Free Radicals and Their Role in Different Clinical Conditions: An Overview

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Abstract

Free Radicals are molecules with an unpaired electron and are important intermediates in natural processes involving cytotoxicity, control of vascular tone, and neurotransmission. Free radicals are very unstable and react quickly with other compounds, and try to capture the needed electron to gain stability. A chain reaction thus gets started. Once the process is started, it can cascade, and finally results in the disruption of a living cell. Generally, harmful effects of reactive oxygen species on the cell are most often like damage of DNA, oxidations of polydesaturated fatty acids in lipids, oxidations of amino acids in proteins, oxidatively inactivate specific enzymes by oxidation of co-factors. Free radicals cause many human diseases like cancer Alzheimer's disease, cardiac reperfusion abnormalities, kidney disease, fibrosis, etc. The free radicals formed in our body are combated by antioxidants that safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Excessive exercise has been found to increase the free radical level in the body and causes intense damage to the Regular physical exercise enhances the antioxidant defense system and protects against exercise induced free radical damage. Apart from the destructive effects of free radical they are also responsible for some vital actions like destroy the bacteria and other cells of foreign matter, kill cancer cells, turning on and off of genes and fight infection, to keep our brain alert and in focus.

Keywords

Radicals, Free Radicals, Reactive oxygen species, Anti-oxidant, Redox signaling

Introduction

Free Radicals are molecules with an unpaired electron. Due to the presence of a free electron, these molecules are highly reactive. 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 [1].

Types of long lived radicals

Stable radicals: The prime example of a stable radical is molecular oxygen O_2. Organic radicals can be long lived if they occur in a conjugated π system, such as the radical derived from α-tocopherol & vitamin E. Thiazyl radicals show remarkable kinetic and thermodynamic stability, with only a very limited extent of π resonance stabilization.

Persistent radicals: Compounds with persistent radicals are long lived due to steric crowding around the radical center and makes them physically difficult to react with another molecule. Examples of these include-Gomberg's triphenylmethyl radical, Fremy's salt (Potassium nitrosodisulfonate, Nitroxides, such as TEMPO(2,2,6,6-Tetramethylpiperidine-1-oxyl),verdazyls, nitronyl nitroxides, azephenylenyls , radicals derived from PTM (perchlorophenylmethyl radical) and TTM (tris(2,4,6-trichlorophenylmethyl radical). The longest-lived free radical is melanin, which may persist for millions of years.

Diradicals: Molecules containing two radical centers are called diradical. Multiple radical centers can also exist in a molecule. Molecular oxygen naturally (i.e. atmospheric oxygen) exists as a diradical (in its ground state as triplet oxygen). The high reactivity of atmospheric oxygen is owed somewhat to its diradical state (although non-radical states of oxygen are actually less stable). The existence of atmospheric molecular oxygen as a triplet-state radical is the cause of its paramagnetic character, which can be easily demonstrated by attraction of oxygen to an external magnet [2-7].

Production route of free radicals

Production of free radicals in the body is continuous and inescapable. The basic causes include the following [8]:

The immune system: Immune system cells deliberately create oxy-radicals and ROS (Reactive oxygen species) as weapons.
Energy production: During energy-producing cell generates continuously and abundantly oxy-radicals and ROS as toxic waste. The cell includes a number of metabolic processes, each of which can produce different free radicals. Thus, even a single cell can produce many different kinds of free radicals.
Stress: The pressures common in industrial societies can trigger the body's stress response to mass produce free radicals. The stress response races the body's energy-creating apparatus, increasing the number of free radicals as a toxic by-product. Moreover, the hormones that mediate the stress reaction in the body - cortisol and catecholamine - themselves degenerate into particularly destructive free radicals.

Pollution and other external substances: Air pollutants such as asbestos, benzene, carbon monoxide, chlorine, formaldehyde, ozone, tobacco smoke, and toluene ,Chemical solvents such as cleaning products, glue, paints, and paint thinners , Over-the-counter and prescribed medications , Perfumes , Pesticides , Water pollutants such as chloroform and other trihalomethanes caused by chlorination ,Cosmic radiation, Electromagnetic fields, Medical and dental x-rays, Radon gas, Solar radiation ,the food containing farm chemicals, like fertilizers and pesticides, processed foods containing high levels of lipid peroxides, are all potent generator of free radicals.
General factors: Aging, Metabolism, Stress
Dietary factors: Additives, alcohol, coffee, foods of animal origin, foods that have been barbecued, broiled, fried, grilled, or otherwise cooked at high, temperatures, foods that have been browned or burned, herbicides, hydrogenated vegetable oils, pesticides, sugar.
Toxins: Carbon tetrachloride, Paraquat, Benzo (a) pyrene, Aniline dyes, Toluene
Drugs: Adriamycin, Bleomycin, Mitomycin C, Nitrofurantoin, Chlorpromazine

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 formed. 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 can occur [8].

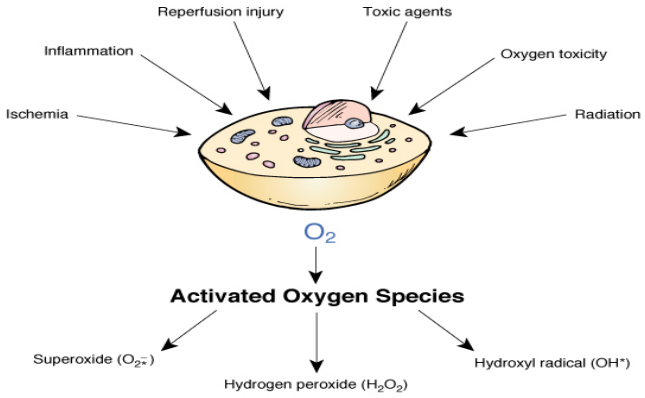

Figure 1: Free radical formation [8]

Steps involving free radical generation

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 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: $2Cl\cdot \rightarrow Cl_2$

The formation of radicals may involve breaking of covalent bonds homolytically, a process that requires significant amounts of energy. For example, splitting H_2 into 2H· has a ΔH° of +435 kJ/mol, and Cl_2 into 2Cl· has a ΔH° of +243 kJ/mol. This is known as the homolytic bond dissociation energy, and is usually abbreviated as the symbol DH°. 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.

Free radical-targets

Free radicals attack three main cellular components.

Lipids

Peroxidation of lipids in cell membranes can damage cell membranes by disrupting fluidity and permeability. Lipid peroxidation can also adversely affect the function of membrane bound proteins such as enzymes and receptors.

Proteins

Direct damage to proteins can be caused by free radicals. This can affect many kinds of protein, interfering with enzyme activity and the function of structural proteins.

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 radicals 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.

Damaging effects

Cells normally defend themselves against ROS damage through the use of enzymes such as superoxide dismutase and catalase. Small molecule antioxidants such as ascorbic acid (vitamin C), uric acid, and glutathione also play important roles as cellular antioxidants. Similarly, polyphenol antioxidants assist in preventing ROS damage by scavenging free radicals. The negative effects of ROS on cell metabolism include roles in programmed cell death and apoptosis, whereas positive effects include induction of host defense genes and mobilization of ion transport systems. In particular, platelets involved in wound repair and blood homeostasis release ROS to recruit additional platelets to sites of injury. These also provide a link to the adaptive immune system via the recruitment of leukocytes. Reactive oxygen species are involved in cardiovascular disease, hearing impairment via cochlear damage induced by elevated sound levels, ototoxicity of drugs such as cisplatin, and in congenital deafness in both animals and humans. [2-5]
Generally, harmful effects of reactive oxygen species on the cell are most often:

- Damage of DNA
- Oxidations of polydesaturated fatty acids in lipids
- Oxidations of amino acids in proteins
- Oxidatively inactivate specific enzymes by oxidation of co-factors

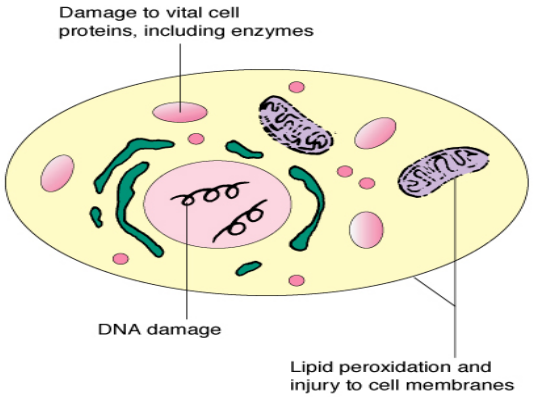

Fig.2. Cellular damage due to free radicals [9]

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 byproduct of the normal metabolism of oxygen and have important roles in cell signaling. However, during times of environmental stress ROS levels can increase dramatically, which can result in significant damage to cell structures. Platelets involved in wound repair and blood homeostasis release ROS to recruit additional platelets to sites of injury. Generally, harmful effects of reactive oxygen species on the cell are most often like -Damage of DNA, oxidations of polydesaturated fatty acids in lipids, oxidations of amino acids in proteins, oxidatively inactivates specific enzymes by oxidation of co-factors. [10-14]

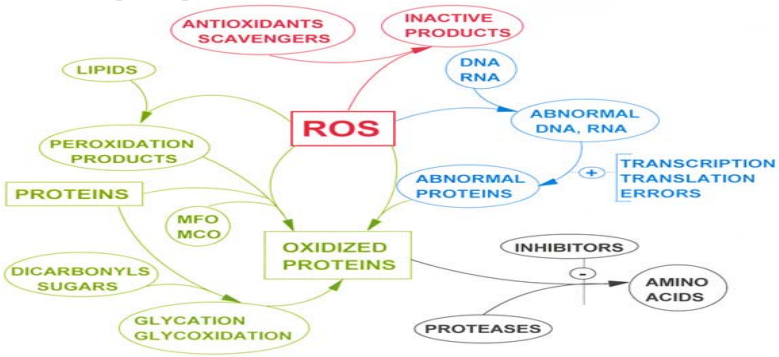

Figure 3: Effects of ROS [8]

Free radicals in beneficial role

- Free radicals perform many critical functions in our bodies in controlling the flow of blood through our arteries, to fight infection, to keep our brain alert and in focus.
- Phagocytic cells involved in body defense produce and mobilize oxygen free radicals to destroy the bacteria and other cells of foreign matter which they ingest.
- Similar to antioxidants, some free radicals at low levels are signaling molecules, i.e. they are responsible for turning on and off of genes.
- Some free radicals such as nitric oxide and superoxide are produced in very high amount by immune cells to poison viruses and bacteria.
- Some free radicals kill cancer cells. In fact certain cancer drugs aim in increasing the free radical amount in body.

Defensive systems against free radicals

All aerobic forms of life maintain elaborate anti-free-radical defense systems, also known as antioxidant systems.

Enzymes: The defense enzyme, superoxide dismutase (SOD), takes hold of molecules of superoxide - a particularly destructive free radical-and changes them to a much less reactive form. SOD and another important antioxidant enzyme set, the glutathione system, work within the cell. Circulating biochemical's like uric acid and ceruloplasmin react with free radicals in the intercellular spaces and bloodstream.

Self repair: The body also has systems to repair or replace damaged building blocks of cells. Most protein constituents in the cell are completely replaced every few days. Scavenger enzymes break used and damaged proteins into their component parts for reuse by the cell.

Nutrients: Vitamins and other nutrients neutralize the oxy radicals' and serves as second line of defense. Among the many substances used are Vitamins C and E, beta-carotene, and bioflavonoids. [15].

Free radical diagnosis-

Free radical can be diagnosed by certain techniques that includes [16]:

i. Electron Spin resonance
ii. Nuclear magnetic resonance using a phenomenon called CIDNP
iii. Chemical labeling-
This includes the use of X-ray photoelectron spectroscopy (XPS) or Absorption spectroscopy.
iv. Use of free radical markers-
Stable, specifc, or nonspecific derivatives of physiological substances can be measured e.g lipid peroxidation products (isoprostanes), amino acid oxidation products (meta-tyrosine, ortho-tyrosine, hydroxyl-Leu dityrosine) , peptide oxidation products (oxidized glutathione).

v. Indirect method-
Measurement of the decrease in the amount of antioxidants(,reduced glutathione-GSH)

Free radicals and human disease

Cancer: Like radiation and carcinogens, free-radical oxidation breaks strands of DNA. The breaks are repaired, but some mistakes occurs leading mutations. These genetic mutations can cause cancers. The age-related increase in cancer rates might have something to do with an age-related rise in oxidative damage to DNA.

Alzheimer's disease: The brain in Alzheimer's disease (AD) is under increased oxidative stress and this may have a role in the pathogenesis of neuron degeneration and death in this disorder. The direct evidence supporting increased oxidative stress in AD is: (1) increased brain Fe, Al, and Hg in AD, capable of stimulating free radical generation; (2) increased lipid peroxidation and decreased polyunsaturated fatty acids in the AD brain, and increased 4-hydroxynonenal, an aldehyde product of lipid peroxidation in AD ventricular fluid; (3) increased protein and DNA oxidation in the AD brain; (4) diminished energy metabolism and decreased cytochrome c oxidase in the brain in AD; (5) advanced glycation end products (AGE), malondialdehyde, carbonyls, peroxynitrite, heme oxygenase-1 and SOD-1 in neurofibrillary tangles and AGE, heme oxygenase-1, SOD-1 in senile plaques; and (6) that amyloid beta peptide is capable of generating free radicals. So free radicals are possibly involved in the pathogenesis of neuron death in Alzheimer's disease (AD).

Cardiac Reperfusion Abnormalities: Oxygen free radicals are highly reactive compounds causing per oxidation of lipids and proteins and are thought to play an important role in the pathogenesis of reperfusion abnormalities including myocardial stunning, irreversible injury, and reperfusion arrhythmias. Free radical accumulation has been measured in ischemic and reperfused myocardium directly using techniques such as electron paramagnetic resonance spectroscopy and tissue chemiluminescence and indirectly using biochemical assays of lipid per oxidation products. Potential sources of free radicals during ischemia and reperfusion have been identified in myocytes, vascular endothelium, and leukocytes. Injury to processes involved in regulation of the intracellular Ca^{2+} concentration may be a common mechanism underlying both free radical- induced and reperfusion abnormalities.

Kidney: Mitochondrial free radical production induces lipid peroxidation during myohemoglobinuria. Iron catalyzed free radical formation and lipid peroxidation are accepted mechanisms of heme protein-induced acute renal failure. However, the source(s) of those free radicals which trigger lipid peroxidation in proximal tubular cells remains unknown. In conclusion, the terminal mitochondrial respiratory chain is the dominant source of free radical.

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 interocular 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.

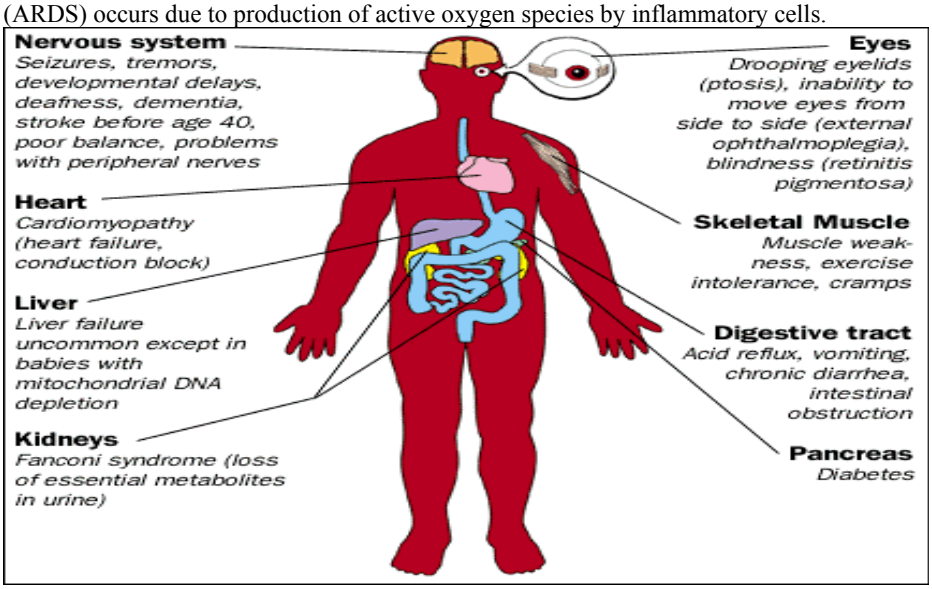

Figure 4: Overview of free radical damage [16]

Anti-oxidant

Substances that inhibit oxidation, and are capable of counteracting the damaging effects of oxidation in body tissue are termed antioxidants. They prevent damage caused by free radicals. They create a barrier from free radial damage that results in decaying process of oxidation. Oxidation causes aging in the skin, so antioxidants like pomegranate, vitamin C, vitamin E, goji berry, ellagic acid, and green tea can reduce the process of aging Antioxidants are intimately involved in the prevention of cellular damage -- the common pathway for cancer, aging, and a variety of diseases. [6-13]. Although there are several enzyme systems within the body that scavenge free radicals, the principle micronutrient (vitamin) antioxidants are vitamin E, beta-carotene, and vitamin C. Additionally, selenium, a trace metal that is required for proper function of one of the body's antioxidant enzyme systems, is sometimes included in this category. The body cannot manufacture these micronutrients so they must be supplied in the diet [1]. The ideal antioxidants should bear certain properties like; they must be effective in low concentration. They must be adequately soluble in oxidizable product. They must be non-toxic and non-irritant at the effective concentration even after prolong storage .They must be odorless, tasteless and should not impart color to the product. Their decomposition product should be non- toxic and non-irritant. They must be stable and effective over wide range of pH. They must be neutral and should not react chemically with other constituent present [9].

Some natural and synthetic antioxidant: Vitamin E d-alpha tocopherol a fat-soluble vitamin present in nuts, seeds, vegetable and fish oils, whole grains (esp. wheat germ), fortified cereals, and apricots. Vitamin C Ascorbic acid is a water-soluble vitamin present in citrus fruits and juices, green peppers, cabbage, spinach, broccoli, kale, cantaloupe, kiwi, and strawberries. Beta-carotene is a precursor to vitamin A (retinol) and is present in liver, egg yolk, milk, butter, spinach, carrots, squash, broccoli, yams, tomato, cantaloupe, peaches, and grains. Because beta-carotene is converted to vitamin A by the body there is no set requirement. [17-24]. Butylated hydroxy Toluene (BHT), butylated hydroxy Anisole (BHA), gallic acid are synthetic antioxidants.

Antioxidants preventing against free radical damage

The vitamins C and E are thought to protect the body against the destructive effects of free radicals. Antioxidants neutralize free radicals by donating one of their own electrons, ending the electron-"stealing" reaction. The antioxidant nutrients themselves don't become free radicals by donating an electron because they are stable in either form. They act as scavengers, helping to prevent cell and tissue damage that could lead to cellular damage and disease.
Vitamin E – The most abundant fat-soluble antioxidant in the body. It is one of the most efficient chain-breaking antioxidants available, is the primary defender against oxidation, and is the primary defender against lipid per oxidation (creation of unstable molecules containing more oxygen than is usual). Vitamin C – The most abundant water-soluble antioxidant in the body. It acts primarily in cellular fluid. It combats free-radical formation caused by pollution and cigarette smoke. Also helps return vitamin E to its active form [1].

Role of antioxidant in preventing cancer and heart disease

Epidemiological observations show lower cancer rates in people whose diets are rich in fruits and vegetables. This has lead to the theory that these diets contain substances, possibly antioxidants, which protect against the development of cancer. There is currently intense scientific investigation into this topic. Thus far, none of the large, well designed studies have shown that dietary supplementation with extra antioxidants reduces the risk of developing cancer. Antioxidants are also thought to have a role in slowing the aging process and preventing heart disease and strokes. Therefore from a public health perspective it is premature to make recommendations regarding antioxidant supplements and disease prevention. [25-27].

Exercise and oxidative damage

Endurance exercise can increase oxygen utilization from 10 to 20 times over the resting state. This greatly increases the generation of free radicals, prompting concern about enhanced damage to muscles, and other tissues. As it is not possible to directly measure free radicals in the body, the by-products that result from free radical reactions can be measured. If the generation of free radicals exceeds the antioxidant defenses then one would expect to see more of these by-products. Regular physical exercise enhances the antioxidant defense system and protects against exercise induced free radical damage. These changes occur slowly over time and appear to parallel other adaptations to exercise. On the other hand, intense exercise in untrained individuals overwhelms defenses resulting in increased free radical damage. Thus, the "weekend warrior" who is predominantly sedentary during the week but engages in vigorous bouts of exercise during the weekend may be doing more harm than good. [28-30].

Antioxidant supplements prevent exercise-induced damage

Vitamin deficiencies can create difficulties due to increased level of free radical in the body. It is hypothesized that vitamin E is involved in the recovery process following exercise. Currently, the amount of vitamin E needed to produce these effects is unknown. So, adequate amount of these vitamins must be regularly taken to reduce damages caused by free radicals.

Amount of antioxidant required

Antioxidants supplements were once thought to be harmless but increasingly we are becoming aware of interactions and potential toxicity. It is interesting to note that, in the normal concentrations found in the body, vitamin C and beta-carotene are antioxidants; but at higher concentrations they are pro-oxidants and, thus, harmful. Also, very little is known about the long-term consequences of mega doses of antioxidants. The body's finely tuned mechanisms are carefully balanced to withstand a variety of insults. Taking chemicals without a complete understanding of all of their effects may disrupt this balance [31 and 32].

Some recommendations regarding usage of antioxidants

One should follow balanced training program that emphasizes regular exercise and should include 5 servings of fruit or vegetables per day. This may help to develop inherent antioxidant systems.
For extremely demanding races (such as an ultra distance event), or when adapting to high altitude, a vitamin E supplement can be taken. One should carefully take the antioxidants so that over supplement does not occur because it is extremely hazardous [33].

Conclusion

Monitoring and rapid detection of free radical is necessary to combat the spread of various diseases. Difficulty in producing free radical scavengers in dosage from illustrates the need for more research about the chemical nature and behavior of free radicals. So if we can intensify our knowledge regarding free radicals & go deep into it we can easily prove the proverb “PREVENTION IS BETTER THAN CURE” but at the same time we should remember, “AN APPLE A DAY KEEPS A DOCTOR AWAY”.
So detailed knowledge regarding the benefits and hazard of free radicals must be known so that in a busy life where everyone is involved in a rat race one can easily combat against the deadly effect of free radicals and can live a healthy life. Moreover this would serve as a concise knowledge about free radicals for the study of students as well as researchers.

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