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A review of oxidants and antioxidants in biological systems

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Abstract

Oxidants are any agent capable of removing electron from another molecule in a course of a redox reaction. When it comes to biological systems free-radicals, especially reactive oxygen species (ROS) and reactive nitrogen species (RNS) are involved in oxidation reactions. Both endogenous and exogenous factors can contribute to free-radical formation. Fortunately, an endogenous balance between free-radicals and antioxidant systems is present in the biological system. Despite the common belief that free-radicals are destructive agents, nowadays it has been proven that free-radicals such as ROS and RNS are greatly involved in signalling pathways and immune function. The destructive effect of antioxidants becomes significantly important only when this intrinsic balance is disturbed in favour of free-radicals, a condition known as oxidative stress. Over-production of free-radicals in oxidative stress can have deleterious effects on the biological systems in virtue of their interaction with important biological molecules such as proteins, lipids, and DNA. Favourably antioxidants are available to prevent the over-accumulation of free radicals and consequently their harmful effects by scavenging them. Various classification attributes have been suggested for antioxidants, amongst which, classification on the basis of their mechanism of action as primary and secondary antioxidant or their classification on the basis of their enzymatic activity as enzymatic and non-enzymatic antioxidants are the most famous ones. The objective of this review article is to provide the basic information required for the understanding of reactive oxygen species and their formation, antioxidants terminology, their classification and the mechanism by which the antioxidants are involved in counteracting harmful effect of oxidant in biological systems.

Keywords

Antioxidants, biological systems, oxidative stress, ROS, RNS.

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Free-radical terminology

Oxidant species are free-radicals with one or more unpaired electron present on the outer atomic or molecular orbital. Exogenous factors such as hyperoxia, smoking, exposure to ozone, and heavy metals or intrinsic factors like a respiratory chain reaction in mitochondria, oxidative reactions occurring in peroxisome for phagocytosis; can result in the formation of free-radicals (Phaniendra *et al.,* 2015). These newly synthesized free-radicals are highly unstable, and thus highly reactive to reach a stable configuration via either donating the unpaired electron or accepting an electron from other molecules. In biological systems, a free-radical lasts for a few milliseconds as it immediately reacts with the neighbouring molecules including but not limited to, proteins; nucleic acids and lipids, resulting in the conversion of non-radical molecules into free-radicals. This initial conversion is the start of an ongoing chain reaction producing millions of free-radicals (Tiwari, 2004). The chain reaction can be stopped by either two free-radicals cross-linkage pairing the unpaired electrons or antioxidant system interventions (Santo *et al.,* 2016).

Classification of oxidants in the biological systems

These oxidants are generally classified into two groups as reactive oxygen species (ROS) and reactive nitrogen species (RNS). In Table 1, a summary of the main endogenous antioxidants is provided (Dontha, 2016).

Table 1: Major endogenous oxidants present in the biological systems (Badarinath *et al.,* 2010).

Major endogenous oxidants	
Reactive oxygen species	**Formula**
Superoxide anion	O_2
Hydrogen peroxide	H_2O_2
Hydroxyl radical	OH
Hypochlorous acid	HOCl
Peroxyl radicals	ROO
Hydroperoxyl radical	HOO
Reactive nitrogen species	
Nitric oxide	NO
Peroxynitrite	$ONOO^-$

ROS generation within the biological systems

Because of intrinsic factors like, mitochondria involvement in respiratory chain reaction, endoplasmic reticulum role in CYP450 system, transition metal ion catalysed reaction and oxidative reactions occurring in peroxisome through phagocytosis the covalent bond can be broken leaving behind one electron from each pair, or in other words free-radical production. For instance, addition of one

electron to molecular oxygen, give rise to superoxide free-radical. As shown in Figure 1 this conversion is mediated by Nicotine Adenine Dinucleotide Phosphate (NAD(P)H) oxidase, Xanthine Oxidase (XAO/XO) or mitochondrial electron transport chain (ETC) (Gupta, 2015). Mitochondria are major sources of ROS; the main sites of superoxide radical production in the respiratory tract. Mitochondria are one of the major endogenous factors contributing to oxidative stress. Normally mitochondria reduce oxygen to water through ETC for adenosine triphosphate (ATP) synthesis. Mitochondrial respiration is the set of metabolic reactions and processes requiring oxygen that takes place in mitochondria to convert the energy stored in macronutrients to ATP, the universal energy donor in the cell. However, approximately 1-3 % of all electrons involved in this chain reaction may leak from the system, reducing oxygen to superoxide. This explains, why mitochondria are believed to be the major site of superoxide free-radical formation in the body. NAD(P)H oxidase in macrophages, leukocytes and monocytes mediates the conversion of oxygen to superoxide upon phagocytosis (Birben *et al.,* 2012). In turn, superoxide is converted into hydrogen peroxide by superoxide dismutase enzyme. Because H_2O_2 is a lipophilic molecule, it can easily diffuse through cell membrane, thus having more deleterious effect on living cells compared to superoxide (Birben *et al.,* 2012).

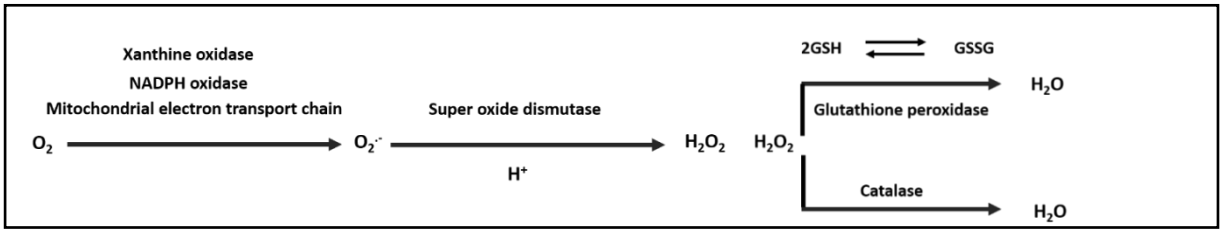

Figure 1: ROS production pathways (Lagouge and Larsson, 2003).

Iron and copper metals have high importance in biological systems by getting involved in molecular oxygen reduction. Figure 2 shows the reduction of hydrogen peroxide into hydroxyl radical by ferrous ion, known as the Fenton reaction. As show in Figure 1, reaction (2) mediates the oxidation of superoxide into molecular oxygen by ferric ion. The net balance of the reactions (1) and (2) gives Haber-Weiss reaction. Haber-Weiss reaction depicts the catalytic involvement of metal ions in the hydroxyl radical formation within the biological systems (Kanti Das *et al.,* 2014).

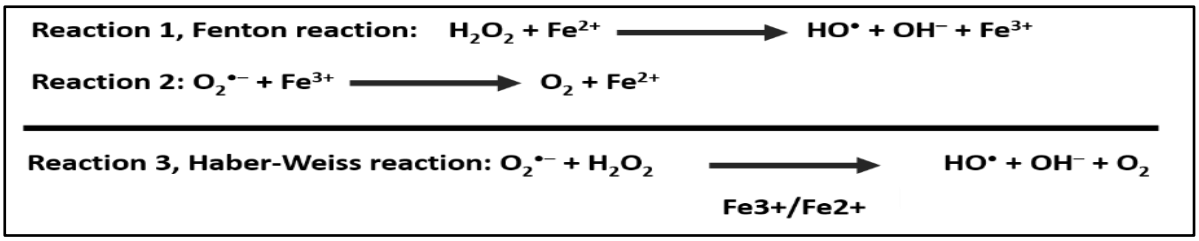

Figure 2: Hydroxyl radical formation in the biological systems (Kanti Das *et al.,* 2014).

When it comes to body physiology, no enzymatic system has been detected to neutralize hydroxyl radical, as it is very reactive within its short life period. To avoid any hydroxyl radical mediated injury of the sort of cell membrane damage, destruction of sugar groups and DNA bases sequence leading to cell death or mutation, its production must be prevented in the first step (Lushchak and Semchyshy, 2012). Amongst all free-radicals, hydroperoxyl (HOO^{-}) is the simplest one playing a significant role in fatty acid peroxidation. Myeloperoxidase is a sort of hem protein converting hydrogen peroxide to hypochlorous acid in the presence of chloride. It is commonly found in neutrophils, explaining its high anti-bactericidal potential (Santo *et al.,* 2016).

RNS generation within the biological systems

The main RNS is known to be nitric oxide (NO) that can be produced endogenously by the conversion of L-arginine amino acid by nitric oxide synthetase (NOS). NO is commonly found in the body, having a significant role in various physiological process, including neurotransmission in central nervous system, smooth muscle relaxation, immune regulation and defence mechanisms. Three types of NOS are present in body. Endothelial NOS (eNOS), expressed in endothelial cells, neuronal NOS (nNOS), mainly expressed in neurons and inducible NOS (iNOS), that is expressed in macrophages upon the release of pro-inflammatory cytokines. eNOS and nNOS are known to be produced whenever needed, thus will disappear second to minutes after enzyme activation. However, iNOS when expressed keeps on producing NO for hours to days and may harm cells (Santo *et al.,* 2016*)*. This excessive NO reacts with superoxide, generating a secondary reactive nitrogen species named peroxynitrite (Gupta, 2015). Although NO is a strong oxidant, it can only react with very specific molecules, in other words NO is a very selective oxidant. On the other hand, peroxynitrite can pass through the cell membrane reach other cells around and react with more vital biomolecules like DNA, proteins and lipids, causing DNA fragmentation, oxidative cleavage of the polypeptide backbone of the proteins, oxidation of the amino acid residue of the

side chain of proteins, generation of protein-protein cross-linkage, and lipid oxidation or peroxidation (Gupta, 2015).

Oxidative stress

Oxidants can affect biological systems in many ways, all of which are dependent on the interaction ability of oxidants with cellular components and the type of oxidants involved. Oxidants in an optimum level are essential molecules in intracellular and intercellular signalling pathways or as a defence mechanism against infections (Poli *et al.,* 2012). Various researches have proven the ability of phagocytes such as, macrophage and neutrophils to generate ROS to kill bacteria. Upon phagocytosis activation, a rapid intake of oxygen, up to 50 times more than normal, by the phagocyte occurs followed by NADPH oxidase stimulation which in turn increases the ROS production in the phagocytes as well (Santo *et al.,* 2016). On the other hand, in a healthy individual some antioxidant systems are present in the body to keep oxidant level in between an acceptable range with a balance between oxidants and antioxidants. If under any circumstances, ROS level exceeds from the predetermined range, in a way that antioxidant system cannot neutralize them, this balance is disturbed in favour of free-radicals, and an oxidative stress state is attained. Various factors may lead to oxidative stress such as suppression of the antioxidant systems or elevated levels of oxidants (Lushchak and Semchyshyn, 2012). Oxidative stress is defined as: "transient or chronic increase in steady-state level of ROS, disturbing cellular core and signalling processes, including ROS-provided ones, leading to oxidative modification of cellular constituents up to the final deleterious effects" (Ďuračková, 2010). Cells under oxidative stress, may respond to this crisis either by adapting to it or by undergoing oxidative damage depending on the intensity of the situation. Generally, cells respond to mild oxidative stress by adaptation, through the elevation of antioxidant production. A serious elevation of oxidant level can induce cell damage, known as oxidative damage by the reaction of free-radicals with important biological molecules like DNA, proteins and lipids in cell membrane, eventually causing dysfunction (Santo *et al.,* 2016).

Antioxidant classification

Antioxidants are key agents in inhibiting oxidative-stress related diseases. For instance, various researches have proven the benefit of plant derived antioxidants in preventing degenerative diseases with an oxidative-stress origin, such as; cancer, Parkinson, Alzheimer and atherosclerosis (Alagumanivasagam *et al.,* 2012). There are different attributes to classify the antioxidants.

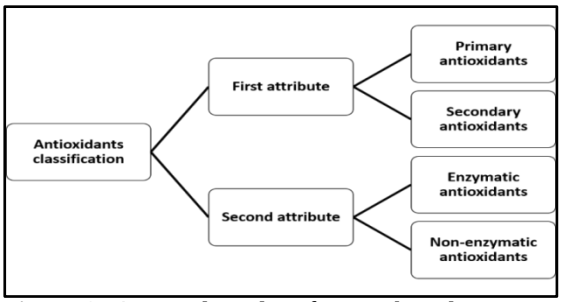

Figure 3: Antioxidant classification based on two different attributes.

The first attribute is based on the function and classifies antioxidants into the primary and secondary groups. The second attribute is based on enzymatic and non-enzymatic antioxidants (Moharram and Youssef, 2015).

Primary antioxidants (enzymatic antioxidants)

Primary antioxidants also known as enzymatic antioxidants react with lipid radicals converting them to more stable molecules, thus inhibiting the progression of chain reaction (Moharram and Youssef, 2015). These antioxidants are produced endogenously as a natural defence against free-radicals. Three main primary antioxidants present in biological systems acting synergistically and complementarily are the first line of defence against ROS known as, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (Bland, 1995). Three types of SOD enzyme have been categorized based on the metal ion found in their neighbour. SOD-1 is a metalloprotein bound to copper and zinc ions localized in the cell's cytosol. SOD-2 is normally found in the mitochondria in association with manganese or iron ions. Lastly, SOD-3 is abundant in the extracellular matrix found close to copper and zinc (Aguilar *et al.*, 2016). As shown in Figure 4 (b) all of these three enzymes mediate the conversion of superoxide into hydrogen peroxide and molecular oxygen (Santo *et al.*, 2016).

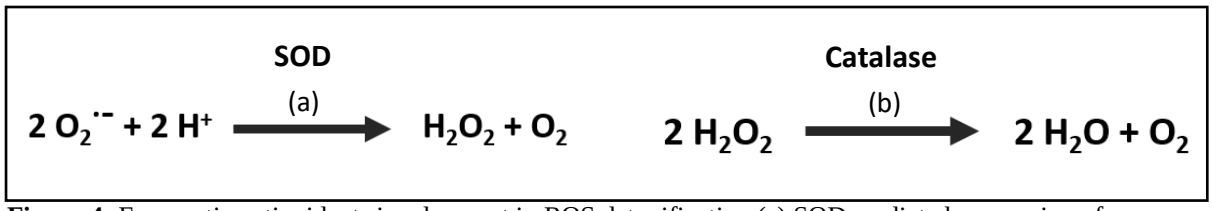

Figure 4: Enzymatic antioxidants involvement in ROS detoxification (a) SOD mediated conversion of superoxide to H_2O_2 (b) Subsequent detoxification of H_2O_2 to water and oxygen.

Catalase is an enzyme which is more or less specific to peroxisome, mediating the conversion of hydrogen peroxide to water and molecular oxygen, as shown in Figure 4 (b). Therefore, it inhibits the accumulation of hydrogen peroxide, thus preventing its involvement in Fenton reaction which normally leads to new free-radical formation (Santo *et al.*, 2016).

Glutathione systems involves the reduced form of glutathione (GSH). GSH is synthesized from glutamate, cysteine and glycine amino acids by two catalytic enzymes called, as γ-glutamylcysteine

ligase (GCL) and GSH synthetase. GSH can scavenge free radicals by direct and indirect enzymatic reactions. In the reaction of such kind, GSH is oxidized to glutathione disulphide (GSSG). Interestingly, glutathione can be recycled from GSSG by glutathione reductase enzyme. Nowadays, the antioxidant capacity of the cells is determined by the GSH/GSSG ratio which is supposed to vary between 10:1 under normal conditions. Glutathione peroxidase (GPx) are a group of enzymes with peroxidase activity. GPx enzymes use glutathione as a reductant agent to mediate reduction of hydrogen peroxide (Chaudière and Ferrari-Iliou, 1999).

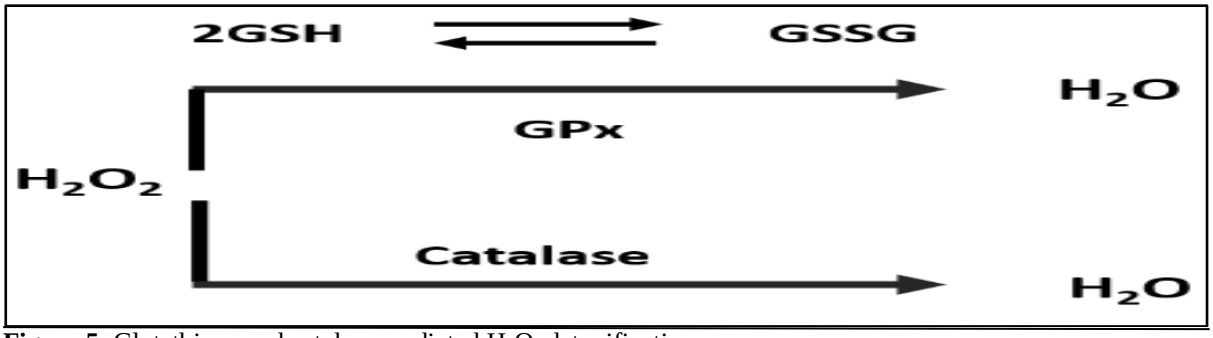

Figure 5: Glutathione and catalase mediated H_2O_2 detoxification.

Secondary antioxidants - non-enzymatic antioxidants

The non-enzymatic antioxidants, also known as secondary antioxidants, are classified into two groups: Endogenous and exogenous. Non-enzymatic endogenous system act as a second line of defence against free-radicals with the enzymatic antioxidants. These non-enzymatic endogenous antioxidants include; Thiols (glutathione, lipoic acid, N-acetyl cysteine), L-arginine, NADPH and NADH, ubiquinone (coenzyme Q10), melatonin, uric acid, bilirubin, metal binding proteins, albumin (copper), ceruloplasmin (copper), metallothionein (copper), ferritin (iron), myoglobin (iron) and transferrin (iron) (Bunaciu *et al.*, 2012). Various classes of non-enzymatic exogenous sources have been suggested amongst which polyphenols are the most important group. Polyphenol group is sub-classified to phenolic acids and flavonoids. Other classes are, vitamins, carotenoids, organosulfur and minerals (Moharram and Youssef, 2015). In vitamin group, vitamin E and C have been reported as antioxidants. Vitamin C cannot be produced endogenously, restricting its source to vegetable and fruits. Vitamin C is known to induce strong antioxidant activity on superoxide, hydroxyl and peroxynitrite radicals by donating them an electron. In contrast with vitamin C, vitamin E is lipophilic, thus playing a great protective effect on cell membrane by inhibiting lipid

peroxidation progression (Bouayed and Bohn, 2010).

Primary antioxidant VS secondary antioxidants

Primary antioxidants have the ability to scavenge millions of free-radicals. Despite primary antioxidants, secondary antioxidants can scavenge only one free-radicals. Meaning that, secondary antioxidants lose their effectivity sooner than primary ones (All antioxidants are not equivalent, 2016).

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