 to organic hydro peroxide. Two third of the enzyme is present in the cytosol and one third in the mitochondria.

ROOH/H2O2 GSH Peroxide ROH/H2O + GSSG

The cystolic and membrane bound monomer GSH phospholipids hydro-peroxides GSHPx and the distinct tetramer plasma GSHPx is able to reduce phospholipids hydro-peroxides without the necessity of prior hydrolysis by phospholipase A2. Glutathione S-transferase catalyse the reaction between the SH group of GSH and potential alkylating agents there by neutralizing their electrophilic sites and rendering them more water soluble. GSH also play a central role in coordinating the synergism of various crucial antioxidants.[30,31]

* 1. **Heme peroxidase**

Heme peroxidase such as horseradish peroxidase, lacto peroxidase and other mammalian peroxidases. The enzyme catalyses the oxidation of a wide variety of electrons donor with the help of H2O2 and thereby scavenges the endogenous H2O2. [32]

H2O2 + AH2 2H2O2 + A

1. **EXOGENOUS ANTIOXIDANTS**

For the effective protection against oxidative insults that we encounter in our daily lives regular consumption of at least some antioxidants in the diet or as supplements, appears to be very crucial. Among the exogenous antioxidants vit C & vit E have been recognized to be especially important and deficiency of these may leads to a number of pathophysiological consequences.

* 1. **Vitamin C and Citrus Bioflavonoids**

Vitamin C (ascorbic acid) exerts an antioxidants effect by undergoing oxidation to dehydroascorbic acid and then being regenerated. Because ascorbic acid is being constantly regenerated, there is always fresh ascorbic acid is available to continue the work of oxygen quenching mainly O2, OH, and various lipid peroxides. The deficiency disease associated with vitamin C is scurvy. Many of symptoms reflect difficulty in forming new good quality connective tissue. However, it has been found consistently that vitamin C acts best in the presence of plant bioflavonoids and as a result vitamin C is often mixed with citrus bioflavonoids prior to being formed in to supplements products. However special attention should be given in the presence of Fe3+ or Cu2+ excess. Vitamin C may act as a strong pro-oxidant and may actually induce lipid peroxidation and oxidative modification of genomic structure. Under such condition Vitamin C may reduce Fe3+ to Fe2+ which in turn facilitate the generation of OH. [33]

* 1. **Vitamin E**

The principle action of Vitamin E is now recognized to be the protection of the phospholipids of the cell membrane from free radical attack. This includes not only the outer cell membrane but also the much larger area of the internal cell membrane. Vitamin E is only one of the several antioxidant nutrients within the cell, but the special connection between vitamin E and membranes is assured by the fact that vitamin E is both fat soluble and hydrophobic and that it also readily finds a location within the membrane between the assembled for phospholipid molecule there. Vitamin E is therefore the antioxidant that is in situ within the membrane ready to deal with free radicals that arise within that exact location. Vitamin E appears a key factor in our overall antioxidant defence but also to be especially significant in the nervous system.[34]

* 1. **Beta-Carotene**

Beta-carotene is at the same time a Vitamin precursor that the body used to make vitamin A Carotenoid and a phytonutrient. It greatly enhances the immune system. It is a powerful antioxidant and free radical scavenger. Beta-carotene is the most efficient neutralizer of singlet oxygen which has particularly high energy and is one of the most destructive ROS molecules.

* 1. **Alpha-Carotene**

Although among the carotenoids beta-carotene is a focus of attention for the supplement industry, most research studies show Alfa-carotene to be more potent an antioxidants. Outstandingly Alpha-carotene was found to be 10 times more potent as an anti-cancer agent then beta-carotene and 38% more potent as an antioxidant then beta-carotene. It seems wise, therefore, to include the Alpha form into the best quality antioxidant formulae.

* 1. **Lycopene and Lutein**

Lycopene is another carotenoid antioxidant and is even more powerful than Alpha-carotene. Lycopene and lutein in small doses may potentially prevent colon carcinogenesis. Lutein was shown to be important in prevention of lung cancer. The carotenoid antioxidants have also been found to be especially important in the natural protection of the eye against macular degeneration. Lutein and lycopene have been found to best show a good level of protection.

* 1. **Minerals**

The Minerals required for forming the superoxide dismutase enzyme do not have to be part of the antioxidant mix. All that is required is that good to generous dietary care or by supplements. In fact, iron and copper may not need to be given and should never be given and should never be given in excess. Excesses of either of these have been reported to actually increase free radical generation by causing an imbalance between these minerals and vitamin C. Magnesium is a special case. It could be included in an antioxidant strategy either alongside the antioxidants otherwise so long as the subject’s intake of it is fully adequate.[35]

1. **INTRACELLULAR CHANGES FOLLOWING THE OXIDATIVE STRESS**

Oxidative stress induced cytotoxic effects appear to be mediated by a perturbation of intracellular free calcium and thiol homeostasis.[36] In a flow cytometric study, it was observed that when skeletal muscle derived L-6 cells subjected to oxidative change, intracellular calcium sharply increased immediately following the challenges such as a response was followed by membrane disintegration as detected by propidium iodide staining of DNA.[37] An early response to oxidative stress is depletion (Via oxidation or covalent adduct formation) of cellular soluble protein and bound thiol (eg. GSH), such depletion can:

1. Decrease plasma membrane Ca2+ ATPase activity and contribute to plasma membrane blabbing (altered permeability) and to impairment of the mitochondrial ability to retain Ca2+.
2. Impair Ca2+ sequestration capacity of the endoplasmic reticulum (an organelle with high Ca2+ affinity in muscle playing a key role in fine tuning of cystolic level of the cation) and perturb Microsomal Ca2+ homeostasis.[38]
3. **HEALTH AND DISEASE**

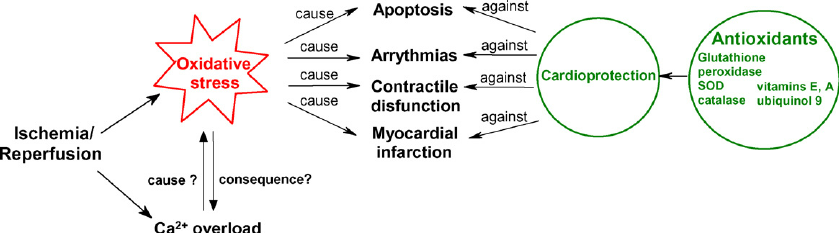
Oxidative damage to DNA proteins and other macromolecules has been implicated in the pathogenesis of a wide variety of diseases, most notably heart disease and cancer. A growing body of animal and epidemiological studies as well as clinical intervention trials suggest that antioxidants may play pivotal role in preventing or slowing the progression of both heart disease and some forms of cancer.

* 1. **Cardiovascular disease**

ROS-induced oxidative stress plays a role in various cardiovascular diseases such as atheroschlerosis, ischemic heart disease, hypertension, cardiomyopathy, cardiac hypertrophy, and congestive heart failure major sources of oxidative stress. Cardiovascular system involves: (1) the enzymes Xanthine Oxidase Reductase (XOR). (2) NAD(P)H oxidase (multi subunit membrane complexes) and (iii) NOS as well as (4) the mitochondrial cytochromes and (5) haemoglobin. NOSs and haemoglobin are also principal sources of RNS including NO· and SNOs which convey NO· bioactivity.[39]

* 1. **Ischemic/reperfusion injury**

Ischemia-reperfusion injury is a clinically relevant problem occurring as damage to the myocardium following blood restoration after a critical period of coronary occlusion. Massive production of ROS during ischemia/reperfusion in turn leads to tissue injury causing thus serious complication in organ transplantation, stroke, and myocardial infarction. During ischemia oversized ATP consumption leads to accumulation of purine catabolite hypoxanthine and xanthine, which upon subsequent reperfusion and influx of oxygen are metabolized by xanthine oxidase to produce enormous amounts of superoxide radical and hydrogen peroxide.[40]



* 1. **Rheumatoid arthritis**

Rheumatoid arthritis is an autoimmune disease that causes chronic inflammation of the joints and tissue around the joints with infiltration of macrophages and activated T cells. The pathogenesis of this disease is linked predominantly with the formation of free radicals at the site of inflammation. Oxidative injury and inflammatory status in various rheumatic diseases was confirmed by increased levels of isoprostanes and prostaglandins in serum and synovial fluid compare in controls Oxidative conditions in synovial tissue are also associated with a higher incidence of p53 mutations.[41]

* 1. **Diabetes**

It is a condition in which there is a decreased uptake of glucose into muscle and adipose tissue leads to chronic extracellular hyper glycaemia resulting in tissue damage and pathophysiological complications involving heart disease, atherosclerosis, cataract formation, peripheral nerve damage, retinopathy and others. Increased oxidative stress has been proposed to be one of the major causes of the hyperglycaemia-induced trigger of diabetic complications. Hyperglycaemia in an organism stimulates ROS formation from a variety of sources. These sources include oxidative phosphorylation, glucose auto oxidation, NAD(P)H oxidase, lipo-oxygenase, cytochrome P450 mono-oxygenase, and nitric oxide synthase (NOS) Diabetic patients have been found to have higher levels of oxidative stress indices It has been shown that under physiological condition glucose may undergo auto oxidation and contribute to ROS formation. ROS are capable of facilitating the glycation reaction that is now believed to be responsible for a most of the diabetic complication.[42]

* 1. **Neurological disorders**

The brain is particularly vulnerable to oxidative damage because of its high oxygen utilisation, its high content of oxydisable polyunsaturated fatty acids, and the presence of redox-active metals (Cu, Fe). Oxidative stress increases with age and therefore it can be considered as an important causative factor in several neurodegenerative diseases, typical for older individuals Alzheimer's disease.[43]

The brains of patients with Alzheimer's disease (AD)show a significant extent of oxidative damage associated with a marked accumulation of amyloid-ẞ peptide (AB), the main constituent of senile plaques in brain, as well as deposition of neurofibrillary tangles and neutrophil threads The direct evidence supporting increased oxidative stress in AD brain include (i) increased Cu, Fe, Al, and Hg content; (ii) increased lipid peroxidation and decreased polyunsaturated fatty acid content, and an increase in4-hydroxynonenal, an aldehyde product of lipid peroxidation in AD ventricular fluid: (iii) increased protein and DNA oxidation; (iv) diminished energy metabolism and decreased cytochrome c oxidase content; (v) advanced glycation end products (AGE), malondialdehyde, carbonyls, peroxynitrite, heme oxygenase-1, and SOD-1 in neurofibrillary tangles (vi) the presence in activated microglia surrounding most senile plaques of nitro tyrosine, formed from peroxynitrite (ONOO-). The elevated production of AB, as a preventive antioxidant for brain lipoproteins under the action of increased oxidative stress and neurotoxicity in ageing is postulated to represent a major event in the development of Alzheimer's disease.[44]

* 1. **Parkinson's disease**

Parkinson's disease (PD) involves a selective loss of neurons in an area of the midbrain called the substantia nigra. A majority of studies explored the effect of oxidative stress that contributes to the cascade of events leading to dopamine cell degeneration in PD. The occurrence of oxidative stress in PD is supported by both post-mortem studies and by studies demonstrating the capacity of oxidative stress to induce nigral cell degeneration. There is evidence that there are high levels of basal oxidative stress in the substantia-nigra-pars-compacta (SNC) in the normal brain, but that this increases in PD patients. One of the earliest detectable changes in the PD brain is a dramatic depletion in substantia-nigra-levels of the glutathione. Since oxidative stress appears to represent a portion of a cascade of biochemical changes leading to dopaminergic death, one of a major problem in understanding the pathogenesis of PD is separating out the effect and extent of oxidative stress from other components of the cascade that they can play a primary role in the initiation of ROS and RNS.[45]

* 1. **Cancer**

Epidemiological evidence consistently relates low antioxidant intake or low blood levels of antioxidants with increased cancer risk. Oxidants are capable of stimulating cell division, which is a critical factor in mutagenesis. When a cell with a damaged DNA strand divides, cell metabolism and duplication becomes deranged. Thus, a mutation can arise which in turn is an important factor in carcinogenesis. It is believed that antioxidants exert their protective effect by decreasing oxidative damage to DNA and by decreasing abnormal increases in cell division. Although antioxidant activity is believed to be responsible for much of the protection against tumour genesis, additional anticancer activities have been observed from several plant-derived substances. Sulphur containing phytochemicals, such as the allyl sulphides found in the allium family (garlic, onions, and leeks), and isothyocyanates and sulphoraphane (cabbage, broccoli, and cauliflower) have been shown to inhibit various steps in tumour development in animal and in vitro studies. Indoles, also found in cruciferous vegetables, and terpenes, natural constituents of citrus oils, may also be protective.[46]

* 1. **Pulmonary Disorder**

Because of its large surface area, the respiratory tract is a major target for free radical insult, not to mention the fact that air pollution is a major source of ROS. Recent studies suggest that free radicals may be involved in the development of pulmonary disorders such as asthma. Cellular damage caused by free radicals is thought to be partly responsible for the bronchial inflammation characteristic of this disease. It has been suggested that increasing antioxidant intake may help to reduce oxidant stress and help to prevent or minimize the development of asthmatic symptoms. Vitamin C, vitamin E, and beta carotene supplementation has been associated with improved pulmonary function. Some evidence suggests glutathione, or possibly N-acetyl cysteine, which is a precursor to glutathione, may be helpful in protecting against pulmonary damage as well.[47]

* 1. **Fibrosis**

Oxygen, paraquat, nitrofurantoins, and bleomycin, produces pulmonary fibrosis. Radical-generating agents such as iron and copper are also associated with liver fibrosis (cirrhosis) and fibrotic changes in other organs such as the heart. The induction of vitreous scarring by intraocular iron or copper is also well known, as is the association of homocystinuria with fibrotic lesions of the arteries. Adult Respiratory Distress Syndrome (ARDS) occurs due to production of active oxygen species by inflammatory cells.

1. **CONCLUSION**

Oxidative stress is an imbalance between reactive oxygen species and the antioxidant defence mechanisms of a cell or tissue, which leads to lipid peroxidation, DNA damage, and the inactivation of many enzymes. The enzymatic antioxidant defence system is the natural protector against lipid peroxidation that includes superoxide dismutase, catalase and glutathione peroxidase. Superoxide dismutase protects against the superoxide radical, which damages the membrane and its biological structure. Catalase primarily decomposes hydrogen peroxide to H₂O at a much faster rate, sharing this function with glutathione peroxidase. Glutathione peroxidase may play an important role in the removal of lipid hydro peroxides. The balance between these enzymes is important for the efficient removal of oxygen radicals from tissues. Therefore, reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and H2O2.

The second line of defence consists of the non-enzymic scavenger’s glutathione, ascorbic acid, and alpha-tocopherol, which scavenge residual free radicals escaping from decomposition by the antioxidant enzymes. Moreover, enzymic antioxidants are inactivated by the excessive levels of free radicals and hence the presence of non-enzymic antioxidants is presumably essential for the removal of these radicals. Glutathione a major non-protein thiol in living organisms plays a central role in coordinating antioxidant defence process. Glutathione reacts directly with reactive oxygen species and electrophilic metabolites, protects the essential thiol group from oxidation, and serves as a substrate for several enzymes including glutathione peroxidase. The lowered glutathione in D-galactosamine induced rats represents the increased utilization of glutathione as a result of oxidative stress. Perturbation in the redox status of glutathione not only impairs cellular defence.

**References**

1. <http://www.phytochemicals.info/freeradicals.pp>
2. Harmon, D..Gerontol,J., 1956, 11:298-300 .
3. Kappus,H.Arch. Toxicology. 1987.60:144.
4. Harsh, M. Cell injuary and cellular adaptation. Text book of pathology, 2005, 5: 38-40.
5. Abheri, D. S., Anisur, R. M., and Ghosh, A.K. Free Radicals and Their Role in Different Clinical Conditions: An Overview. International Journal of Pharma Sciences and Research, 2010, 1(3): 185-192.
6. http://www.uv.es/frag/oxidative stress.htm.
7. Marian, V., Dieter, L., Jan, M., Mark, T.D., Cronin. Free radicals and antioxidants in normal physiological functions and human disease. The International Journal of Biochemistry & Cell Biology. 2007,39: 44-84.
8. Hellsten, Y. The role of Xanthine oxidase in exercise. Elsevier Science publisher, 1994:221-234.
9. Edmonds, S.E. Blake, D.R.Hypoxia,oxidative stress and exercise in rheumatoid arthritis. Elsevier science publishers B.V., Amstradam, 1994:389-422.
10. Lilus,E.M., Manila, P.Photon emission of phagocytes in relation to stress and disease Experiential, 1992,48:1082-1091.
11. Otta. D.M.E., Lindstrom Seppa.p.,Sen, C.K. Cytochrome Paso dependent enzyme and oxidant-mediated responses in rainbow trout exposed to contaminated sediments. Environ Safe. 1994,27:265-280.
12. Karuzine Archkov. A.L. The oxidative inactivation of cytochrome P450 in mono-oxigenase reaction. Free Rad Biol Med., 1994, 16:74-97.
13. Beckman, J.S., Crow, J.P. Pathological implication of nitric oxide superoxide and peroxynitrite formation. Biochem Soc Trans., 1993, 21:330-334.
14. Hogg.N., Darley. UsmarV.M., Wilson M.T.Moncada,S. The oxidation of alpha tocopherol in human low density lipo-protien by the simultanious generation of super oxide and nitric oxide. FEBS Lett.1993, 326:199-203.
15. Jenkins,R.R., Halliwell,B. Metal binding agents possible role in exercise. In exercise and oxygen toxicity. Elsevier science publishers, Amstradam BV, 1994:59-76.
16. Buettner, G.R.Ascorbate autoxidation in the presence of is iron and copper chelates. Free Rodical Res Commun. 1986,1:349-353.
17. Sies.H. Biochemistry of the peroxisomes in the liver cells. Angew Chemint Ed Engl.1974.13:706-718.
18. Kukreja R.C. Kontos,H.A., Hess. M.L., Ellis, E.F. PGH synthase and lipoxigenase generate superoxide in the presence of NADH of NADPH. Cire Res. 1986, 59:612-619.
19. Uday, B., Dipak, D..Ranajit, K. B. Reactive oxygen species: Oxidative damage And pathogenesis.CURRENT SCIENCE, 1999,77(5):658-666.
20. Carwin, H. The rational design, mechanistic study and therapeutic application chemical compounds. Comprehensive medicinal chemistry, 1990, 2/3):734-738.
21. Gutteridge, J.M.C., Halliwell, B.Oxygen and suphur radicals in chemistry and Med. 1986,22:47.
22. MARK P. Antioxidants. CLINICAL NUTRITION INSIGHTS, 1998, 1:1-4,
23. Ohno HS., Fujii, K., Yamashita, J.H.Kizaki, T.,Oh-ishi,S.,Tanaguchi ,N. Superoxide dismutases in exercise and disease. Elsevier science publishers, 1994. 127-162.
24. Sulekha, M., Satish, Y.,Sunita, Y.,Rajesh, K.N. Antioxidants: A Review. Journal of Chemical and Pharmaceutical Research 2009. 1(1):102-104.
25. Shalini,S. International Journal of Physiotherapy.2012.2(7):7-15.
26. Hercilia M. H.,Jofre J.S. F., Rui .C.,Irineu, T. V., Bonfim, A.S., Junior. International Journal of Physiotherapy 2006. 3(2): 144-153.
27. Halliwel.B.in oxygen radicles and diseaseprocess. Hardwardaccademie publisher, Netherlands, 1997:1-14.
28. Eqbal M.A.D., Aminah A. Halimah,A.S. Natural Antioxidants, Lipid Profile, Lipid Peroxidation, Antioxidant Enzymes of Different Vegetable Oils, Advance Journal of Food Science and Technology., 2011..3(4): 308-316.
29. Ralf. D. Metabolism and functions of glutathione inbrain. Progress in Neurobiology. 2000, 62:649-671.
30. Chih .c.L., Wu-hsiung, H. Augusta, A., Leslie, M. K... Ted H. C. Expression of glutathione peroxidase and catalase in copper-deficient rat liver and heart.. Nutritional Biochemistry. 1995. 6:256-262.
31. Anna P., Giorgio F., B., Fiorella P. Analysis of glutathione: implication in redox and detoxification. ClinicaChimicaAcia2003, 333:19-39.
32. Noeleen, B. L. Brendan O'C., Ciarán, O.Fi.,MaryJ O The phylogeny of the mammalian heme peroxidases and the evolution of their diverse functions. BMC Evol Biol. 2008.,8: 101.
33. Dominique P., Pascal, C., Fernand, L., Catherine. R. Antioxidant activity of some ascorbic and cinnamic acids derivatives. IL Farmaco., 1998.53:85-88
34. Estany S., J.R. Palacio,J.R., Barnadas, R.,Sabes, miborra, A., Martinez P. Antioxidant activity of N-acetylcysteine, flavonoids and tocopherol on endometrial cells in culture. Journal of Reproductive Immunology, 2007. 75: 1-10.
35. Lawrence, G., Plaskett, B,A., Antioxidants our indispensible protection. Biomedical information services. 2003:1-11.
36. Pascoe, G.A.,Redd,D.J. Cell calcium, vitaminE, and thiol redox system in cytotoxicity. Free radical Bio Med. 1989, 6:209-224.
37. Sen, Y.P., Hanninen, O. Changes in intracellular calcium and membrane disintegration following exposure of myogenic cells to oxidative stress. XXXII Congress of the international union of physiological Sciences. 1993. 214:345-356.
38. Orolv, S.N., Sen, C.K.,Kolosova, I.A. Evidence for the involvement of G protein in the modulation of sodium and calcium cluxes in myogenic L6 and arotic smooth muscle cells. Biochem Biophys Res Commun. 1993,191:802-810.
39. Stampfer, M.J., Henneken, C.H., Manson, J.E. Vitamin consumption and the risk of coronory disease in women. N Engl J Med. 1993, 328:1449-1449.
40. Das, D.K., Maulik, N. Protection against free radical injury in the heart and cardiac performance. Elsevier science publishers 1994:359-388.
41. De flora,S., Izzotti, A., Antioxidant activity and other mechanisms of thiol involved in chemoprevention of mutation and cancer, AMJ MED. 1991.91(3):122-130.
42. Simon, P. W. Zrmn Y. J.James, V. H. Protein glycation and oxidative stress in Diabetes mellitus and ageing. Free Radical Biology & Medicine. 1991, 10:339- 352.
43. Tanea, T. R. Lipid peroxidation and neurodegenerative disease. Free Radical Biology & Medicine. 2011, 57:1302-1319.
44. Joshua, A. S., John, C., Breitner, M.A., Lovell, W. R. M. Free radical-mediated damage to brain in Alzheimer's disease and its transgenic mouse models. Free Radical Biology & Medicine, 2008, 45:219-230.
45. Ceballos.I., Javoy-Agrid,F. Delacourte. A.Parkinson's disease: neurodegenerative disorder due to brain anti-oxidant system deficiency. Antioxidants in therapy and preventive medicine Plenumpress New York, 1990, 264:493-498.
46. Luo, M.; Zhou, L.; Huang, Z.; Li, B.; Nice, E.C.; Xu, J.; Huang, C. Antioxidant Therapy in Cancer: Rationale and Progress. Antioxidants 2022, 11, 1128. https://doi.org/ 10.3390/antiox11061128
47. Swatz, H.M.Electron spin resonance studies at carcinogenesis. Advance cancer research Academic Press, Newyork 1972:227-252.