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Mechanism of oxygen free radical generation and Endogenous Antioxidants

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Abstract: Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions. Oxidation processes are important because it can control the production of free radicals and the unbalanced mechanism of antioxidant protection that can cause diseases and accelerated ageing. Free radicals can also initiate the oxidation of biomolecules such as protein, amino acids, lipid and DNA which will lead into cell injury and death. Reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen, and hydrogen peroxide are often generated as byproducts of biological reactions or from exogenous factors.

Keywords: Free radicals, Reactive Oxygen Species, Endogenous Antioxidants, Mechanism, Antioxidant

Introduction:

Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals." Free radicals are capable of attacking the healthy cells of the body causing them to lose their structure and function. It is essential in many living organism for the production of energy to fuel biological processes. It is one of the most important routes for producing free radicals in foods, drugs and even in living system. Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction. Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases. Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants.[1]

Plant extracts and plant products such as flavonoids and other polyphenolic constituents have been reported to be effective radical scavengers and inhibitors of lipid peroxidation.[2]

Different phytoconstituents and herbal products which are safer then synthetic medicines and beneficial in the treatment of diseases caused by free radicals. It also protects the body by preventing the free radicals to cause tissue injury. Phytoconstituents are conferring less side effect and compatible to body physiology. Therefore, it is demand of the modern era to use such phytoconstituents or phytomedicines.[3]

FREE REDICALS:

Free Radicals are molecules with an unpaired electron. They contain an odd number of electrons. Due to the presence of a free electron, these molecules are highly reactive. They are very unstable, react quickly with other compounds and try to capture the needed electron to gain stability.[4, 5]

$$A \rightarrow \text{minus one electron} \rightarrow A^{+\bullet}$$

$$B \rightarrow \text{plus one electron} \rightarrow B^{-\bullet}$$

They are important intermediates in natural processes involved in cytotoxicity, control of vascular tone, and neurotransmission. Radiolysis is a powerful method to generate specific free radicals and measure their reactivity.[4,6]

They are well documented for playing a dual role in our body as both deleterious and beneficial species. In low/moderate concentrations free radicals are involved in normal physiological functions but excess production of free radicals or decrease in antioxidant level leads to oxidative stress. It is a harmful process that can be mediates damage to cell structures, including lipids, proteins, RNA and DNA which leads to number of diseases.[6]

At low or moderate concentration some of the free radicals plays beneficial physiological role in vivo this include defence against infectious agents by phagocytosis, energy production, cell growth, function in different cellular signaling systems and the induction of a mitogenic response at low concentrations.[6]

Free radicals may be either oxygen derived (ROS, reactive oxygen species) or nitrogen derived (RNS, reactive nitrogen species). The oxygen derived molecules are O^{-}_{2} (superoxide radical), HO (hydroxyl radical) and H_2O_2 oxygen. The nitrogen derived molecules are NO_2 (nitrogen dioxide) and N_2O_3 (dinitrogen trioxide).[7]

FORMATION OF FREE RADICALS:

Normally, bonds don't split to leave a molecule with an odd, unpaired electron. But when weak bonds split, free radicals are produced. Free radicals are very unstable and react quickly with other compounds trying to capture the needed electron to gain stability. When the "attacked" molecule loses its electron, it becomes a free radical itself, beginning a chain reaction. All this happens in nanoseconds. Once the process is started, it can cascade, finally resulting in the disruption of a living cell. Some free radicals may arise normally during metabolism and by immune system's cells purposefully to neutralize viruses and bacteria. Normally, the body can handle free radicals, but if antioxidants are unavailable, or if the free radical production becomes excessive, damage may occur (Figure 1).[4]

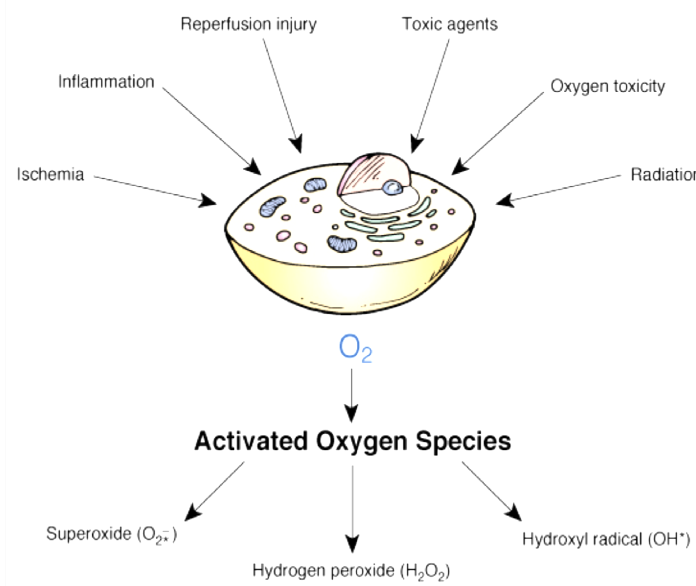

Figure: 1 Free radical formation

SOURCES OF FREE RADICAL PRODUCTION:

There are a number of sources for the production of free radicals. Some of them are mitochondrial electron transport chain, endoplasmatic reticulum, inflammatory cells, enzymes, auto-oxidation and haem proteins.[8]

STEPS INVOLVING FREE RADICAL GENERATION:[4]

In chemistry, free radicals take part in radical addition and radical substitution as reactive intermediates. Chain reactions 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- It involves free radical reactions in which the total number of free radicals remains the same.

Termination- Reactions are those which result in the net decrease in the number of free radicals. Typically two free radicals combine to form a more stable species.

For example: $2Cl\cdot \rightarrow Cl_2$

REACTIVE OXYGEN SPECIES:

Reactive oxygen species (ROS) are a type of oxygen derived free radical whose role in cell injury is well established. They are produced normally in cell during mitochondrial respiration and energy generation but they are degraded and removed by cellular defence system. When the production of ROS increases or the scavenging systems are ineffective and this results is an excess of these free radicals leading to a condition called oxidative stress.**14,17** [9, 10, 11]

Types of ROS include the hydroxyl radical, the superoxide anion radical ($O2^{\bullet -}$), hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical and various lipid peroxides. All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes and other small molecules resulting in cellular damage.[1, 12]

High altitude exposure results in decreased oxygen pressure and an increased formation of reactive oxygen and nitrogen species (RONS) which is often associated with increased oxidative damage to lipids, proteins and DNA.[13,]

MECHANISM OF OXYGEN FREE RADICAL GENERATION:[14-17]

Normally, metabolism of the cell involves generation of ATP by oxidative process in which biradical oxygen (O_2) combines with hydrogen atom (H) and in the process forms water (H_2O). This reaction of O_2 to H_2O involves 'four electron donation' in four steps involving transfer of one electron at each step. Oxygen free radicals are the intermediate chemical species having unpaired oxygen in their outer orbit. These are generated within mitochondrial inner membrane where cytochrome oxidase catalyses the O_2 to H_2O reaction. Three intermediate molecules of partially reduced species of oxygen are generated depending upon the number of electrons transferred.

a) Superoxide oxygen ($\cdot O_2$) – one electron
b) Hydrogen peroxide (H_2O_2) – two electron
c) Hydroxyl radical (OH^-) – three electron

These are generated from enzymatic and non enzymatic reactions as under:

a) Superoxide oxygen ($\cdot O_2$)

Superoxide anion $\cdot O_2$ may be generated by direct auto oxidation of O_2 during mitochondrial electron transport reactions. Alternatively, $\cdot O_2$ is produced enzymatically by xanthine oxidase and cytochrone P-450 in the mitochondria or cytosol. $\cdot O_2$ so formed is catabolised to produce H_2O_2 by superoxide dismutase (SOD).

b) Hydrogen peroxide (H_2O_2):

H_2O_2 is reduced to water enzymatically by catalase (in the peroxisomes) and glutathione peroxidase GSH (both in the cytosol and mitochondria).

The **Haber-Weiss reaction** generates •OH (hydroxyl radicals) from H2O2 (hydrogen peroxide) and superoxide (•O2$^-$). This reaction can occur in cells and is therefore a possible source for oxidative stress. The reaction is very slow but catalyzed by iron. The first step of the catalytic cycle involves reduction of ferric ion to ferrous:

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

The second step is the **Fenton reaction**:

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

Net reaction:

$$\bullet O_2^- + H_2O_2 \rightarrow \bullet OH + OH^- + O_2$$

c) Hydroxyl radical (OH^-)

OH^- radical is formed by 2 ways in biologic processes by radiolysis of water and by reaction of H_2O_2 with ferrous (Fe^{++}) ions; the latter termed as Fenton reaction.

OTHER OXYGEN FREE RADICAL:[14]

1. Nitric oxide (NO), a chemical mediator generated by various body cell (endothelial cell, nervous, macrophages ect), combines with superoxide and forms peroxynitrate (ONOD) which is a potent free radical.
2. Halide reagent (chlorine or chloride) released in the leucocytes reacts with superoxide and forms hypochlorous acid (HOCl) which is a cytotoxic free radical.
3. Endogenous sources of free radicals include some environmental agents such as tobacco and industrial pollutants.

   The various ROS and corresponding neutralizing antioxidants are given in table 1.

TABLE: 1. VARIOUS ROS AND CORRESPONDING NEUTRALIZING ANTIOXIDANTS:[1]

ROS	**NEUTRALIZINGANTIOXIDANTS**
Hydroxyl radical	vitamin C, glutathione, flavonoids, lipoic acid
Superoxide radical	vitamin C, glutathione, flavonoids, SOD
Hydrogen peroxide	vitamin C, glutathione, beta carotene, vitamin E, CoQ10, flavonoids, lipoic acid
Lipid peroxides	beta carotene, vitamin E, ubiquinone, flavonoids, glutathione peroxidase

ANTIOXIDANTS:

Antioxidants or free radicals scavengers are any substance that delay or inhibits oxidative damage to a target molecule. At a time one antioxidant molecule can react with single free radicals and are capable to neutralize free radicals by donating one of their own electrons, ending the carbon-stealing reaction. Antioxidants prevent cell and tissue damage as they act as scavenger. Cells produce defence against excessive free radicals by their preventive & repair mechanisms, physical defenses and antioxidant defenses.

A variety of components act against free radicals to neutralize them from both endogenous and exogenous in origins. These include: endogenous enzymatic antioxidants, non enzymatic, metabolic and nutrient, metal binding proteins like ferritin, lactoferrin, albumin & ceruloplasmin and phytoconstituents & phytonutrients. [1, 3]

EXOGENOUS ANTIOXIDANTS:

They are externally supplied to the body through food & plays important role to protect the body.

ENDOGENOUS ANTIOXIDANTS:

The body produces different antioxidants (endogenous antioxidants) to neutralize free radicals and protect the body from different diseases led by the tissue injuries.

The body has developed several endogenous antioxidant defense systems classified into two groups i.e. **enzymatic and non enzymatic**.

ENZYMATIC-

The enzymatic defense system includes different endogenous enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and non enzymatic defense system consists of vitamin E, vitamin C and reduced glutathione (GSH).

SOD is an important endogenous antioxidant enzyme that acts as the first line defense system against ROS which scavenges superoxide radicals to H_2O_2.

NON ENZYMATIC-

Vitamins C and E are non-enzymatic endogenous antioxidants that also exist within normal cells and react with free radicals to form radicals themselves which are less reactive than such free radicals entities. They break radical chain reactions by trapping peroxyl and other reactive radicals.

Non-enzymatic antioxidants also can be divided into- **metabolic antioxidants and nutrient antioxidants**.

Metabolic Antioxidants- They are the endogenous antioxidants produced by metabolic reactions in the body i.e. lipoid acid, glutathione, L-ariginine, coenzyme Q10, melatonin, uric acid, bilirubin, metal-chelating proteins, transferrin etc.

Nutrient Antioxidants- The nutrient antioxidants belonging to **exogenous** type cannot be produced in the body but provided through diet or supplements viz. **trace metals** (selenium, manganese, zinc), flavonoids, omega-3 and omega-6 fatty acids etc.

1.3.4 PHYTOMEDICINE AS ANTIOXIDANTS:[3]

Plant extracts and phytoconstituents are found effective as radical scavengers and inhibitors of lipid peroxidation. It includes Alkaloids, Flavonoids, Tannins, Phytosterols, Phenolic Acids, Flavanones, Volatile and essential oil, Carotenes and xanthophylls etc.

OXIDATIVE STRESS AND HUMAN HEALTH: [3]

Oxidative stress is a harmful condition that occurs when there is an excess of ROS and/or a decrease in antioxidant levels. This may cause tissue damage by physical, chemical and psychological factors that lead to tissue injury in human and causes different diseases.

Oxygen derived free radical reactions have been implicated in the pathogenesis of many human diseases including-

1. Neurodegenerative disorder: e.g. alzheimer's disease, parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, memory loss and depression.
2. Cardiovascular disease: e.g. atherosclerosis, ischemic heart disease, cardiac hypertrophy, hypertension, shock and trauma.
3. Pulmonary disorders: e.g. inflammatory lung diseases such as

asthma and chronic obstructive pulmonary disease.

4. Diseases associated with premature infants including bronchopulmonary, dysplasia, periventricular leukomalacia, intraventricular hemorrhage, retinopathy of prematurity and necrotizing enterocolitis.
5. Autoimmune disease: e.g. rheumatoid arthritis.
6. Renal disorders: e.g. glomerulonephritis and tubulointerstitial nephritis, chronic renal failure, proteinuria and uremia.
7. Gastrointestinal diseases: e.g. peptic ulcer, inflammatory bowel disease and colitis.
8. Tumors and cancer: e.g. lung cancer, leukemia, breast, ovary, rectum cancers etc.
9. Eye diseases: e.g. cataract and age related of ratina, maculopathy.
10. Ageing process.
11. Diabetes.
12. Skin lesions
13. Immunodepression.
14. Liver disease & pancreatitis.
15. AIDS.
16. Infertility.

Conclusion:

Formation of reactive oxygen species (ROS) is an unavoidable consequence in aerobic organisms during respiration. It has been shown that overproduction of unstable ROS leads to unwanted reactions with other groups or substances in the body, resulting in cell or tissue injury. In addition, numerous studies have revealed that uncontrolled lipid peroxidation is involved in the occurrence of many diseases, including Parkinson's, arthritis, myocardial infarction, Alzheimer's, cancer, cardiovascular disease, and liver damage. Therefore, during the last few decades, human nutrition and biochemistry research focused on antioxidants derived from foods that could prevent or diminish ROS-induced damage.

Reference:

1. Khandelwal K.R., "A practical pharmacognosy techniques and experiments" Nirali prakashan; 2008; pp. 149-156.
2. Ratheesh M., et al,"Anti-inflammatory activity of ruta graveolens Linn on carrageenan

induced paw edema in wistar male rats" African journal of biotechnology; 2007 Vol. 6; issue 10; pp. 1209-1211.

3. Anupam A. Suralkar, et al, "In vivo animal models for evaluation of anti-inflammatory activity" *pharmainfo.net; latest review*; 2008, vol. 6; issue 2;
4. Hussaainn Rehman S.A., et al,"Dose-dependent anti-inflammatory effect of silymarin in experimental animal model of chronic inflammation" *African journal of pharmacy and pharmacology* 2009 ; vol. 3; issue5; pp. 242-247.
5. Sharma U. S., Sharma U.K., et al, "Anti-inflammatory activity of Cordia dichotoma forst f. seeds extracts" *International Journal of Pharmaceuticals Analysis*, 2010; vol.2; issue 1; pp. 01-04.
6. Santosh S. bhujbal, et al,"Anti-inflammatory activities of an isolatd flavanoid fraction from Celosia argentea Linn." *Journal of medical plant research*, 2008, Vol. 2, issue 3, pp. 52-54.
7. Anupam A. Suralkar,et al, "In vivo animal models for evaluation of anti-inflammatory activity" *pharmainfo.net; latest review*, 2008, Vol. 6; issue 2 .
8. Rehman hussain, et al, "Dose-dependent anti-inflammatory effect of silymarin in experimental animal model of chronic inflammation" *African journal of pharmacy and pharmacology*, 2009, Vol. 3, issue 5, pp. 242-247.
9. Meena P.D., et al, " anticancer and antimutagenic properties of *Acacia nilotica*, on 7,12-Dimethylbenz(a)anthracene- induced skin papillomagenesis in swiss albino mice", *Asian Pacific Journal of cancer Prevention*, 2006, Vol 7, pp 627-632
10. Cerutti, P. A. Oxidant stress and carcinogenesis. Eur. J. Clin. Invest. 1991, 21, 1-11.
11. Blomhoff, R. Dietary antioxidants and cardiovascular disease. Curr. Opin. Lipidol.2005; 16: 47–54.
12. You- seong jeong, et al, "Effect of different concentration of formalin on paw edema and pain behaviors in

rats" *J Korean med sci*; 2002; vol. 17; pp. 81-85.

13. Dashputre N.L.,et al, "Evaluation of anti-ulcer activity of methanolic extract of Abutilon indicum Linn. Leaves in experimental rats" *International journal of pharmaceutical sciences and Drug research,* 2011; Vol.3 ; issue 2; pp. 97-100.
14. Gupta J K, et al, "Evaluation of anti-ulcer activity of Leucas lavandylifolia on mucosal lesion in rat", *Asian journal of pharmaceutical and clinical research,* 2010 vol. 3, issue 2, pp.118-120.
15. Ranju Pal, et al, "Antioxidant and free radical scavenging activity of ethanolic extract of the root of *Morinda citrifolia*" *African journal of pharmacy and pharmacology*; 2012, vol. 6; issue 5; pp. 278-282.
16. Cerutti, P. Oxy-radicals and cancer. Lancet 1994, 344, 862-863.
17. Qian, Z.J., Jung, W.K., Kim, S.K. Free radical scavenging activity of a novel antioxidative peptide purified from hydrolysate of bullfrog skin, Rana catesbeiana shaw. Bioresour. Technol.2008;99: 1690–1698.