Review Article

An Overview of Antioxidant and free Radicals- A Review Article

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Abstract

The term "antioxidant" refers to any molecule capable of deactivating free radicals or reactive oxygen species. Free radicals are atoms, molecules or ions with unpaired electrons that are highly unstable. Free radicals derive from three elements: oxygen, nitrogen and sulfur. Free radical can be classified into oxygen-centered radicals and oxygen-centered non radicals. Superoxide anion ($•O_2^{\ -}$), hydroxyl radical (•OH), alkoxyl radical (RO•), peroxyl radical ($ROO^•$), nitric oxide ($NO^•$), nitric dioxide ($NO_2^•$) and peroxynitrite ($OONO^−$) are the various example of free radicals. Oxygen centered non-radicals are hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$), Hypochlorous acid and Ozone. Free radicals causes' oxidative damage of cellular DNA, protein and lipids, resulting in the development of various diseases such as cancer, cardiovascular diseases, type 2 diabetes mellitus, cataract, rheumatoid arthritis, or different neurodegenerative diseases.

The body resists the functions of reactive oxygen species by utilizing enzymatic antioxidant e.g superoxide dismutases, glutathione peroxidases, glutathione reductase and catalase. Non-enzymatic antioxidants such as vitamin E, vitamin C, carotenoids and polyphenols can hunt the reactive oxygen species. Endogenous compounds such as glutathione, ubiquinol, urate, bilirubin are also engaged in the detoxification of ROS.

Keywords: Antioxidant, free radical, reactive oxygen species, reactive nitrogen species

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Introduction

The term "antioxidant" refers to any molecule capable of deactivating free radicals or reactive oxygen species. Antioxidants are established nature's approach to provide adequate defense against reacting oxygen species attack. An individual with insufficient supply of antioxidants can be at the risk of oxidative stress which leads to accelerated tissue and organ damage [1]. Enzymatic and nonenzymatic are two different types of antioxidant found in biological systems [2, 3].

Alive organisms have evolved highly complex antioxidant systems, which work synergistically with each other to protect the body systems against free radical damage [4]. Approximately 1–3% of oxygen consumed by the human body is converted into ROS. Free radicals are atoms, molecules or ions with unpaired electrons that are not stable also called reactive oxygen species (ROS). Reactive oxygen species function not only as signaling molecules but also as redox regulators of protein function [5]. Free radicals derive from three elements: oxygen, nitrogen and sulfur, thus create reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive sulfur species (RSS) [6]. Up to a certain limit, they involve in physiological processes within a cell, but at high concentrations, they produce undesirable modifications to cell components, such as lipids, proteins, and DNA [5, 7-11] (**Figure 1**).

Free radical can be classified into oxygen-centered radicals and oxygen-centered non radicals. Superoxide anion ($•O_2^{\ -}$), hydroxyl radical (•OH), alkoxyl radical (RO•), peroxyl radical ($ROO^•$), nitric oxide ($NO^•$), nitric dioxide ($NO_2^{\cdot}$) and peroxynitrite ($OONO^−$) are the various example of free radicals.Oxygencentered non-radicals are hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$), Hypochlorous acid and Ozone [12, 13]. Other significant intracellular sources of ROS include microsomal cytochrome P450 enzymes, flavoprotein oxidases and peroxisomal enzymes involved in fatty acid metabolism (**Figure 2**)

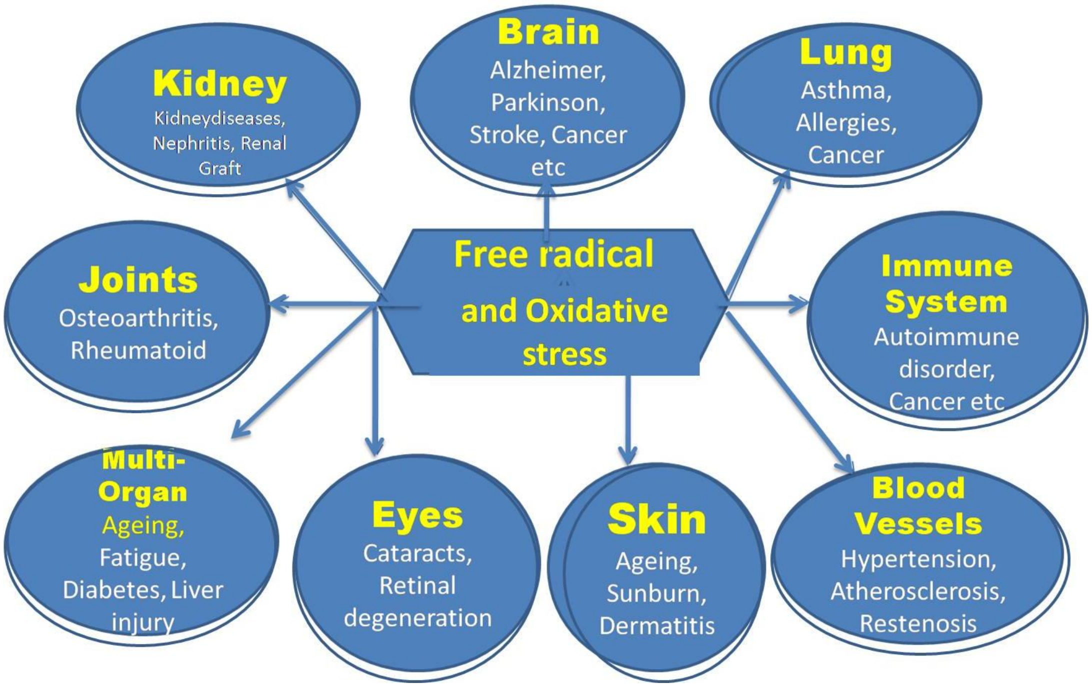


Figure 1 Effects of free radicals on different organs of human

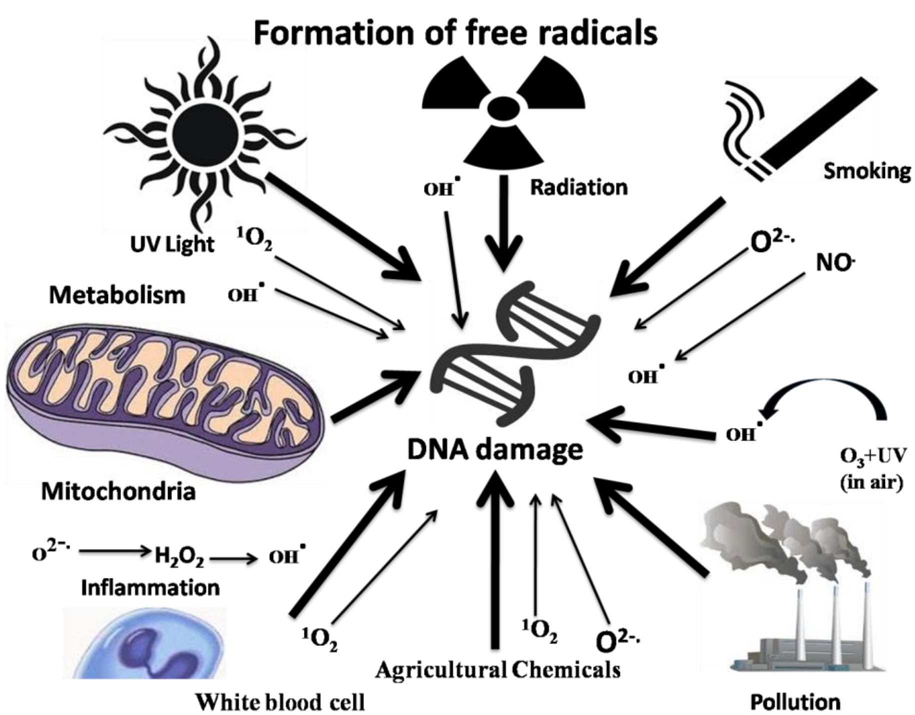


Figure 2 Diverse sources of free radicals

Superoxide anion

The superoxide anion plays a significant role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical, or singlet oxygen. Superoxide anion is an initial free radical formed during process mediated by nicotine adenine dinucleotide phosphate [NAD(P)H] oxidase or xanthine oxidase. Usually, electrons are transferred through mitochondrial electron transport chain for reduction of oxygen to water and approximately 1 to 3% of all electrons escape from the system to produce superoxide (Reaction 1). Mitochondria generate energy using 4 electron chain reactions, reducing oxygen to water. [14].

$$NADPH + 2O_2 \leftrightarrow NADP^{+} + 2O_2^{-\bullet} + H^{+} \rightarrow \text{(Reaction 1)}$$

The hydroxyl radical

Hydroxyl radical is the most reactive ROS that can damage proteins, lipids and carbohydrates and DNA. It can also initiate lipid peroxidation by taking an electron from polyunsaturated fatty acids. •OH, is the neutral form of the hydroxide ion. The hydroxyl radical has a high reactivity, making it a very dangerous radical with a very short *in vivo* half-life of approximately, 10^{-9} s [15]. The redox state of the cell is largely linked to an iron (and copper) redox couple and is maintained within strict physiological limits. The iron is released by superoxide and it has been reported in dehydratase-lyase family [16]. The released Fe^{2+} participate in the Fenton reaction, which yield highly reactive hydroxyl radical. (Reaction 2)

$$Fe^{+2} + H_2O_2^{\bullet-} \rightarrow Fe^{+3} + HO^{-} + HO^{\bullet} \rightarrow \text{(Reaction 2)}$$

Nitric oxide

NO• is generated within biological system by nitric oxide synthases (NOSs), which metabolise arginine to citruline with the formation of NO• *via* a five electron oxidative reaction [17] (**Figure 3**). Nitric oxide (NO•) is an plentiful reactive radical that acts as an important oxidative biological signaling molecule involved in neurotransmission, blood pressure regulation, defence mechanisms, smooth muscle relaxation and immune regulation [18]. However, since it is soluble in both aqueous and lipid media, it readily diffuses through the cytoplasm and plasma membranes. Nitric oxide reacts with oxygen and water to form nitrate and nitrite anions, which causes nitrosative stress [19]. During the oxidative burst, nitric oxide and the superoxide anion may react together to produce peroxynitrite anion (ONOO−), which is also a powerful oxidizing agent. Nitric oxide readily binds transition metal ions like Fe^{2+} of haem groups in the enzyme soluble guanylate cyclase (sGC) [20].

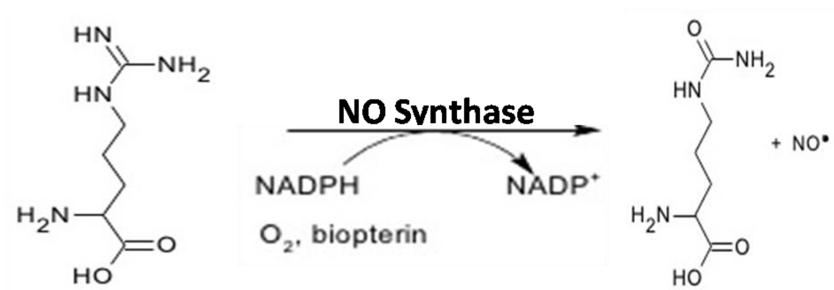


Figure 3 Formation of Nitric oxide radical from arginine.

Nitric dioxide

($NO_2^{\cdot}$) is formed from the reaction of peroxyl radical and NO, polluted air. Nitric dioxide adds to double bonds and abstract labile hydrogen atoms initiating lipid peroxidation and production of free radicals. [21].

Peroxynitrite

Reaction of $NO^{\bullet}$ and superoxide anion can generate peroxynitrite ($O2^{\cdot-} + NO^{\bullet} \rightarrow OONO^{-}$). Peroxynitrite is a cytotoxic species and causes injury to low-density lipoprotein (LDL). This appears to be an important tissue damaging species [21] and also involved in neurodegenerative disorders and kidney diseases [22]. Nitrotyrosine, which is formed from peroxynitrite-mediated reactions [22].

Peroxyl and Alkoxyl radicals

Peroxyl radicals ($ROO^{\bullet}$) are formed by a direct reaction of oxygen with alkyl radicals ($R^{\bullet}$), the reaction between lipid radicals and oxygen is one good example $R^{\bullet} + O_2 \leftrightarrow ROO^{\bullet} \rightarrow$ (Reaction 3). Decomposition of alkyl peroxides (ROOH) also results in peroxyl ($ROO^{\bullet}$) and alkoxyl ($RO^{\bullet}$) radicals. Peroxyl and alkoxyl radicals are frequently observed in lipid peroxidation. Few peroxyl radicals break down to liberate superoxide anion and also can react with each other to generate singlet oxygen [23].

$$R^{\bullet} + O_2 \leftrightarrow ROO^{\bullet} \rightarrow \text{(Reaction 3)}$$

Singlet oxygen

Molecular oxygen in the ground state is a triplet (two electrons have the same spin). Tayakama [24] reported that metastable phosphatidylcholine hydroperoxides present in the living organism produced singlet oxygen during their breakdown in the presence of Cu^{2+} in the dark. Singlet oxygen can be formed from hydrogen peroxide, which reacts with superoxide anion, or with HOCl or chloroamines in cells and tissues [26]

Compared with other reactive oxygen species, singlet oxygen is rather mild and nontoxic for mammalian tissue [25]. However, singlet oxygen has been known to be involved in cholesterol oxidation (Girotti *et al* 2000). Oxidation of cholesterol by singlet oxygen results in formation of 5α–OOH (3β–hydroxy–5α–cholest–6–ene–5–hydroperoxide), [26]. Oxidation and degradation of cholesterol by singlet oxygen was observed to be accelerated by the co-presence of fatty acid methyl ester. In the human organism, singlet oxygen is both a signal and a weapon, with therapeutic potency against various pathogens such as microbes, viruses, and cancer cells [25]

Hydrogen peroxide (H_2O_2)

Hydrogen peroxide is generated through a dismutation reaction from superoxide anion by superoxide dismutase (Reaction 4). Amino acid oxidase and xanthine oxidase are enzymes that can produce hydrogen peroxide from superoxide anion. Hydrogen peroxide is a weak oxidizing and reducing agent and it is considered as a less reactive species. Hydrogen peroxide can generate the hydroxyl radical in the presence of metal ions and superoxide anion. Hydrogen peroxide can produce singlet oxygen through reaction with superoxide anion or with HOCl or chloroamines in living systems. Hydrogen peroxide can degrade certain heme proteins, such as hemoglobin, to release iron ions.

$$2O_2^{-\bullet} + H^{+} \rightarrow O_2 + H_2O_2 \rightarrow \text{(Reaction 4)}$$

Ozone

Ozone is an unwanted oxidant and most toxic air pollutant [27]. Ozone can form in urban air as a result of photochemical reactions and pollution. The ozone has capacity to cause oxidation of biomolecules either directly or via free radical mechanisms [28].

Hypochlorous acid

HOCl is produced by myeloperoxidase at sites of inflammation [28]. The enzyme oxidizes chloride ions in the presence of H_2O_2 (Reaction 5). Although HOCl is not a free radical, it is a potent oxidizing and chlorinating agent. HOCl attacks primarily amines and sulfhydryl groups in proteins and chlorinates purine bases in DNA [29].

$$H_2O_2 + Cl^{-} \rightarrow HOCl + H_2O \rightarrow \text{(Reaction 5)}$$

Enzymatic sources

The enzymatic sources of ROS under sub cellular levels are xanthine oxidases, cyclo-oxygenases (COX), lipoxygenases (LOX), NO synthases (nitric oxide synthase) and mitochondrial oxidases [30].

Monoamine oxidase

This enzyme present in mitochondrial membrane and causes oxidative deamination and thus produces H_2O_2 in matrix and cytosol [31].

NADPH oxidase/Respiratory Burst Oxidase

NADPH oxidase is a multicomplex enzyme located in plasma membrane present of neutrophils and produces $O_2^{-\bullet}$ and plays an important role in host defenses by generating superoxides$^{\bullet}$ and also helps in signaling. It contains several components including cytochrome b558, p47, p67, p40 and rac 1.Upon stimulation, cytoplasmic subunits activate gp91 and cause respiratory bursts that activate superoxide, and release them into the phagosomes [32].

Xanthine oxidoreductase

This enzyme catalyzes hypoxanthine into xanthine, and then into uric acid (**Figure 4**). Xanthine Oxidoreductase (XOR) produce large amount of H_2O_2 and $O_2^{\ -}$. It is also found that XOR can transform nitrates into nitrites and NO. It also catalyzes the NO with O^{2-} and form highly reactive peroxynitrites [33, 34].

The following chemical reactions are catalyzed by xanthine oxidase:

$$\text{Hypoxanthine} + H_2O + O_{2\leftrightarrow}\ \text{xanthine} + H_2O_2 \rightarrow \text{(Reaction 6.a)}$$
$$\text{Xanthine} + H_2O + O_2 \leftrightarrow \text{uric acid} + H_2O_2 \rightarrow \text{(Reaction 6.b)}$$

Xanthine oxidase can also act on certain other purines, pterins, and aldehydes. For example, it efficiently converts 1-methylxanthine to 1-methyluric acid, but has little activity on 3-methylxanthine [35]. Under some circumstances it can produce superoxide ion.

$$RH + H_2O + 2\ O_2 \leftrightarrow ROH + 2\ O_2^{-} + 2\ H^{+}$$

XO has also been found to produce the strong one-electron oxidant carbonate radical anion from oxidation with acetaldehyde.

Here is a diagram highlighting the pathways catalyzed by xanthine oxidase.

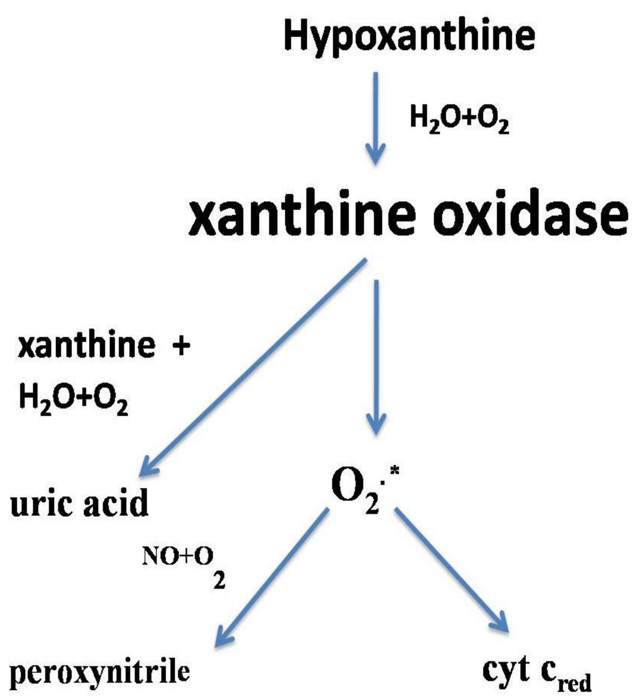


Figure 4 The pathways catalyzed by Xanthine oxidase

ROS generation by arachidonic acid

ROS is generated intracellularly during the metabolism of arachidonic acid and various enzymes like cyclooxygenase, lipooxygenase, cytochrome P450 oxidase are involved.

Cytochrome P450 oxidase

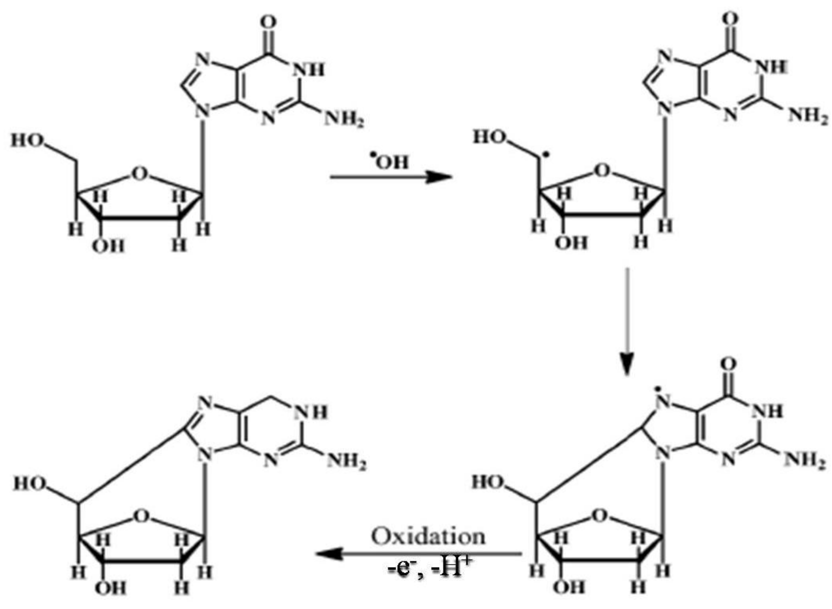
This is heme-containing enzyme; it is present in mitochondria and participates in metabolism of cholesterol, steroids, hormones, catabolism of bile acids. It transfers 2e-, one is bound to oxygen and the second is reduced to water.

Myeloperoxidase

This is heme-containing enzyme, present in neutrophils and eosinophils and catalyzes the H_2O_2 with various substrates to form highly reactive hypochloric acids [36, 37].

Effects of Oxidative Stress on DNA

Free radicals involves in degradation of bases of single or doublestranded DNA and lead to sugar-bound modifications, mutations, deletions or translocations and cross-linking with proteins. All types of these DNA modifications (**Figure 5**) are highly significant to carcinogenesis, aging, neurodegenerative, cardiovascular, and autoimmune diseases. Formation of 8-OH-G is the best-known DNA damage occurring via oxidative. Promoter regions of genes contain consensus sequences for transcription factors. Formation of 8-OH-G DNA in transcription factor binding sites can modify binding of transcription factors and alter the expression of related genes. Besides 8-OH-G, 8,59-cyclo-29-deoxyadenosine (cyclo-dA) has also been shown to inhibit transcription from a reporter gene in a cell system. The binding of TATA-binding protein may be impaired by the presence of cyclo-dA [38].



8, 59-cyclo-29-deoxyadenosine

Figure 5 The schematic diagram is adopted from [38, 39]

Ascorbic Acid (Vitamin C)

Ascorbic acid includes mainly two compounds namely, L-ascorbic acid and L-dehydroascorbic acid effective against superoxide radical anion, hydrogen peroxide, hydroxyl radical and singlet oxygen [40]. Vitamin C reacts with superoxide and inhibits the formation of nitrosamines during protein digestion and protect damages to DNA and cellular proteins [41]. The antioxidant mechanisms of ascorbic acid are based on hydrogen atom donation to lipid radicals and removal of molecular oxygen [42].

Tocoferol (Vitamin E)

Tocoferol is the most effective antioxidant at high levels of oxygen, protecting cellular membranes from lipidic peroxidation, free radical scavenging, maintenance of membrane integrity and immune function [43].It triggers apoptosis of cancer cells and inhibits free radical formations [44]. Vitamin E is composed of eight isoforms, with four tocopherols (a, b, c and d tocopherol) and four tocotrienols (a, b, c and d tocotrienols). Tocoferol confers the antioxidant activity mainly because of chroman head group present. Vitamin E donates electron to peroxyl radical, which is produced during lipid peroxidation.

Retinoids (Vitamin A)

Retinoids are the natural and synthetic derivatives of vitamin A. Vitamin A or retinol is a carotenoid produced in the liver and results from the breakdown of b-carotene. The retinoids originate from retinyl esters, carotenoids and it can trap peroxyl free radicals [45] and peroxidation peroxidation to lipids [46, 47].

Lycopene

The prominent carotenoid in serum is the antioxidant red pigment called lycopene which is fat soluble. Lycopene can prevent carcinogenesis especially in upper digestive tract neoplasm and atherogenesis by protecting critical cellular biomolecules, including lipids, lipoproteins, proteins, and DNA. Lycopene is most efficient biological antioxidizing agent, because it can bind to chemical species that react easily to oxygen [48].

Carotenoids

Carotenoids like lycopene, b-carotene and xanthophyls are pigments found in plant ssytem. The b-carotene has been found to react with peroxyl (ROO^-), hydroxyl (^-OH) and superoxide ($O_2{}^{2\bullet}$) radicals [49]. Studies have revealed that an increased consumption of a diet rich in carotenoids is correlated with a lower risk of age-related diseases. Carotenoids contain conjugated double bonds and has the ability to delocalize unpaired electrons [50].

Vitamin K

Vitamin K is a group of fat-soluble compounds, essential for posttranslational conversion of protein-bound glutamates into carboxyglutamates in various target proteins. The antioxidant properties of Vitamin K are mainly due to the 1, 4-naphthoquinone structure within it. The K1 and K2 are two natural isoforms of this vitamin [51].

Thiol antioxidants

The major thiol antioxidant is the tripeptide glutathione (GSH), acts either by donating a hydrogen atom or an electron and it is considered to be the major thiol-disulphide redox buffer of the cell [52, 53]. The antioxidant capacity of thiol compounds is due to the sulphur atom, which manage the loss of a single electron [54]. The glutathione can act as a co-factor for several detoxifying enzymes and scavenge hydroxyl radical and singlet oxygen directly [53].

Lipoic acid

Lipoic acid is present in meat, liver and heart [55]. Lipoic acids can prevent oxidative damages of proteins. Lipoic acids play an important role in reducing blood glucose concentration and also prevent oxidative damages of proteins. Lipoic acid regenerates GSH in liver, kidney, and lung tissue and also regenerates vitamins C and E. Lipoic acids improve age-related decline in memory and brain related ailments, including Alzheimer's disease and Parkinson's disease [56].

Polyphenols

Polyphenols are heterogeneous group of compounds divided into different classes based on their chemical structure. Phenolic acids are composed of hydroxycinnamic and hydroxybenzoic acids. They have antioxidant activity as chelators and free radical scavengers against hydroxyl, peroxyl radicals, superoxide anions and peroxynitrites. The polyphenols, particularly flavonoids present in green and black tea is having major importance in health and disease particularly like as anticancer and anti-inflammatory agents. The main flavonoids identified in tea are catechin, including (-)-epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and (-)-

epicatechin (EC) [57]. Green tea contains gallic acid (GA) and other phenolic acids such as chlorogenic acid and caffeic acid, and flavonols such as kaempferol, myricetin and quercetin [58] whereas black tea contains mostly the polymerized catechins such as theaflavins and thearubigins.

Melatonin (N-acetyl-5-methoxytryptamine)

This is neurohormone that is synthesized in the pineal gland. Melatonin scavenges free radicals in oxygen metabolism, and protecting against free radical-induced damage to DNA, proteins and membranes.

Coenzyme Q

CoQ, also known as ubiquinone is a redox-active substance plays an important role in the respiratory chain and in other cellular metabolism. Coenzyme acts by preventing the formation of lipid peroxyl radicals. CoQ in its reduced form as the hydroquinone (ubiquinol) is a potent lipophilic antioxidant and is capable of recycling and regenerating other antioxidants such as tocopherol and ascorbate.

Enzymatic antioxidants

Superoxide dismutase (SOD), (EC 1.15.1.1) (**Table 1**).This enzymatic antioxidant catalyzes the conversion of superoxide anions to dioxygen and hydrogen peroxide. Three forms of SOD are present in human system: cytosolic Cu, Zn-SOD, mitochondrial Mn-SOD and extra cellular- SOD [59]. Superoxide dismutase neutralizes superoxide ions by going through successive oxidative and reductive cycles of transition metal ions. Extra cellular superoxide dismutase is a tetrameric secretary glycoprotein contains copper, zinc, and is having a high affinity for certain glycosaminoglycans like heparin and heparin sulphate [60].

Table 1 Enzymatic antioxidant, their cellular location and the reactions the carried

Enzymatic antioxidant	Cellular location	Substrate	Reaction
Mn/Cu/Zn SOD	Mitochondrial matrix(Mn SOD) and cytosol (Cu/Zn SOD)	$O_2^{\bullet -}$	$O_2^{\bullet -} \rightarrow H_2O_2$
CAT	Peroxisomes cytosol	H_2O_2	$H_2O_2 \rightarrow O_2 + H_2O$
GSHPx	Cytosol	H_2O_2	$H_2O_2 + GSH \rightarrow GSSH + H_2O$
Pyx	Cytosol	H_2O_2	$H_2O_2 + TrxS_2 \rightarrow Trx(SH)_2 + H_2O$

Catalase (EC1.11.1.6)

Ctalase is present in the peroxisome of aerobic cells that converts hydrogen peroxide to water and molecular oxygen (Table 1). One molecule of catalase can convert approximately 6 million molecules of hydrogen peroxide to water and oxygen within a minute [60] having one of the highest turnover rates for all enzymes.

Glutathione peroxidse

Glutathione peroxidase has two forms Viz. selenium-dependent and selenium-independent (Table 1) [60].The glutathione metabolism is one of the most important antioxidative defense mechanisms present and there are four different Se-dependent glutathione peroxidases present in humans [61] and these enzymes add two electrons to reduce peroxides by forming selenoles (Se-OH) and these seleno-enzymes allow them to eliminate peroxides. Selenium-dependent glutathione peroxidase acts glutathione (GSH) and catalyzes the conversion of hydrogen peroxide to water, while simultaneously oxidizing GSH. It also competes with catalase for hydrogen peroxide as a substrate and is the major source of protection against low levels of oxidative stress [61]. Another form of glutathione peroxidises (GSHPX), enzymes that require selenium (has selenocysteine at the active site) for their action remove H_20_2. Glutathione reductase, an FAD containing enzyme, regenerates GSH from GSSG [62].

Conclusion

This article summarizes the functions of both enzymatic and non enzymatic antioxidant, free radicals, including their different reaction mechanisms. Biological systems are under a continuous influence of oxidative stress because of excessive generation of ROS. Antioxidants play a very important role in the body defense system against reactive oxygen species (ROS), which are the harmful byproducts generated during normal cell aerobic respiration. To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated antioxidant protection system. Diet forms an important component of the antioxidant protection system, it supplies the major antioxidants such vitamin C, vitamin E, carotenoids and polyphenols. Therefore, food rich in these components should form a part of the daily diet.

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