Chapter

Antioxidant Categories and Mode of Action

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Abstract

Oxidative stress has received a considerable scientific attention as a mediator in the etiology of many human diseases. Oxidative stress is the result of an imbalance between free radicals and antioxidants. Cells can be damaged by free radicals that are considered to play a main role in the aging process and diseases development. Antioxidants are the first line of defense against the detrimental effects of free radical damage, and it is essential to maintain optimal health via different mechanisms of action. Types of antioxidants range from those generated endogenously by the body cells, to exogenous agents such as dietary supplements. Antioxidant insufficiency can be developed as a result of decreased antioxidant intake, synthesis of endogenous enzymes, or increased antioxidant utilization. To maintain optimal body function, antioxidant supplementation has become an increasingly popular practice through improving free radical protection. In this chapter, we first elucidate the oxidative stress, and then define the antioxidant and its categories. Finally, introduce the antioxidants mode of actions for cell protection from free radicals.

Keywords: oxidative stress, antioxidants, reactive oxygen species, antioxidant enzymes, free radicals, antioxidant mechanisms

1. Introduction

Oxidative stress refers to the imbalance between oxidants and antioxidants within the body due to antioxidant deficiency or increased reactive oxygen species (ROS), reactive nitrogen species (RNA), and reactive sulfur species (RSS) production, which lead to potential cellular damage [1, 2]. ROS is a collective term that encompasses all highly reactive forms of oxygen, including free radicals. ROS categories include hydroxyl radical ($OH^{\bullet}$), perhydroxyl radical ($HO_2^{\bullet}$), hypochlorous acid (HOCl), superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), nitric oxide radical ($NO^{\bullet}$), hypochlorite radical ($OCl^{\bullet}$), peroxynitrite (ONOO), and different lipid peroxides. RNS are derived from nitric oxide by the reaction with $O_2^{\bullet -}$ to form $ONOO^-$, while RSS are easily produced from thiols through a reaction with ROS [3, 4].

Due to unpaired electrons of free radicals, these free radicals show high activity to react with other molecules in order to be neutralized. The free radicals have important functions in cell signaling, apoptosis, ion transportation, and gene expression [4]. Chemical reactivity of inactivated free radicals can damage all cellular macromolecules including carbohydrates, proteins, lipids, and nucleic acids. In general, cells are able to protect themselves against ROS damage via intracellular enzymatic reactions, metal

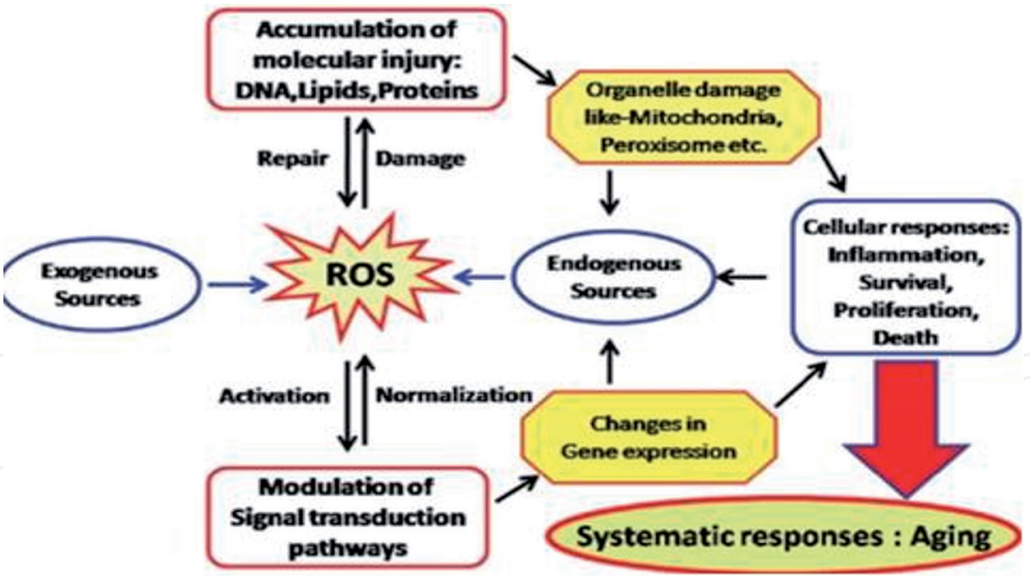

Figure 1.
Reactive oxygen species (ROS) generation by endogenous and exogenous sources can lead to oxidative damage and accumulation of proteins, lipids and DNA, when defensive (repair) mechanisms of the body become weak. These ROS also modulate the signal transduction pathways, which result in organelle damage, and changes in gene expression followed by altered responses of the cells, which finally results into aging. Adapted from Pandey and Rizvi [5].

chelating, and free radical scavenging actions to keep the ROS homeostasis at a low level. In addition, dietary antioxidants can assist to keep an adequate antioxidant status in the body. Nevertheless, during environmental stress and cell dysfunction, levels of ROS can increase dramatically and cause significant cellular damage in the body. Consequently, oxidative stress significantly contributes to the pathogenesis of different diseases, such as heart disease, inflammatory disease, cancer, diabetes mellitus, Alzheimer's disease, autism, and to the aging process (**Figure 1**) [3–5]. The chapter clarifies oxidative stress. Then classify the antioxidants and their applications. Finally, we describe antioxidants' mode of action and how they prevent the cell damage.

2. Oxidative stress

2.1 Oxidative damage to proteins

Protein oxidation can lead to amino acid modification, fragmentation of the peptide chain, aggregation of cross-linked reaction products, and increased electrical charges. Oxidized proteins are more susceptible to proteolysis, and a raise in oxidized proteins may be responsible for the loss of selected physiological and biochemical roles. Free radical damage to proteins may play a role in the causation of cataracts and aging (**Figure 2**) [1, 6].

2.2 Oxidative damage to lipids

Lipids have an important structural and functional role in cell membranes. After cell death, membrane lipids are susceptible to peroxidation and this process can cause misinterpretation of some lipid peroxidation assays. In particular, polyunsaturated fatty acids are susceptible targets for ROS attack. The important reactive moiety and initiator for ROS chain reaction and lipoperoxidation of polyunsaturated is $OH^{\bullet}$ [7]. Because of lipid peroxidation, several compounds are produced, such as alkanes, malondialdehyde, and isoprostanes. These compounds are utilized

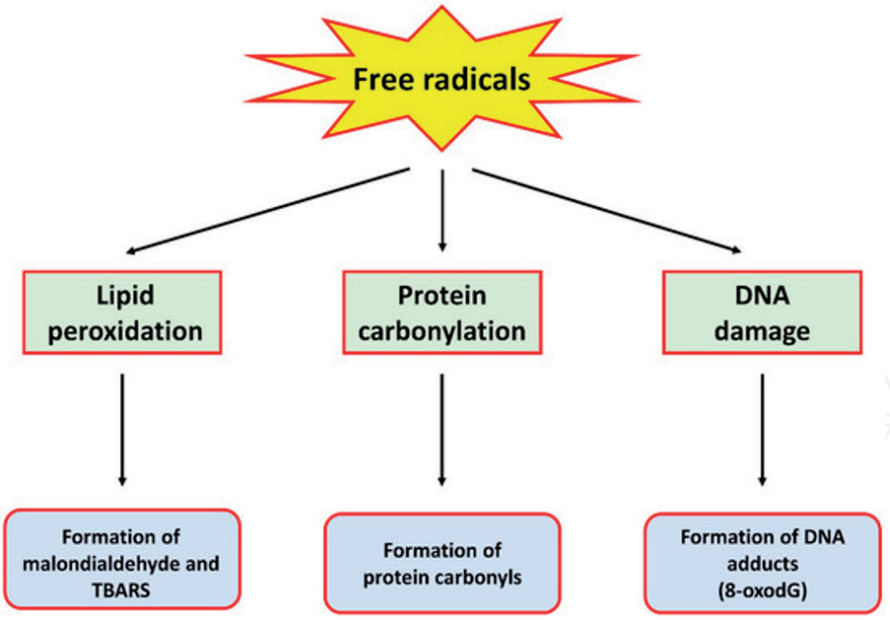

Figure 2.
A schematic diagram illustrating the detrimental effects of free radicals on biomolecules. Adapted from Law et al. [1].

as indicators in lipid peroxidation assay, and have been confirmed in diseases including neurogenerative diseases, heart disease, and diabetes (**Figure 2**) [1, 8].

2.3 Oxidative damage to DNA

Activated oxygen and agents that produce oxygen-free radicals, for example, ionizing radiations, promote damage in DNA that leads to deletion, mutations, and other fatal genetic effects. Through this DNA damage, both sugar and base moieties are susceptible to oxidation, leading to base degradation, single-strand breakage, and cross links to proteins. Free radical damage to DNA is associated in the causation of cancer and accelerated aging (**Figure 2**) [1, 5, 9].

2.4 Oxidative damage to carbohydrates

According to carbohydrates, the production of oxygen-free radicals during early glycation could contribute to glycoxidative damage. Through the primary stages of nonenzymatic glycosylation, fragmentation of sugar forms short-chain species like glycoaldehyde whose chain is too short to cyclize and is thus prone to autoxidation, producing the superoxide radical that can lead to the formation of β-dicarbonyls, which are well-known mutagens [10]. Carbohydrates free radical oxidation mechanisms are comparable to those of lipids. Low molecular carbohydrates, such as glucose, mannitol, and deoxyribose, are well known to interact with $HO^{\bullet}$, forming oxidized intermediates, which does not affect food quality [11].

3. Antioxidants

Antioxidants are inhibitors of oxidation, even at small concentrations; therefore, antioxidants have different physiological functions in the body. In addition, antioxidants act as free radical scavengers, by reacting with the reactive radicals and demolishing them to become less active, less dangerous, and long-lived substance than those radicals that have been neutralized. Antioxidants may be able

to neutralize free radicals via accepting or donating electron(s) to remove the unpaired status of the radical [4]. Also, antioxidants can be defined as compounds able to inhibit oxygen-mediated oxidation of different substances from simple molecule to polymer and complicated bio-system [8].

The US Food and Drug Administration (FDA) defined antioxidants as substances utilized to preserve food by retarding deterioration, rancidity, or discoloration owing to oxidation. Whereas antioxidants are important to the food industry to prevent rancidity, antioxidants are also important to biologists and clinicians as they may assist to protect the human body against diseases from ROS danger by regulating ROS-related enzymes [8]. Cellular level of free radicals may be decreased by antioxidants either via inhibiting the activities or expression of free radical generating enzymes such as NAD(P)H oxidase and xanthine oxidase (XO), or by promoting the activities and expression of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [12–14].

Since 1990s, antioxidant research has increased dramatically due to its potential role in disease prevention and health promotion. In biological systems such as animal models and clinical trials, the antioxidant action of pure compounds, foods, and dietary supplements has been extensively examined [4, 15, 16]. Numerous study models have been determined in chemical and/or biological systems to examine the mechanism of action of antioxidants, as well as the identification and recognition of new antioxidants, particularly from natural substances. Further research in animal models and cell cultures has provided critical information on the bioavailability, metabolism, and toxicity issues of antioxidants, suggesting probable clinical applications of these substances. Nevertheless, animal models and human research are expensive and not suitable for early antioxidant screening of foods and dietary supplements. Therefore, cell culture models have been utilized for early screening and study proceeding to animal research and human clinical trials [4].

Antioxidants can protect the cells and organs of the body against the harmful effect of the oxidative stress through various defense mechanisms by both enzymatic and nonenzymatic reactions, which work synergistically and together with each other. To prevent lipid peroxidation in food, nonenzymatic antioxidants are often added. The use of antioxidants for food and therapeutic purposes must be characterized carefully, because several lipid antioxidants can exert a prooxidant effect to other molecules under particular circumstances [5, 7].

The feature of a perfect antioxidant is that it should be readily absorbed, eliminate free radicals, and chelate redox metals at physiologically suitable levels. In addition, it should work in both aqueous and membrane domains, and have a positive effect on gene expression [7].

4. Antioxidant categories

Antioxidants can be classified in several ways [17, 18].

1. Based on their activity, they can be classified as enzymatic and nonenzymatic antioxidants. Dangerous oxidative products can be converted to H_2O_2 and then to water by enzymatic antioxidants that are able to break down and get rid of free radicals in a multistep process in the presence of cofactors such as copper (Cu), zinc (Zn), manganese (Mn), selenium (Se), and iron (Fe).

2. Vitamin C, vitamin E, plant polyphenol, carotenoids, and glutathione are nonenzymatic antioxidants, which act by interrupting free radicals chain reactions.

3. Based on solubility, antioxidants can be classified as water-soluble or lipid-soluble antioxidants. Vitamin C is a type of water-soluble vitamin found in cellular fluids such as cytosol or cytoplasmic matrix.

4. According to size, antioxidants can be categorized as small or large-molecule antioxidants. The small molecule antioxidants neutralize the ROS in a process named radicals scavenging and carry them away. Vitamin C, vitamin E, carotenoids, and glutathione (GSH) are the main antioxidants in this category. Large molecule antioxidants include enzymes (SOD, CAT, and GPx) and sacrificial proteins (albumin) that absorb ROS and prevent them from attacking other essential proteins.

5. Kinetically antioxidants can be categorized as below:

    a. Antioxidants that are able to break chains through reacting with peroxyl radicals containing weak O–H or N–H bonds, phenol, naphthol, hydroquinone, aromatic amines, and aminophenols.

    b. Antioxidants with a capability to break chains by reacting with alkyl radicals: quinines, nitrones, and iminoquinones.

    c. Antioxidants that terminate cyclic chain such as aromatic amines, nitroxyl radicals, and variable valence metal compounds.

    d. Hydroperoxide decomposing antioxidants such as sulfide, phosphide, and thiophosphate.

    e. Metal-deactivating antioxidants include diamines, hydroxyl acids, and bifunctional compounds.

    f. Synergism action of a number of antioxidants including phenol sulfide in which the phenolic group reacts with the peroxylradical's sulfide group with hydroperoxide.

6. Based on their occurrence, antioxidants are categorized as natural or synthetic [19, 20].

    a. Natural antioxidants

They are classified as chain-breaking antioxidants, which react with radicals and convert them into more stable products. Generally, antioxidants of this group are phenolic in structure and include the following:

1. Antioxidant minerals: these are antioxidant enzymes cofactors like selenium, copper, iron, zinc, and manganese. Absence of the cofactors will definitely enhance many macromolecules metabolism such as carbohydrates.

2. Antioxidant vitamins: these are important and required for most body metabolism functions such as, vitamin C, E, and B.

3. Phytochemicals: these are phenolic compounds derivatives that are neither vitamins nor minerals. Examples include flavonoids, catechins, carotenoids, carotene, lycopene, and herbs and spices such as diterpene, rosmariquinone, thyme, nutmeg, clove, black pepper, ginger, garlic, curcumin, and derivatives.

b. Synthetic antioxidants

These are phenolic compounds that carry out the role of capturing free radicals and stopping the chain reaction. These compounds include butylated hydroxyl anisole (BHA), butylated hydroxyltoluene (BHT), propyl gallate (PG), metal chelating agent (EDTA), tertiary butyl hydroquinone (TBHQ), and nordihydroguaiaretic acid (NDGA).

4.1 Antioxidant enzymes

There are several enzymes that catalyze reactions to neutralize free radicals and ROS. These enzymes form the body's endogenous defense mechanisms from free radicals to protect the cell. The enzyme antioxidants GPx, CAT, and SOD are the best-known substances of the antioxidant protection system, and they are responsible for the free radical change [21]. Enzymes are important components of the protection and defense mechanisms, by decreasing ROS generation via removing potential oxidants/transferring ROS/RNS into relatively stable compounds [5]. For optimum catalytic activity, these enzymes require micronutrient cofactors such as Se, Fe, Cu, Zn, and Mn [21].

4.1.1 Superoxide dismutase (SOD)

Irwin Fridovitch of Duke University and Joe McCord discovered antioxidant enzyme (SOD) (EC 1.15.1.1) in 1967, which belongs to the group of oxidoreductases. SOD is an important cellular defense against free radical damage. Therefore, medical scientists have begun to look seriously at free radicals [3]. SOD antioxidant enzymes are metal-containing proteins that catalyze the dismutation of the highly reactive superoxide anion to O_2 and to the less reactive species H_2O_2 (Eq. (1)). The result is that peroxide can be destroyed by reaction of CAT or GPX [22, 23].

$$O_2^- + O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \quad (1)$$

In mammals, there are three forms of SOD; the active site of the enzyme contains one or two different atoms of a transition metal in a certain oxidation state. SODs are categorized by their metal cofactors into known forms: cytosolic SOD, extracellular SOD [CuZnSOD], and mitochondrial SOD [MnSOD]. Each form is produced by distinct genes and distinct subcellular localization, but catalyzes the same reaction. This distinct subcellular localization of the three SOD forms is especially significant for compartmentalized redox signaling [24].

CuZnSOD enzymes have two identical subunits of about 32 kDa, though a monomeric structure is found in a high concentration of protein from *E. coli*. Each subunit includes a metal cluster, an active site, and a Cu and a Zn atom bridged By a histamine residue. The Cu and Zn which are important for SOD enzymatic activity. Zn contributes in appropriate protein folding and stability. Cu is not replaceable with another metal, while Zn is replaceable with cobalt and Cu, and it is not essential for enzyme action at low pH. CuZnSOD plays a major function in the first line of antioxidant defense [25].

MnSOD is a homotetramer 96 kDa; each subunit contains one Mn atom, those cycles from Mn^{3+} to Mn^{2+} and back to Mn^{3+} during the two-step dismutation of superoxide. In mitochondria, the main source of oxygen radicals is the respiratory chain. It was shown that this enzyme is greatly stimulated and decreased by cytokines, while oxidants moderately influenced it [26–28].

Extracellular SOD (ECSOD) is a tetrameric protein, containing Cu and Zn having a high affinity for certain glycosaminoglycans such as heparin and heparin

sulfate [7]. ECSOD is found primarily in the extracellular membrane and to a lesser extent, in the extracellular fluids. It protects against the inactivation of NO liberating from the endothelium by $O_2^{\bullet-}$ through diffusion to smooth muscle, thus preserving endothelial function. Studies have shown that ECSOD plays an essential role in various oxidative stress-dependent pathophysiologies, such as hypertension, ischemia reperfusion injury, and lung injury. In addition, a number of lines of research propose a role for ECSOD in aging. ECSOD plasma levels decrease with aging, and in old rats, gene transfer of ECSOD improves endothelial function. However, it is still unknown whether ECSOD expression or activity in blood vessels is adjusted by aging and whether endogenous ECSOD is engaged in regulation of vascular functions during aging [29].

4.1.1.1 Application

SOD enzymes enhance the rejuvenation and cellular repair, while decreasing the damage caused by free radicals. SOD is necessary to generate sufficient amounts of skin building cells named fibroblasts and plays an essential role in preventing the progress of amyotrophic lateral sclerosis (ALS), which causes death if it affects the nerve cells in the spinal cord and brain. In addition, this enzyme is also utilized for inflammatory diseases treatment, burn injuries, prostate problems, corneal ulcer, arthritis, and reversing the long-term consequences of radiation and smoke exposure. Furthermore, it prevents wrinkle formation if the skin lotion contains this enzyme. Also, it enhances wound healing, reduces scars, and lightens skin pigmentation caused by UV rays.

Moreover, SOD facilitates nitric oxide moving into hair follicles. This is beneficial for people with a genetic predisposition or free radicals for premature hair loss. SOD is a very potent antioxidant, in that it combats the effect of free radicals on the hair follicles. Because of nitric oxide's ability as a blood vessel relaxant, allowing more blood to reach the hair follicle, and SOD ability to remove free radicals, hair loss can be prevented or reversed. Maintaining overall well-being and health, as well as free radical protection, can be achieved by taking dietary supplement that provides an adequate supply of SOD [3].

4.1.2 Catalase (CAT)

Catalase (EC 1.11.1.6) is an enzyme responsible for H_2O_2 degradation that is generated by oxidases involved in β-oxidation of fatty acids, respiration, and purine catabolism [3]. It is present in nearly all animal cells as a protective enzyme. The highest levels of CAT activity are measured in the liver, kidney, and red blood cells.

Human CAT composes four identical subunits of 62 kDa, each subunit containing four distinct domains and one prosthetic heme group, and has a molecular mass of about 240 kDa [30]. CAT enzyme reacts with H_2O_2 to form water and molecular oxygen and with H donors such as methanol, ethanol, formic acid, or phenols with peroxidase activity. CAT protects cells from H_2O_2 generated within them. Therefore, it has an essential role in the acquisition of tolerance to oxidative stress in the adaptive response of cells. Various disease conditions and abnormalities are associated with the deficiency or mutation of CAT enzyme [30, 31].

4.1.2.1 Application

In the food industry, CAT enzyme is used to remove H_2O_2 from milk prior to cheese production, and to prevent food from oxidizing in food wrappers. In addition,

CAT enzyme is used in the textile industry for H_2O_2 removal from fabrics, to make sure the material is peroxide free. Recently, esthetics industries have begun to use CAT enzyme in facial masks, as the combination of CAT enzyme with H_2O_2 on the face can be used to increase cellular oxygenation in the upper layers of the epidermis [3].

4.1.3 Glutathione perioxidases (GPx)

Glutathione peroxidase (EC 1.11.1.9) contains a single selenocysteine residue in each of the four identical subunits, which is important for enzyme activity. GPx (80 kDa) is an imperative intracellular enzyme that catalyzes H_2O_2 to water and lipid peroxides to their corresponding alcohols mainly in the mitochondria and sometimes in the cytosol. In mammals, there are five GPx isoenzymes. Though their expression is ubiquitous, the level of each isoform differs depending on their tissue type. Mitochondrial and cytosolic glutathione peroxidase (GPx1 or cGPx) reduces fatty acid hydroperoxides and H_2O_2 at the expense of glutathione [32].

GPx1 is the main ubiquitous selenoperoxidase present in most cells; found in the cytosolic, mitochondrial, and peroxisomal compartments. It is an important antioxidant enzyme required in the detoxification of H_2O_2 and lipid hydroperoxides and preventing DNA, protein and lipids damage by harmful accumulation of intracellular H_2O_2 [33]. GPx1 uses GHS as an obligate co-substrate in the reduction of H_2O_2 to water [32]. Phospholipid hydroperoxidase glutathione (PHGPX) is found in most tissues and can directly reduce the phospholipid hydroperoxides, fatty acid hydroperoxides, and cholesterol hydroperoxides that are produced in peroxidized membranes and oxidized lipoproteins [30].

GPx4 is found in both the cytosol and the membrane fraction, and is highly expressed in renal epithelial cells and tests. Cytosolic GPx2 or extracellular GPx3 is inadequately found in nearly all tissues except for the gastrointestinal tract and kidney. In recent, GPx5, a new kind, expressed particularly in mouse epididymis, is selenium independent [34].

Several studies underlined the clinical importance of GPx. In addition, GPx, especially GPx1, have been implicated in the progression and prevention of many frequent and complex diseases, including cancer and cardiovascular disease [34, 35].

4.1.3.1 Application

GPx is an important antioxidant enzyme in the body. Glutathione (GHS), the master antioxidant, is important for GPx levels due to the closely linked relationship; GHS is a tripeptide that protects the cells against the negative effects of pollution and functions as the body's immune system booster. GHS plays an essential role in red blood cells to remain intact and protects white blood cells, which are responsible for the immune system. An antioxidant's role is specifically essential for the brain because it is sensitive to the presence of free radicals. To increase the body's protection from free radicals, it is imperative to combine certain antioxidants such as glutathione, vitamin C and E, Se, and GPx [3].

4.2 Nonenzymatic antioxidants

In previous decades, there has been increasing evidence that large amounts of antioxidants present in our diet contribute to the antioxidant defense system by preventing oxidative stress and specific human diseases. Phytochemicals, the plant-derived compounds, are one of the classes of the dietary factors, which play an essential role in functions of the body. Food materials contain a number of natural compounds reported to have antioxidant characteristics due to the presence

of hydroxyl groups in their structure. Synthetic and natural antioxidants prevent the oxidative damage to the most important macromolecules such as lipids, proteins, and nucleic acids found in human body through scavenging the free radicals formed in different biochemical processes [36]. These antioxidants consist of small molecules including vitamin C, E, and β-carotene or natural antioxidants such as flavonoids, tannins, coumarins, phenolics, and terpenoids [37]. Because of oxidative stress, the free radicals that have been produced react with lipids, proteins and nucleic acids and lead to stimulation of apoptosis, which causes various neurological, cardiovascular, and physiological disorders [38].

In addition to phytochemical antioxidants, which can protect the body from oxidative damage, there are other antioxidants for example polyphenols, lycopene, and lutein [39]. Even though there has been a considerable concentration on antioxidant function of phytochemicals for several years, it is distinguished that phytochemicals have nonantioxidant effects important for health such as cell signaling and gene expression [40].

4.2.1 Glutathione

Glutathione (γ-glutamyl-cysteinyl-glycine; GSH) is a tripeptide and is the most abundant intracellular antioxidant protecting normal cells from oxidative injury due to its role as a substrate of ROS scavenging enzymes. Glutathione is primarily present in its reduced form (GSH) in normal conditions, with only a small amount being found in the fully oxidized state (GSSG) [41]. Glutathione functions as a nonenzymatic antioxidant through free radical scavenging in cells and serves as a cofactor for several enzymes, include GPx, glutathione reductase (GR), and glutathione transferase (GST) [42, 43].

4.2.1.1 Application

Recently, there is a new era of therapeutic applications of glutathione through the association of decreased GSH levels with the common features of aging and a wide range of pathological conditions, including neurodegenerative disorders. Remarkably, depletion and alterations of GSH in its metabolism appear to be crucial in the onset of Parkinson's disease [44].

4.2.2 Vitamin E

Vitamin E, C, and β-carotene are the main antioxidant vitamins for tissues against free radical damage. Vitamin E, a major lipid soluble antioxidant, functions as the most important membrane-bound antioxidant, neutralizing free radicals, and preventing oxidation of lipids within membranes [45]. Vitamin E is the free radical scavenger in the prevention of chronic diseases [46]. α-Tocopherol is the main form of vitamin E with antioxidant and immune functions. α-tocopherol has been revealed to be a more effective inhibitor of peroxynitrite-induced lipid peroxidation and inflammatory reactions [47]. *In vitro* tocotrienols have excellent antioxidant activity and have been proposed to restrain ROS more effectively than tocopherols [48].

4.2.2.1 Application

The main function of vitamin E is to protect against lipid peroxidation through evidence suggesting that α-tocopherol and vitamin C function together in a cyclic type of process. It has been reported that vitamin E supplementation in hypercholesterolemic patients has shown to increase autoantibody levels against oxidized LDL, and prevent ischemic heart disease [49].

4.2.3 Vitamin C

In extracellular fluids, vitamin C, a water-soluble vitamin, is the most important antioxidant and can protect biomembranes against lipid peroxidation injury through eliminating peroxyl radicals in the aqueous phase before peroxidation initiation. Vitamin C is an effective antioxidant located in the aqueous phase of cells; it simply loses electrons to give stability to reactive species such as ROS [45]. In addition to vitamin C's biological functions as a superoxide and hydroxyl radicals' scavenger, it also functions as an enzyme cofactor [42].

4.2.3.1 Application

Vitamin C plays an essential function in the defense against oxidative damage particularly in leukocytes, as well as the possible effect it may have on the treatment of chronic degenerative diseases, autoimmune diseases, and cancer [42, 45].

4.2.4 Carotenoids

Carotenoids are structurally and functionally different natural pigments found in many fruits and vegetables. A combination of carotenoids and tocopherols antioxidants in the lipid phase of biological membranes may enhance better antioxidant protection than tocopherols alone. Antioxidant characteristics of carotenoids include scavenging single oxygen and peroxyl radicals, thiyl, sulfonyl, sulfur, and NO_2 radicals and giving protection to lipids from superoxide and hydroxyl radical attack [49].

4.2.4.1 Application

Carotenoids and some of their metabolites are proposed to play a protective function in several ROS-mediated disorders, include cardiovascular, cancer, and myocardial infarction among smokers. Carotenoid-rich food and supplementation decrease morbidity in nonsmokers and reduce the risk of prostate cancer [42].

4.2.5 Vitamin A

Vitamin A, a lipid soluble vitamin, is important for human health and has free radicals scavenging features that aid it to act as a physiological antioxidant in protecting a number of chronic diseases such as cardiovascular disease and cancer. All transretinol, the parent compound, are the most abundant dietary form of vitamin A that occurs naturally in the form of fatty acid esters such as retinyl palmitate, while retinal and retinoic acid are the minor natural dietary components of vitamin A [45]. Vitamin A was first labeled as an inhibitor of the effect of linoleic acid on the oxidation processes. At present, vitamin A and carotenoids are known for their antioxidant actions depending on their capability to interact with radicals and prohibit cell lipid peroxidation [9].

4.2.5.1 Application

Vitamin A is important for life in mammals; it cannot be synthesized in body and has to be supplied by food. Due to its role as antioxidant, vitamin A has a new role in preventive nutrition against neurodegenerative diseases. Recently, vitamin A has increased the interest in supplementation via food [50].

4.2.6 Uric acid

Uric acid, hyperuricemia, is a potent free radical scavenger and estimated ~60% of free radical scavenging capacity in plasma [51]. Uric acid is a physiological antioxidant and an effective preventer of the production of ROS species during the action of xanthine oxidase (XO) in catalysis reaction of xanthine and hypoxanthine [42]. A study illustrated the urate ability to scavenge oxygen radicals and protect the erythrocyte membrane from lipid oxidation, characterized further by Ames et al. through the effect of uric acid in protection of cells from oxidants, which related to a variety of physiological situation [51]. Nevertheless, it is probable that the increase in serum level of uric acid is a response to protect against the detrimental effects of extreme free radicals and oxidative stress [52].

4.2.6.1 Application

Studies showed that serum uric acid levels are highly predictive of mortality in patients with coronary artery disease, heart failure, or diabetes. In addition, high uric acid level is associated with deleterious effect on vascular function. Recently, it has been found that patients with high serum uric acid level had impaired flow-mediated dilation, which was normalized by therapy for 3 months with the xo inhibitor allopurinol [53].

4.2.7 Lipoic acid

Lipoic acid is a strong antioxidant, and it reveals a great capability of antioxidant when given natural or as a synthetic drug. Lipoic acid is a short-chain fatty acid, composed of sulfur in their structure that is known for its contribution in the reaction that catalyzes the oxidation decarboxylation of α-keto acids, for example pyruvate and α-ketoglutarate, in the citric acid cycle. Lipoic acid and its reduced form, dihydrolipoic acid (DHLA), are capable of quenching free radicals in both lipid and aqueous domains. Lipoic acid and DHLA have been revealed to have antioxidant, cardiovascular, antiaging, detoxifying, anti-inflammatory, anticancer, and neuroprotective pharmacological properties [40, 54].

4.2.7.1 Application

Regarding the pathology of diabetes, there are many potential applications for lipoic acid. In type I diabetes, destruction of pancreatic β-cells leads to loss secretion of insulin, while the major problem in type II diabetes is insulin resistance of peripheral tissues. Lipoic acid has potential preventive or ameliorative effect in both type I and type II diabetes [54].

4.2.8 Flavonoids

Flavonoids are low in molecular weight and are the main type of phenolic compounds in plants. They are structured by 15 carbon atoms, organized in a C6-C3-C6 configuration. Due to their high redox potential, flavonoids are, in particular, important antioxidants that allow them to function as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, they include a metal chelating potential [55].

4.2.8.1 Application

Flavonoids are generally found in many fruits and vegetables. When human increasingly consumed it, flavonoids have been linked with a decrease in the incidence of diseases such as prostate [56, 57] or breast cancer [58, 59].

4.2.9 Tannins

Tannins are relatively high-molecular compounds, which comprise the third essential group of phenolics and can be divided into condensed and hydrolysable tannins. Condensed tannins are produced by the polymerization of flavonoid units. The mainly studied condensed tannins are based on flavan-3-ols: (−)-epicatechin and (+)-catechin. Hydrolysable tannins are heterogeneous polymers containing phenolic acids, in particular, gallic acid (3,4,5 trihydroxyl benzoic acid) and simple sugar [42, 55].

4.2.9.1 Application

Because of tannin features, such as being the potential metal ion chelators, protein-precipitating agents, and biological antioxidants, tannins have different effects on biological systems. As a consequence of the diverse tannins biological roles and structural variation, it has been difficult to modify models that would let a precise prediction of their effects in any system. Therefore, the tannin structure modification and activity relationship are important to predict their biological effect [42].

5. Antioxidants: mechanisms of action

Generally, the antioxidants defend against free-radicals-induced oxidative damage by various mechanisms as discussed in below sections.

5.1 Preventive antioxidants

ROS such as H_2O_2, $O_2^{\bullet}$, and $OH^{\bullet}$ are produced irreversibly during metabolism. Therefore, methods have been extensively studied to reduce the damage enhanced by oxidative stress. Intracellular antioxidant enzymes produced in the cell are an essential protective mechanism against free radicals formation. SOD, CAT, GPx, GR, GST, thioredoxin reductase, and hemeoxygenase are the most important antioxidants enzymes. SODs convert $O_2^{\bullet}$ into H_2O_2, which is then converted into water by CAT, GPx, and Fenton reaction. Thus, two toxic species are converted into a harmless product (**Figure 3**) [5].

During metabolism, peroxides are formed and then eliminated via both GST and GPX. GRd regulates the equivalent of GSH and oxidized glutathione (GSSG), and the ratio of GSH/GSSG is a known index of oxidative stress [60]. The action of GRd plays an imperative role in increasing GSH concentration, which maintains the oxido-redox condition in the organism [14]. Consequently, the oxidative stress role has been reported in the progress and clinical symptom of autism. Recently, a comparison study between autism and control individuals showed decrease in GSH/GSSG ratio and increase in free radical generation in autism compared to control cells [60]. In addition, GPx is presented throughout the cell, while CAT is frequently limited to peroxisomes. In the brain, which is very sensitive to free radical damage, it has seven times more GPx activity than CAT activity. Moreover, CAT's highest levels are found in the liver, kidney, and erythrocytes, where it decomposes the most of H_2O_2 [61].

5.2 Free radical scavengers

5.2.1 Scavenging superoxide and other ROS

Superoxide ($O_2^{\bullet -}$), a predominant cellular free radical, is contributed in a huge number of deleterious alterations often linked to a low concentration of antioxidants

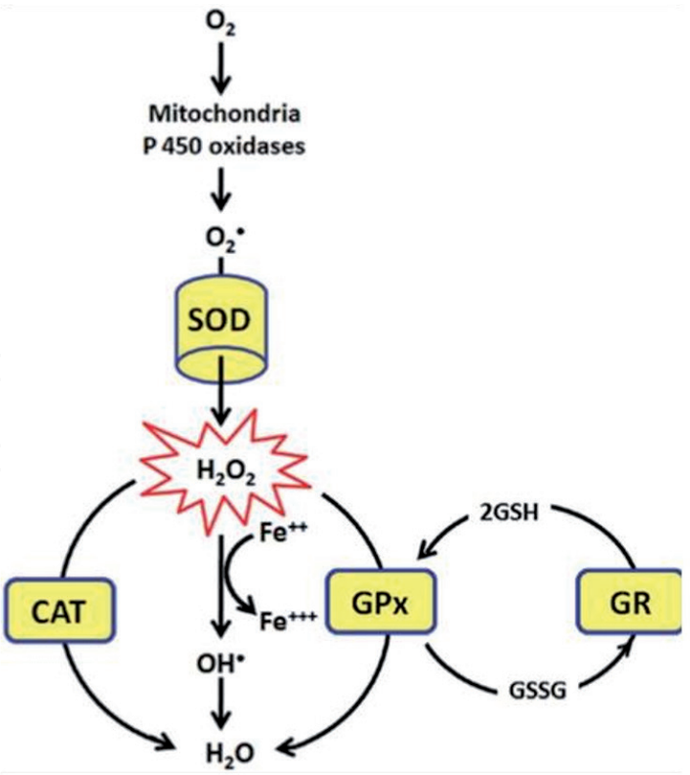

Figure 3.
Antioxidant enzyme system, $O_2^{\bullet}$, is dismutaed toH_2O_2 by SOD enzyme. The resulted H_2O_2 is converted into water by CAT and GPx. In this way, two toxic species, $O_2^{\bullet}$ and H_2O_2, are converted into the harmless product water. GPx neutralized H_2O_2 via taking hydrogen from two GSH molecules forming two molecules of water and GSSG. GR then regenerates GSH from GSSG. CAT, the essential part of enzymatic defense, neutralizes H_2O_2 into water. By Fenton reaction, H_2O_2 is also converted to the highly reactive $OH^{\bullet}$ and then to water through oxidation of Fe^{2+} to Fe^{3+}. Adapted from Pandey and Rizvi [5].

and associated with a raise in peroxidative processes. Though $O_2^{\bullet-}$ itself is not reactive to biomolecules, it assists in production of stronger $OH^{\bullet}$, and $ONOO^{-}.O_2^{\bullet-}$ is formed in large quantities, in phagocytes via NADPH oxidase enzyme during pathogen-killing process. In addition, it is a byproduct of mitochondrial respiration [3].

5.2.2 Scavenging hydroxyl radical and other ROS

Hydroxyl radical ($OH^{\bullet}$) is an extremely active and more toxic radical on biologic molecules such as DNA, lipids, and proteins than other radical species. In general, $OH^{\bullet}$ is considered to be formed from the Fe^{2+} or Cu^{+}/H_2O_2 Fenton reaction system, through incubation $FeSO_4$ and H_2O_2 in aqueous solution. Therefore, antioxidants activity as $OH^{\bullet}$ scavenger can be accomplished by direct scavenging or prohibiting of $OH^{\bullet}$ generation by the chelation of free metal ions or altering H_2O_2 to other nontoxic compounds [3].

5.2.3 Metal ion (Fe^{2+}, Fe^{3+}, Cu^{2+}, and Cu^{+}) chelating

Even though trace minerals are essential dietary components, they can function as prooxidants (through enhancing formation of free radicals). Fe^{2+} and Cu^{+} react with H_2O_2, which is a product produced by the dismutation of the $O_2^{\bullet-}$ via SOD, to form extremely reactive $OH^{\bullet}$ (Eq. (2)). Dissimilarly, iron and copper's reaction with H_2O_2 forms more singlet oxygen than $OH^{\bullet}$. Fe^{2+} and Cu^{+} are oxidized to Fe^{3+} and Cu^{2+}, respectively. Cellular reductant such as NADH and oxidized metal ions Fe^{3+}, and Cu^{2+} are reduced and permit the recycling to react with another molecule of H_2O_2 to produce $OH^{\bullet}$ radical in the presence of vitamin C (Eq. (3)). $OH^{\bullet}$ is strongly

reactive and can directly react with proteins and lipids to produce carbonyls (aldehydes and ketones), cross linking, and lipid peroxidation. Chelating metal ions are able to decrease their action, thus reducing the ROS formation.

$$Fe^{2+} \text{ (or } Cu^{+}) + H_2O_2 \; Fe^{3+} \text{ (or } Cu^{2+}) + OH^{\bullet} + OH^{-} \quad (2)$$

$$Fe^{3+} \text{ (or } Cu^{2+}) + \text{vit C } H^{-} \; Fe^{2+} \text{ (or } Cu^{+}) + \text{vitamin C}^{-\bullet} + H^{+} \quad (3)$$

Studies showed that Se antioxidant is able to chelate Cu^{+} (formed in situ with Cu^{2+}/ascorbic acid) extremely efficiently and prevent the damage of DNA by $OH^{\bullet}$ radical (formed via Cu^{+}/H_2O_2) [3].

5.3 Free radical generating enzyme inhibitors

It has been reported that the main sources of free radicals in different physiological and pathological conditions is associated with a number of enzymes. NADPH oxidases are a type of plasma membrane linked enzymes that have an ability to transfer one electron from the cytosolic donor NADPH to a molecule of extracellular oxygen, forming $O_2^{\bullet-}$ [62]. Uric acid is formed by xanthin oxidase enzyme through catalyzing the oxidation of hypoxanthine and xanthin to uric acid yielding $O_2^{\bullet-}$ and H_2O_2 and increase the oxidation level in an organism [63]. In addition, $O_2^{\bullet-}$ is also formed as a by-product of mitochondrial respiration as well as several other enzymes, for example NADH oxidase, monooxygenases and cyclooxygenases. $O_2^{\bullet-}$ is biologically quite toxic and is produced in significant amounts by the enzyme NADPH oxidase to be used in oxygen dependent killing mechanisms for invading pathogens. During the respiratory burst, it is an important control of reactive oxygen derivatives production for the defense of an organism against invading microorganisms, without causing an important loss of tissue functions [3]. Nonetheless, excessive ROS enhance oxidative stress such as low density lipoprotein (LDL) oxidation. A direct link between elevated phagocytic NADPH oxidase activities and increased circulating oxidized LDL in metabolic syndrome patients has been found. As a result, both modulation of NADPH oxidase to prohibit ROS overproduction and antioxidants supplementation have been reported as active strategies to prevent the deleterious effect of oxidative stress in hemodialysis patients [64]. In recent years, many natural antioxidants have revealed potential to inhibit enzymes that promote $O_2^{\bullet-}$ generation as well as the development of new therapeutic agents for oxidative stress-related diseases [3].

5.4 Prevention of lipid peroxidation

Lipid peroxidation is defined as oxidative deterioration of lipids composed of C-C double bonds such as unsaturated fatty acids, glycolipids, cholesterol, cholesterol ester, phospholipids. ROS damage the unsaturated fatty acids, which include numerous double bonds and the methylene-CH2-groups with particularly reactive hydrogen atoms, and begin the radical peroxidation chain reactions [65]. Antioxidants are able to directly react and quench peroxide radicals to stop the chain reaction. Lipid peroxidation and DNA damage are related to different chronic diseases, such as cancer, and atherosclerosis. Antioxidants can scavenge ROS and peroxide radicals, therefore prohibiting or treating certain pathogenic situations. Scientific attention has been concentrated in lipid peroxidation for recognizing natural antioxidants and studying their mechanism of action. Researches on antioxidants such as vitamins, polyphenols and flavones against free radical enhanced lipid peroxidation have been assumed in

many systems such as lipid, red blood cells and LDL. The antioxidant activity of these polyphenols depends considerably on molecules structure, the initiation conditions and the microenvironment of the reaction medium [3].

5.5 Prevention of DNA damage

In vivo, the $OH^{\bullet}$ and $ONOO^{-}$ radicals produced from nitric oxide and $O_2^{\bullet -}$ are able to react directly with plasmid DNA macromolecules to cleave one DNA strand, leading to oxidative DNA damage. Cell death and mutation as a result of DNA damage are associated with neurodegenerative and heart diseases, cancer and aging. Consequently, DNA or plasmid damage has received attention and been utilized as models for the study and identification of antioxidants [66]. A study has been progressed include DNA damage caused by Cu^{+} induced $OH^{\bullet}$, through metal-free plasmid DNA mixed with Cu^{2+}, ascorbic acid and H_2O_2 at pH 7. The reaction includes reduction of Cu^{2+} to Cu^{+} in situ with ascorbic acid. The $OH^{\bullet}$ radical formed via Cu^{+}/H_2O_2 cleaves one DNA strand, causing the ordinarily supercoiled plasmid DNA to unwind [3].

5.6 Prevention of protein modification

Besides lipid peroxidation and DNA damage, protein modification through nitration or chloration of amino acids also is caused by ROS. *In vivo*, peroxynitrite, O═N—O—O^{-}, is a powerful oxidant and nitrating agent formed through the reaction of $O_2^{\bullet -}$ with free radical nitric oxide via a diffusion-controlled reaction. In cells, $ONOO^{-}$ is a much stronger oxidizing agent than $O_2^{\bullet -}$ and is able to damage a wide range of different molecules such as DNA and proteins. $ONOO^{-}$ and its protonated form peroxynitrous acids (ONOOH) are capable of exerting direct oxidative modifications during one or two electron oxidation processes [67]. *In vivo*, $ONOO^{-}$ reacts nucleophically with CO_2 to produce nitrosoperoxy carbonate, which is the predominant pathway for $ONOO^{-}$. These modifications often cause the alteration of protein function or structure, in addition to enzyme activities inhibition. Proteins containing nitrotyrosine residues have been detected in various pathogenic conditions, such as diabetes, hypertension, and atherosclerosis, all linked with promoted oxidative stress, including increased formation of $ONOO^{-}$. Antioxidants and antioxidant enzyme are utilized to prevent the protein modification of $ONOO^{-}$. Antioxidants or enzyme such as CAT is able to remove H_2O_2 and also inhibit HOCl formation; similarly, SOD or antioxidants, like polyphenols, may scavenge $O_2^{\bullet -}$ and inhibit $ONOO^{-}$ formation [3].

6. Conclusion

This chapter briefly summarized types of antioxidants, and their mode of action. The harmful products formed during normal cellular functions are oxygen radical derivatives that are the most important free radical in the biological system. For normal physiological functioning, it is important to maintain a tolerated antioxidant status by increasing intake of natural antioxidants. Studies have shown that different types of antioxidants, including natural and synthetic antioxidants, can help in disease prevention. The antioxidant compounds may directly react with the reactive radicals to destroy them via accepting or donating electron(s) to directly remove the unpaired status of the radical. Moreover, they may indirectly reduce the production of free radicals by inhibiting the efficacy or expressions of free radical creating enzymes or by stimulating the activities and expressions of other antioxidant enzymes. Thus, it is essential to know the antioxidant mechanisms of action with the free radicals.

Conflict of interest

The authors declare they have no financial or other conflict of interests related to this chapter.

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References

[1] Law BMH, Waye MMY, So WKW, Chair SY. Hypotheses on the potential of rice bran intake to prevent gastrointestinal cancer through the modulation of oxidative stress. International Journal of Molecular Sciences. 2017;**18**:1-20

[2] Aziz MA, Ghanim HM, Diab KS, Al-Tamimi RJ. The association of oxidant-antioxidant status in patients with chronic renal failure. Renal Failure. 2016;**38**(1):20-26

[3] Krishnamurthy P, Wadhwani A. Antioxidant enzymes and human health. In: El-Missiry MA, editor. Antioxidant Enzyme. Croatia: In Tech; 2012. pp. 3-18. DOI: 10.3109/0886022X.2015.1103654

[4] Lü JM, Lin PH, Yao Q , Chen C. Chemical and molecular mechanisms of antioxidants: Experimental approaches and model systems. Journal of Cellular and Molecular Medicine. 2009;**14**(4):840-860

[5] Pandey KB, Rizvi SI. Markers of oxidative stress in erythrocytes and plasma during aging in humans. Oxidative Medicine and Cellular Longevity. 2010;**3**(1):2-12

[6] Kumar S. Free radicals and antioxidants: Human and food system. Advanced in Applied Science Research. 2011;**2**(1):129-135

[7] KurutasEB. The importance of antioxidants which play the role in cellular response against oxidative/ nitrosative stress: Current state. Kurutas Nutrition Journal. 2016;**15**:71-93

[8] Bagchi K, Puri S. Free radicals and antioxidants in health and disease: A review. Eastern Mediterranean Health Journal. 1998;**4**(2):350-360

[9] Zadák Z et al. Antioxidants and vitamins in clinical conditions. Physiological Research. 2009;**58**(1): S13-S17

[10] Benov L, Beema AF. Superoxide-dependence of the short chain sugars-induced mutagenesis. Free Radical Biology and Medicine. 2003;**34**:429-433

[11] Polumbryk M, Ivanov S, Polumbryk O. Antioxidants in food systems. Mechanism of action. Ukrainian Journal of Food Science. 2013;**1**(1):15-40

[12] Shebis Y et al. Natural antioxidants: Function and sources. Food and Nutrition Sciences. 2013;**4**:643-649

[13] Panchatcharam M et al. Curcumin improves wound healing by modulating collagen and decreasing reactive oxygen species. Molecular and Cellular Biochemistry. 2006;**290**:87-96

[14] Shih PH, Yeh CT, Yen GC. Anthocyanins induce the activation of phase II enzymes through the antioxidant response element pathway against oxidative stress-induced apoptosis. Journal of Agricultural and Food Chemistry. 2007;**55**:9427-9435

[15] Williamson G, Manach C. Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. American Journal of Clinical Nutrition. 2005;**81**:243S-2255S

[16] Lotito SB, Frei B. Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: Cause, consequence, or epiphenomenon? Free Radical Biology and Medicine. 2006;**41**:1727-1746

[17] Shahidi F, Zhong Y. Novel antioxidants in food quality preservation and health promotion. European Journal of Lipid Science Technology. 2010;**112**:930-940

[18] Nimse SB, Pal D. Free radicals, natural antioxidants, antheir reaction mechanisms. Royal Society of Chemistry Advances. 2015;**5**:27986-28006

[19] Mathew BB, Tiwari A, Jatawa SK. Free radicals and antioxidants: A review. Journal of Pharmacy Research. 2011;**4**(12):4340-4343

[20] Hurrell RF. Influences of vegetable protein sources on trace element and mineral bioavailability. Journal of Nutrition. 2003;**133**(9):2973S-2977S

[21] Butnariu M, Grozea I. Antioxidant (antiradical) compounds. Journal of Bioequivalence and Bioavailability. 2012;**4**(6):4-6

[22] Vitale M, Di Matola T, Ďascoli F. Iodide excess induces apoptosis in thyroid cells trough a p53-independent mechanism involving oxidative stress. Endrocriology Scoeity. 2000;**141**:598-605

[23] Fernandez V, Barrientos X, Kiperos K, Valenzuela A, Videla LA. Superoxide radical generation, NADPH oxidase activity and cytochrome P-450 content of rat liver microsomal fractions in an experimental hyperthyroid state: Relation to lipid peroxidation. Endocrinology. 1985;**117**:496-501

[24] Fukai T, Ushio-Fukai M. Role in redox signaling, vascular function, and diseases. Antioxidants and Redox Signaling. 2011;**15**(6):1583-1606

[25] Ghafourifar P, Cadenas E. Mitochondrial nitric oxide synthase. Trends in Pharmacological Sciences. 2005;**26**(4):190-195

[26] Maggi-Capeyron MF, Cases J, Badia E, et al. A diet high in cholesterol and deficient in vitamin E induces lipid peroxidation but does not enhance antioxidantenzyme expression in rat liver. Journal of Nutritional Biochemistry. 2002;**13**:296-301

[27] Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the enrire antioxidant defence grid. Alexandria Journal of Medicine. 2017;**54**(4):287-293. https://doi.org/10.1016/j.ajme.2017.09.001

[28] MacMillan-Crow LA, Crow JP, Thompson JA. Peroxynitrite-mediated inactivation of manganese superoxide dismutase involves nitration and oxidation of critical tyrosine residues. Biochemistry. 1998;**37**:1613-1622

[29] Fukai T. Extracellular SOD and aged blood vessels. American Journal of Physiology—Heart and Circulatory Physiology. 2009;**297**(1):H10-H12

[30] Buschfort C, Muller MR, Seeber S, et al. DNA excision repair profiles of normal and leukemic human lymphocytes: Functional analysis at the single cell level. Cancer Research. 1997;**57**:651-658

[31] Góth L, Rass P, Páy A. Catalase enzyme mutations and their association with diseases. Molecular Diagnostics. 2004;**8**:141-149

[32] Esworthy RS, Ho YS, Chu FF. The GPx1 gene encodes mitochondrial glutathione peroxidase in the mouse liver. Archives Biochemistry Biophysics. 1997;**340**:59-63

[33] JB DH et al. Lack of the antioxidant enzyme glutathione peroxidase-1 (GPx1) does not increase atherosclerosis in C57BL/J6 mice fed a high fat diet. Journal of Lipid Research. 2006;**47**(6):1157-1167

[34] Imai H, Narashima K, Arai M, Sakamoto H, Chiba N, Nakagawa Y. Suppression of leukotriene formation

in RBL-2H3 cells that overexpressed phospholipid hydroperoxide glutathione peroxidase. Journal of Biological Chemistry. 1998;**273**:1990-1997

[35] Rayman MP. Selenium in cancer prevention: A review of the evidence and mechanism of action. Proceedings of the Nutrition Scoiety. 2005;**64**:527-542

[36] Shui GH, Leong LP. Analysis of polyphenolic antioxidants in star fruit using liquid chromatography and mass spectrometry. Journal of Chromatography A. 2004;**1022**:67-75

[37] Perumalla VS, Hettiarachchy NS. Green tea and grape seed extracts potential applications in food safety and quality. Food Research International. 2011;**44**(4):827-839

[38] Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: A review of upstream and downstream antioxidant therapeutic options. Current Neuropharmacology. 2009;**7**(1):65-74

[39] Moon JK, Shibamoto T. Antioxidant assays for plant and food components. Journal of Agricultural and Food Chemistry. 2009;**57**(5):1655-1666

[40] El Barky AR, Hussein SA, Mohamed TM. The potent antioxidant alpha lipoic acid. Journal of Plant Chemistry and Ecophysiology. 2017;**2**(1):1-5

[41] Pocsi I, Prade RA, Penninckx MJ. Glutathione, altruistic metabolite in fungi. Advances in Microbial Physiology. 2004;**49**:1-76

[42] Skowyra M. Antioxidant properties of extracts from selected plant materials (*Caesalpinia spinosa*, *Perilla frutescens*, *Artemisia annua* and Viola wittrockiana) in vitro and in model food systems [thesis]. Department of Chemical Engineering, Universitat Politècnica de Catalunya; 2014

[43] Sun SY. N-acetylcysteine, reactive oxygen species and beyond. Cancer Biology & Therapy. 2010;**9**(2):109-110

[44] Homma T, Fujii J. Application of glutathione as anti-oxidative and anti-aging drugs. Current Drug Metabolism. 2015;**16**(7):560-571

[45] Arredondo ML. Relationship between vitamin intake and total antioxidant capacity in elderly adults. Universitas Scientiarum. 2016;**21**(2):167-177

[46] Herrera E, Barbas C. Vitamin E: Action, metabolism and perspective. Journal of Physiology and Biochemistry. 2001;**57**:43-56

[47] McCormick CC, Parker RS. The cytotoxicity of vitamin E is both vitamer and cell specific and involves a selectable trait. Journal of Nutrition. 2004;**134**:3335

[48] Schaffer S, MullerbWE, Eckert GP. Tocotrienols: Constitutional effects in aging and disease. Journal of Nutrition.2005;**135**:151

[49] Rahman K. Studies on free eadicals, antioxidants, and co-factors. Clinical Interventions in Aging. 2007;**2**(2):219-236

[50] Sauvant P, Cansell M, Hadj A, Atgie C. Vitamin a enrichment: Caution with encapsulation strategies used for food applications. Food Research International. 2012;**46**(2):469-479

[51] Ames BN, Cathcart R, Schwiers E, Hochstein P. Uric acid provides an antioxidant defense in humans against oxidant-and radical-caused aging and cancer: A hypothesis. Proceedings of the National Academy Sciences of the United States of America. 1981;**78**:6858-6862

[52] Johnson RJ. Essential hypertension, progressive renal disease, and uric acid: A pathogenetic link?

Journal of Anerican Nephrology. 2005;**16**:1909-1919

[53] Mercuro G et al. Effect of hyperuricemia upon endothelial function in patients at increased cardiovascular risk. The American Journal of Cardiology. 2004;**94**:93293-93295

[54] Packer L, Witt EH, Tritschler HJ. Alpha lipoic acid as a biological antioxidant. Free Radical Biology and Medicine. 1995;**19**(2):227-250

[55] Walter M, Marchesan E. Phenolic compounds and antioxidant activity of rice. Brazilizn Archives of Biology and Technology. 2011;**54**(1):371-377

[56] Tsao R, Yang R. Optimization of a new mobile phase to know the complex and real polyphenolic composition: Towards a total phenolic index using high performance liquid chromatography. Journal of Chromatography. 2003;**1018**(1):29-40

[57] Jaganathan SK et al. Role of pomegranate and citrus fruit juices in colon cancer prevention. World Journal Gastroenterology. 2014;**20**(16):4618-4625

[58] Sharmila G et al. Chemopreventive effect of quercetin, a natural dietary flavonoid on prostate cancer in in vivo model. Clinical Nutrition (Edinburgh, Scotland). 2014;**33**(4):718-726

[59] Yiannakopoulou EC. Effect of green tea catechins on breast carcinogenesis: A systematic review of in-vitro and in-vivo experimental studies. European Journal of Cancer Prevention: The official Journal of the European Cancer Prevention Organisation. 2014;**23**(2):84-89

[60] James SJ, Rose S, Melnyk S, et al. Cellular and mitochondrial glutathione redox imbalance in lymphoblastoid cells derived from children with autism. Federation of American Societies For Experimental Biology Journal. 2009;**23**:237-283

[61] Marklund SL, Westman NG, Lundgren E, et al. Copper and zinc containing superoxide dismutase, catalase, and glutathione peroxidase in normal and neoplastic human cell lines and normal human tissues. Cancer Research. 1982;**42**:1955-1961

[62] Lin HC, Tsal SH, Chen CS, et al. Structure-activity relationship of coumarin derivatives on xanthine oxidase-inhibiting and free radical-scavenging activities. Biocmeical Pharmacology. 2008;**75**:1416-1425

[63] Schroder K, Vecchione C, Jung O, et al. Xanthine oxidase inhibitor tungsten prevents the development of atherosclerosis in ApoE knockout mice fed a Western-type diet. Free Radical Biology and Medicine. 2006;**41**:1353-1360

[64] Castilla P, Davalos A, Teruel JL, et al. Comparative effects of dietary supplementation with red grape juice and vitamin E on production of superoxide by circulating neutrophil NADPH oxidase in hemodialysis patients. American Journal of Clinical Nutrition. 2008;**87**:1053-1561

[65] Porter NA, Caldwell SE, Mills KA. Mechanisms of free radical oxidation of unsaturated lipids. Lipids. 1995;**30**:277-290

[66] Zhao F, Tang YZ, Liu ZQ. Protective effect of icariin on DNA against induced oxidative damage. Journal of Pharmacy and Pharmacology. 2007;**59**:1729-1732

[67] Pacher P, Beckman JB, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiolagical Review. 2007;**87**:315-424