ROS mediated damage and benefits of antioxidants to use as therapy

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Abstract— In all the aerobic organisms, endogenous and exogenous processes generate reactive oxygen species (ROS), and their harmful effects are nullified by the antioxidant defense system at some extent. Oxidative stress occurs due to imbalance between ROS production and antioxidant defence systems. ROS exposure damages the functional biological components of the cells which causes several pathological defects. There are reports of these defects, suggest that oxidative stress induced damages are involved in diseases like: heart disease, lung disease, chronic kidney disease, neurodegenerative diseases, and cancer. Antioxidants act as a therapy and can cure the pathological defects induced by oxidative stress at some level. The purpose of this paper is to provide a subjective knowledge on this topic.

Index Terms— Antioxidants, Reactive oxygen species, free radicals, oxidative stress, damage, diseases, defence system

1 INTRODUCTION

OXYGEN is the vital component for the life of aerobic organisms however certain redox mediated chemical modifications convert this stable compound to highly unstable compounds. Reactivity of molecular oxygen (O_2) is increased by these modifications and it is capable to initiate various biological events (Nita and Grzybowski, 2016). In the living organisms, modifications of O_2 produce reactive oxygen species (ROS) during the normal metabolic processes. Collectively ROS is a broad terminology which encompasses superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet -}$) and more other O_2 derived chemical species. ROS has inherent property of damaging the biological components that leads to the pathological defects. (Cross *et al*., 1987; Finkel 2011). Oxidative stress is developed in the system due to shifting of balance between ROS generation and antioxidant defense system in the favor of oxidants (Schieber and Chandel, 2014). Cellular system is equipped with number of antioxidant proteins that scavenge the ROS and mitigate the oxidative stress related defects. Dietary intake of antioxidants in the form carotenoids, polyphenols and vitamins improve the health quality.

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2 ROS generation

Reactive Oxygen Species (ROS) are generated inside the cells through cellular metabolism. Evolution of superoxides, hydrogen peroxide and other stressors are similar as other normal processes carried out in the cells. These are generated as a byproduct during cellular metabolism like mitochondrial respiratory chain and NADPH oxidase activities. Functionally hydrogen peroxide works in both ways having positive and negative impacts on cellular health. Functional modulation of H_2O_2 depends on its availability and concentration (Holmstrom and Finkel, 2014). Elevated H_2O_2 level triggers the oxidation of redox regulated proteins which are primarily not considered for redox functions although these are modulated by ROS mediated thiol modifications. Many of the phosphatases, ki-

nases and transcription factors are activated by ROS dependent thiol modifications within the protein (Brigelius and Flohe, 2011). The big question for its dual function is how one can predict its actual concentration that will be required for normal physiology and pathology. Stochastically it is easy to answer but realistically, it can only be assumed. It is kind of a tough task because of its high reactivity, diffusibility and molecular conversion into other chemical species. In normal physiological condition basal H_2O_2 level is estimated to be of nanomolar concentration (~ 1-10 nM) which is elevated transiently to ~ 500-700 nM at the time of signaling (Stone and Yang, 2006). Signaling through H_2O_2 is always a contradictory debate among the redox scientific groups.

3 Mitochondria contribution towards ROS

Mitochondria, special cell organelles, contribute majorly for ROS generation. Single electron reduction of molecular oxygen (O_2) leads to the formation of superoxide anion ($O_2^{\bullet-}$) inside the mitochondria. There are eight sites present in the mitochondria that engage in ROS production. Out of eight, three sites located in inner mitochondrial membrane: complex I, II and III of mitochondrial respiratory chain are well characterized for superoxide generation (Murphy, 2009; Glasauer and Chandel, 2013).

Superoxides selectively target the iron sulfur cluster containing proteins and these types of proteins are abundantly present inside the mitochondria (Fridovich, 1997). SOD2 or MnSOD (superoxide dismutase) protein is present inside the mitochondrial matrix that dismutates the superoxides to hydrogen peroxide (H_2O_2). Complex III can release $O_2^{\bullet-}$ to inter mitochondrial space from where it can be leaching out to cytosol through voltage dependent anion channels (Murphy, 2009). SOD1 proteins of cytosol and intermembrane space of mitochondria detoxify these diffused superoxides and convert them into H_2O_2 (Brand, 2010). So, inside the cells free radical compounds are generated continuously and converted into varying forms with the help of redox proteins.

4 NAPDH Oxidases

NADPH oxidases (NOXs) are present in the plasma membrane and the membranes of other cell organelles like endoplasmic reticulum, mitochondria etc. These proteins are also associated with the membranes of phagocytic cells and produce superoxides by one electron reduction of O_2 in the presence of NADPH (Nicotinamide adenine dinucleotide phosphate) (Morgan *et al*., 2011). In mammalian cells NOX enzymes of phagocytes are mainly linked with neutrophils and macrophages where it is termed as Phox (NOX of phagocytes). In normal physiological condition this oxidase (Phox) is present in inactive form, while it becomes active against exposure of foreign particles or microbial invasion and inflammatory mediators. Activation of Phox leads to the production of ROS.

The phagocytic oxidases are multi subunit enzymes consisting of catalytic subunits and regulatory subunits. Activity of this oxidase is governed by the association of these subunits and is specific for the NADPH (as electron donor). NOXs or Phox transfers the electrons of NADPH to O_2 for the generation of $O_2^{\bullet-}$ which is further catalyzed by other cytosolic antioxidant proteins to different secondary free radicals like H_2O_2 (hydrogen peroxide), $OH^{\bullet}$ (hydroxyl radical) and nonradicals- HOCl (hypochlorous acid), O_3 (ozone) etc. (Nauseef, 2008). Myeloperoxidase (MPO) and eosinophil peroxidase use the superoxides generated by NOX as substrate and produce other by-products - hypochlorous, hypobromus and hypothiocynaos acids. These oxidant products are specifically reactive with thiols and methionine residues (Winterbourn, 1985; Pattison and Davies, 2006).

5 Xanthine Oxidase

Xanthine oxidase (XO), a member of oxidoreductase family, is a Mo (molybdenum) containing hydroxylase (Hille, 2002). XO is mostly found in the eubacteria, archaea and eukaryotes. In eukaryotes, it forms a homodimer of 290 kDa. Each Monomer of XO contain four active sites which constitute of a Mo-metal center, two iron-sulfur cluster (2FE-2S) and a Flavin adenine dinucleotide (FAD) for redox regulations (Hille and Nishino, 1995). XO generates superoxides and hydrogen peroxide radicals by catalyzing the wide variety of aromatic heterocyclic compounds such as

hypoxanthine, xanthine and aldehydes to uric acid (Hille, 2005). These enzymes are primarily involved in the catabolism of purines in eukaryotes. In humans, XO is expressed in the tissues of several organs like kidney, lung and myocardium, though its higher expression has been reported in the visceral organs (splanchnic system) (Harrison, 2002). TNFα, IL-1β, IFN-γ are well known inflammatory cytokines are linked to induce the expression of this oxidase (Kelley *et al*., 2006). Xanthine oxidases are transcribed as Xanthine dehydrogenase (XDH) precursors. Post-translational modifications and limited proteolysis convert the XDH precursor to XO. XDH contains the Mo (IV) center associated with Fe/S center along with $FADH_2$ reduces the NAD^+ to NADH and produces uric acid from substrate hypoxanthine (Garattini *et al*., 2003). During some specific conditions as: limited proteolysis, minimal oxygen availability, inflammation or reversible oxidation of cysteine 535 and 992, XDH can be converted to XO (Parks *et al*., 1999). Oxidase form of XDH i.e. XO shows more affinity for O_2 than its initial substrate NAD^+. Biasness for O_2 as a substrate for XO generates $O_2^{\bullet-}$ and hydrogen peroxide by one and two electron transfer respectively from xanthine or hypoxanthine (Stipek *et al*., 1994; Harris and Massey, 1997). Therefore, XO is the major source of ROS production inside the tissue and vascular system and eliciting ROS mediated defects. Superoxides generated from its activity coupled with reduced •NO (reduced nitric oxide) form $ONOO^-$ (peroxynitrite) (Aslan *et al*., 2004).

6 Cellular proteins coping the system against stress

Redox balance of cellular system is required for normal physiological processes and metabolism. Till date we are aware of several proteins that contribute their functions for balancing the redox system. To mitigate this defect, cellular defence system is equipped with various antioxidant proteins. Antioxidant proteins target the ROS according to their specificity and detoxify them.

7 Superoxide dismutases (SOD)

Superoxides are generated in various metabolic processes by one electron reduction. Superoxides are mainly evolved with the respiratory electron transport chain and neutrophilic action. Superoxides and their byproducts are highly reactive for biological components and show pathogenesis in many cardiovascular diseases; hypercholesterolemia, atherosclerosis, hypertension, diabetes and heart failure (Fukai and Ushio-Fukai, 2011). Incorporation with other chemical compounds, like nitric oxide (NO), which is a relatively weaker oxidant, can convert them into highly reactive oxidants ($ONOO^-$) peroxinitrite radical. Superoxide dismutases provide the principal cellular defense against the $O_2^{\bullet-}$. SODs are the metallo-proteins that dismutate the superoxides ($O_2^{\bullet-}$) to hydrogen peroxide (H_2O_2) and oxygen (O_2). There are three isoforms of SODs are present in mammals namely SOD1 (Cu/Zn SOD), SOD2 (Mn SOD) and SOD3 (extra cellular Cu/Zn SOD). SOD isoforms are the products of different genes and catalyze the same reactions in different cell compartments.

The mechanism underlying the catalysis of $O_2^{\bullet-}$ to H_2O_2 by SODs involve the alternating reduction and oxidation of metal ion at the active center of protein in the concerned SODs. SOD1 (Cu/Zn-SOD) is primarily an intracellular protein existing as homodimer of 32 kDa. SOD1 is mainly localized in the cytosol while its smaller fractions are also present in the intermembrane space of mitochondria (Okado and Fridovich, 2001). Immunocytochemical studies in rat hepatocytes showed that SOD1 is present in the nucleus, lysosome and peroxisome in addition to cytosol or mitochondrial space (Chang *et al*., 1988). SOD2 is Mn containing mitochondrial matrix protein existing as 96 kDa homotetramer. It is synthesized in the cell cytoplasm and enters the mitochondrial matrix by a signal peptide to scavenge the $O_2^{\bullet-}$ of the mitochondrial matrix.

Extra cellular Cu or Zn SOD (ecSOD), termed as SOD3 is composed of two disulfide linked dimers of molecular weight 135 kDa. It is mainly present in the extracellular vascular space such as blood vessels, lungs, kidney, uterus and in trace amounts in the heart (Folz and Crapo, 1994).

8 Catalases

Hydrogen peroxide (H_2O_2) is continuously generated inside the living cells by dismutation of superoxides or as byproducts of other reactions. Catalases are ubiquitous heme containing enzymes found almost in all aerobic organisms that detoxify the hydrogen peroxide burst by con-

verting to water (H_2O) and oxygen (O_2). In mammalian cells, it is mainly present in the peroxisomes. Active form of mammalian catalase contains four monomers of 60 kDa homotetramer subunits. Single monomer subunit of catalase is insufficient for degradation of H_2O_2, so the activity of catalase depends upon its active homotetrameric structure (Kirkman and Gaetani, 2006).

It is a better-known peroxidase although its reactivity with less concentration of peroxides is very low due to high K_m value, around > 10mM. Other peroxidases like glutathione peroxidases and peroxiredoxins show low K_m than catalase, so function as better peroxidases at lower levels of peroxides in the mammalian system (Rhee *et al*., 2003). Catalysis of catalases take place in two steps. In the first step H_2O_2 oxidizes the iron (Fe^{3+}) of heme to intermediate oxyferryl (Fe^{4+}=O) group with porphyrin cation ($^{\bullet\alpha+}$por) radical. This intermediate compound-I ($^{\bullet\alpha+}$por) radical oxidizes the second H_2O_2 (peroxides) or alkyl peroxides into simpler or nonoxidative form. H_2O2 is catalyzed into H_2O and O_2 and alkyl peroxides are converted into aldehyde and water (Kirkman and Gaetani, 2006).

9 Glutathione

Glutathione often called as GSH, is a tripeptide low molecular weight thiol (γ-L-glutamyl-L-cysteinylglycine) primarily involved in the protection of cellular system against oxidative stress. It is the most abundant antioxidant estimated to be 1-10 mM inside the cell (Meister and Anderson, 1983; Forman, 2016). Cytosol is the primary reservoir of total cellular GSH (85-90 %) and left 10-15% is distributed in other organelles like mitochondria, nuclear matrix and peroxisomes (Lu, 2000). Initially its function has been linked to the antioxidant only but with passing time, novel roles appeared. Signal transduction, expression of genes, cellular apoptosis by glutathione depletion (Franco *et al*., 2008), glutathionylation of proteins for functional regulation (Chandel *et al*., 2016 Peskin *et al*., 2016) and metabolism of nitric oxide (Jones, 2004).

10 Glutathione Peroxidases (GPx)

Glutathione peroxidase is a seleno-cysteine (Se-Cys) based antioxidant protein involved in the reduction of peroxides. Reduction of high reactive peroxides to the less reactive compounds requires thiol which is derived from two molecules of glutathione (Warner and Wispe, 1997). Glutathione is an important cellular component that oxidizes non-enzymatically even in the absence of GPx to maintain cellular redox homeostasis. Altered function of GPxs leads to the accumulation of peroxides radicals which leads to further cellular defects including tissue injury, cytokine-mediated inflammations (Meyer *et al*., 1994). There are four GPx proteins, termed as Gpx1, Gpx2, Gpx3 and Gpx4. Gpx1-3 are tetrameric while Gpx4 is monomeric protein. Gpx1, often called as cytosolic Gpx, can catalyze the reduction of lipid peroxides, organoperoxides (Grossmann and Wendel, 1983). This is a tetrameric protein and each subunit constitutes of ~ 22 kDa of molecular weight. Gpx2 is also a cytosolic tetrameric protein that share a common substrate as Gpx1. It is more homologous to Gpx1. In mammalian system it is found in the liver and gastrointestinal tract (Chu *et al*., 1993). Gpx3 is an extracellular glycoprotein, majorly found in blood plasma sharing 50% sequence homology with Gpx1 (Takahashi *et al*., 1990). Gpx4 is a phospholipid hydroperoxide glutathione peroxidase (PHGpx), which shows some structural differences and sequence homology with other Gpxs. It is a monomeric protein of ~ 22 kDa of molecular weight catalyzes almost all types of peroxides and their fatty acid derivatives (Thomas *et al*., 1990).

11 Peroxiredoxins

Peroxiredoxins are ubiquitous thiol-based antioxidant proteins. Peroxiredoxins (Prxs) are better known peroxidases member of the oxidoreductase enzyme class which detoxify free radicals like hydrogen peroxide, hydroperoxides, peroxynitrites etc. It is believed that proteins of this class evolved from thioredoxin like precursors (Copley *et al*., 2004).

12 Antioxidants and their functions

In aerobic life, oxidation is an autonomous metabolic process in which numerous forms of free radicals are generated. Cellular redox system with its antioxidant proteins are fully devoted in balancing the free radicals load. Antioxidants are the chemical species that inhibit or delay the de-

fects of reactive oxygen species and promote healthy life (Halliwell, 2007).

Antioxidants can be defined in many ways like as biochemists concern, it is a chemical species that quench the reactive oxygen species or free radicals into stable or inert compounds. From a nutritionist point of view, antioxidants are the compounds that contain bioactive compounds like polyphenols, flavonoids, carotenoids, vitamins etc. for the health benefits (Finley *et al*., 2011).

ROS imbalance is reported for the various pathobiological defects and chronic diseases, for example cancer and cardiovascular diseases. Oxidation of vital components like DNA, protein, lipids attributes for these defects. There is a rich history of consumption of food-based antioxidants to avoid health defects. In 18th and 19th century people were aware and used to consume citrus food (containing vitamin C) to prevent scurvy (Lind, 1983), unpolished rice (vitamin B1) to prevent beriberi (Fletcher, 1907) and consumed liver from meat source (vitamin A) to prevent night blindness (Wolf, 1978).

13 Components of antioxidants:

13.1 Carotenoids: Carotenoids represent a huge family compound, sharing its presence in most of the plant pigments for color. Carotenoids are also present in ample amounts in our dietary foods like vegetables and fruits. Tomato, carrot, berries are some of the examples which are the good source of carotenoids. Carotenoids take part in the form of hydrocarbons, α and β-carotene, lycopene, xanthophyll, lutein and zeaxanthin in dietary foods. These compounds are known for the health benefits against several diseases. A few carotenoid compounds are also present in human blood and tissues (Krinsky and Johnson, 2005). β-carotene, lycopene, lutein and zeaxanthin are the most common examples of carotenoids. β-carotene and lycopene are the fat-soluble carotenoids, found in low density lipoproteins (LDL). Lutein and zeaxanthin are the members of xanthophyll compounds present in the high- and low-density lipoproteins (Clevidence and Bieri, 1993).

13.2 Polyphenols

Polyphenols are one of the most abundant phytochemicals produced as a secondary metabolite by the plants (Crozier *et al*., 2006). Like most of the plant components, these are not directly involved in the growth of plants. It has some ancillary roles like defence against pathogens, as a signaling molecule to uptake nutrients etc. (Scalbert *et al*., 2005). Structurally polyphenols are aromatic rings containing hydrocarbons with one or more hydroxyl groups and classified in two major classes: glycosides and aglycones. Glycosides are sugars containing polyphenols. Aglycones are non-sugar single compounds (Jaganath and Crozier, 2009). Flavonoids are one of the most important naturally occurring polyphenols derived from plant sources. These compounds are reported for their vast pharmaceutical and nutraceutical values. Fruits, vegetables and their derived products are considered as good sources of polyphenols. Flavonoids are also further subclassified as flavones, flavonols, flavanones, chalcones and isoflavones (Spencer *et al*., 2008). Non flavonoids are low molecular weight (C1-C6) phenolic acids, also present in our dietary food sources like in berries.

13.3 Vitamins

Majorly vitamin C and E act as a primary antioxidant capable of scavenging radicals generated within cells or plasma before they can damage DNA, proteins or lipids. Normal cellular metabolism in chloroplasts, mitochondria and peroxisomes generates reactive oxygen species (ROS) as a byproduct which is enhanced by a variety of environmental stresses, including drought, starvation, wounding, high salt, high light, exposure to pollutants, etc. leading to oxidative stress. In both plants and animals' ascorbic acid interacts enzymatically and non-enzymatically with damaging oxygen radicals (ROS) and their derivatives to form non-toxic, non-radical products, i.e. DHA and 2, 3-diketogulonic acid (Dalton *et al*., 1995). Due to its antioxidant nature ascorbate functions as a recycler for other antioxidants. It is involved in the regeneration of lipophilic, membrane associated alpha-tocopherol (vitamin E) radical at the surface of biological membranes, thus contributing to the ability of alpha-tocopherol to break the chain of lipid peroxidation in lipid bilayers (Buettner. 1993). Ascorbate reduces tetrahydrobiopterin radical in cultured endothelial cells due its antioxidant nature for proper action of endothelial nitric oxide synthase (Baker *et al*., 2001; Patel *et al*., 2002).

Vitamin E is the fat-soluble compound with distinctive antioxidant activity essential for health, first discovered in 1922 by Evan and Bishop (Niki and Traber. 2012). The richest dietary sources of vitamin E are edible vegetable oils and fat-containing foods (Zingg, 2007). It inhibits the production of reactive oxygen species molecules when fat undergoes oxidation and during the propagation of free radical reactions (Burton *et al*., 1983).

13.4 Selenium

Is an essential trace element naturally present in many foods which plays a critical role in reproduction, thyroid hormone metabolism, DNA synthesis, and protection from oxidative damage and infection (Sunde *et al*., 2012). Seafoods and organ meats are the richest food sources of selenium. In animal and human tissues selenium is active in the form of seleno-methionine where it incorporates non-specifically with the amino acid methionine in body proteins (Terry *et al*., 2012).

13.5 Dietary food components for health benefits

Dietary intake of antioxidants from our food is considered as remedy to avoid the defects of ROS and RNS. It contains nutritional value as well as phytochemicals in the form of polyphenols, vitamins, carotenoids etc. that are capable of scavenging ROS and RNS (Yu, 1994).

Curcumin is the active compound extracted from turmeric. It is a well-known ROS scavenger. Antioxidant activity of curcumin is comparable with vitamin C and E. ROS accumulation greatly reduces the cell viability of osteoblasts and induces the caspase mediated cell apoptosis. ROS (H_2O_2) mediated cell toxicity in osteoblasts leads to bone dysfunction. Curcumin enhances the cell viability by restricting the oxidative stress mediated cell apoptosis. Efficacy of curcumin is effective in bone dysfunctioning or osteoporosis (Dai *et al*., 2017).

Kiwi fruit is considered as a good source of vitamin-C and polyphenols. Studies on kiwi fruit have demonstrated that deficiency of vitamin-C and other oxidative stress related defects can be overcome by its consumption (Carr *et al*., 2013). Various neuronal defects that become detrimental due to oxidative stress is lowering down by the consumption of kiwi fruit (Xue *et al*., 2017). Quercetin is an active compound (flavonol) isolated from kiwi fruit that has a protective impact against oxidative stress and is used as an antioxidant. Kissper is a peptide isolated from kiwi fruit, known for its antioxidant and anti-inflammatory effects. Results of this peptide suggested that it can create pores in synthetic lipid bilayer. In-vivo and in-vitro data suggested that kissper can be used as a therapeutic agent to cure intestinal inflammation and lipopolysaccharide induced ROS generation (Ciacci *et al*., 2013).

In-vitro and in-vivo studies suggest that naturally occurring antioxidants reduce the effects of oxidants. Various in-vitro methods like DPPH, FRAP, ABTS and various fluorescence-based probes are available to test the antioxidant efficiency or activity which tells at what rate oxidants would be quenched, but it is very difficult to understand if it really works similar in the living system. When we come to the mechanism of antioxidants functions in the living system, there are lots of contradictory statements, which are not conclusive. Indeed, there is no evident baseline for the antioxidant theory and disease control because of varying mode of catalytic action of antioxidants (Galati *et al*., 2002; Fang *et al*., 2005).

14 Conclusion

All ROS production cause several types of damage to the organs and also leads to age related diseases. Antioxidants are effective to neutralize and control damage repair. Several studies have done about antioxidant effectiveness about to age related diseases. Now days peoples are awarded about number of antioxidants and their benefits. further more and more studies are required to know about all aspects of antioxidants and dose or concentration to use as threptic for age related diseases that happen by oxidative stress.

15 REFERENCES

[1] Nita M, Grzybowski A. The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. Oxidative Medicine and Cell Longevity 2016; 2016: 3164734-3164757.

[2] Cross CE, Halliwell B, Borish ET, Pryor WA, Ames BN, Saul RL, McCord JM, Harman D. Oxygen radicals and human disease. Annals of Internal Medicine 1987; 4:526-545.

[3] Finkel T. Signal transduction by reactive oxygen species. Journal of Cell Biology 2011; 1:7-15.

[4] Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. Current Biology 2014; 24:453-462.

[5] Holmstrom KM, Finkel T. Cellular mechanisms and physiological consequences of redox-dependent signaling. Nature Reviews Molecular Cell Biology 2014; 6:411-421.

[6] Brigelius-Flohe R, Flohe L. Basic principles and emerging concepts in the redox control of transcription factors. Antioxidants and Redox Signaling 2011; 15:2335-2381.

[7] Stone JR, Yang S. Hydrogen peroxide: a signaling messenger. Antioxidants and Redox Signaling 2006; 8:243-270.

[8] Murphy MP. How mitochondria produce reactive oxygen species. Biochemical Journal 2009; 417:1-13.

[9] Glasauer A, Chandel NS. ROS. Current Biology 2013; 23:100-102.

[10] Fridovich I. Superoxide anion radical (O2•−), superoxide dismutases, and related matters. Journal of Biological Chemistry 1997; 30:18515-18517.

[11] Brand M. The sites and topology of mitochondrial superoxide production. Experimental Gerontology 2010; 45:466-472.

[12] Morgan B, Sobotta MC, Dick TP. Measuring E(GSH) and H_2O_2 with roGFP2-based redox probes. Free Radical Biology and Medicine 2011; 51:1943-1951.

[13] Nauseef WM. Biological roles for the NOX family NADPH oxidases. Journal of Biological Chemistry 2008; 283:16961-16965.

[14] Winterbourn CC. Comparative reactivities of various biological compounds with myeloperoxidase-hydrogen peroxide-chloride, and similarity of the oxidant to hypochlorite. Biochimica et Biophysica Acta 1985; 2:204-210.

[15] Pattison DI, Davies MJ. Reactions of myeloperoxidase-derived oxidants with biological substrates: gaining chemical insight into human inflammatory diseases. Current Medicinal Chemistry 2006; 13:3271-3290.

[16] Hille R. Molybdenum and tungsten in biology. Trends in Biochemical Sciences. 2002; 27:360-367.

[17] Hille R, Nishino T. Flavoprotein structure and mechanism. 4. Xanthine oxidase and xanthine dehydrogenase. Federation of American Societies for Experimental Biology Journal 1995; 11:995-1003.

[18] Hille R. Molybdenum-containing hydroxylases. Archives of Biochemistry and Biophysics 2005; 1:107-116.

[19] Harrison R. Structure and function of xanthine oxidoreductase: where are we now? Free Radical Biology and Medicine 2002; 6:774-797.

[20] Kelley EE, Hock T, Khoo NK, Richardson GR, Johnson KK, Powell PC, Giles GI, Agarwal A, Lancaster JR Jr, Tarpey MM. Moderate hypoxia induces xanthine oxidoreductase activity in arterial endothelial cells. Free Radical Biology and Medicine 2006; 6:952-959.

[21] Garattini E, Mendel R, Romão MJ, Wright R, Terao M. Mammalian molybdo flavoenzymes, an expanding family of proteins: Structure, genetics, regulation, function and pathophysiology. Biochemical Journal 2003; 372:15-32.

[22] Parks DA, Skinner KA, Tan S, Skinner HB. Xanthine oxidase in biology and medicine. In: Gilbert DL, Colton CA, editors. Reactive Oxygen Species in Biological Systems: Selected Topics. New York: Kluwer Academic/Plenum Publishers 1999; 397-420.

[23] Stipek S, Novak L, Crkovska J, Zima T, Platenik J. Xanthine oxidoreductase. Biochemical, Biological and Pathogenic Functions 1994; 95:289-295.

[24] Harris CM, Massey V. The oxidative half-reaction of xanthine dehydrogenase with NAD; reaction kinetics and steady-state mechanism. Journal of Biological Chemistry 1997; 45:28335-28341.

[25] Aslan M, Freeman BA. Oxidant-mediated impairment of nitric oxide signaling in sickle cell disease-mechanisms and consequences. Cellular and Molecular Biology 2004; 1:95-105.

[26] Fukai T, Ushio-Fukai M. Superoxide dismutases: role in redox signaling, vascular function and diseases. Antioxidant and Redox Signaling 2011; 15:1583-606.

[27] Okado-Matsumoto A, Fridovich I. Subcellular distribution of superoxide dismutases (SOD) in rat liver Cu, Zn-SOD in mitochondria. Journal of Biological Chemistry 2001; 42:38388- 38393.

[28] Chang LY, Slot JW, Geuze HJ, Crapo JD. Molecular immunocytochemistry of the CuZn superoxide dismutase in rat hepatocytes. Journal of Cell Biology 1988; 107:2169-2179.

[29] Folz RJ, Crapo JD. Extracellular superoxide dismutase (SOD3): tissue-specific expression, genomic characterization, and computer-assisted sequence analysis of the human EC SOD gene. Genomics. 1994; 22:162–171

[30] Kirkman HN, Gaetani GF. Mammalian catalase: a venerable enzyme with new mysteries. Trends in Biochemical Sciences. 2006; 1:44-50.

[31] Rhee SG, Chang TS, Bae YS, Lee SR, Kang SW. Cellular regulation by hydrogen peroxide. Journal of the American Society of Nephrology 2003; 8:211-215.

[32] Meister A, Anderson ME. Glutathione. Annual Review of Biochemistry 1983; 52:711-760.

[33] Forman HJ. Glutathione - from antioxidant to post-translational modifier. Archives of Biochemistry and Biophysics 2016; 595:64-67.

[34] Lu SC. Regulation of glutathione synthesis. Current Topics in Cell Regulation 2000; 36:95- 116.

[35] Franco R, DeHaven WI, Sifre MI, Bortner CD, Cidlowski JA. Glutathione depletion and disruption of intracellular ionic homeostasis regulate lymphoid cell apoptosis. Journal of Biological Chemistry 2008; 283:36071-36087.

[36] Chandel A, Das KK, Bachhawat AK. Glutathione depletion activates the yeast vacuolar transient receptor potential channel, Yvc1p, by reversible glutathionylation of specific cysteines. Molecular Biology of the Cell 2016; 24:3913-3925.

[37] Peskin AV, Pace PE, Behring JB, Paton LN, Soethoudt M, Bachschmid MM, Winterbourn CC. Glutathionylation of the active site cysteines of peroxiredoxin 2 and recycling by glutaredoxin. Journal of Biological Chemistry 2016; 291:3053-3062.

[38] Jones, DP. Redox potential of GSH/GSSG couple: assay and biological significance. Methods in Enzymology 2002; 348:93-112.

[39] Warner BB, Wispe JR. Transgenic Models for the Study of Lung Injury and Repair: Integration of Molecular, Functional and Cellular Approaches. II. Eukaryotic Antioxidant Systems. In: Massaro C, Clerch L, editors. Oxygen, Gene Expression, Cellular Function. NY: Marcel Dekker Inc. 1997; 76-79.

[40] Meyer M, Pahl HL, Baeuerle PA. Regulation of the transcription factors NF-κB and AP-1 by redox changes. Chemico-biological interactions. 1994; 91(2-3):91-100.

[41] Grossmann, A. Wendel A. Non-reactivity of the selenoenzyme glutathione peroxidase with enzymatically hydroperoxidized phospholipids. European Journal of Biochemistry 1983; 135:549-552.

[42] Chu FF, Doroshow, JH, Esworthy RS. Expression, characterization, and tissue distribution of a new cellular seleniumdependent glutathione peroxidase, GSHPx-GI. Journal of Biological Chemistry 1993; 268:2571-2576.

[43] Takahashi K, Akasaka M, Yamamoto Y, Kobayashi C, Mizoguchi, J, Koyama J. Primary structure of human plasma glutathione peroxidase deduced from cDNA sequences. Journal of Biochemistry 1990; 2:145-148.

[44] Thomas JP, Maiorino M, Ursini F, Girotti AW. Protective action of phospholipid hydroperoxide glutathione peroxidase against membrane-damaging lipid peroxidation. In situ reduction of phospholipid and cholesterol hydroperoxides. Journal of Biological Chemistry 1990 1:454-461.

[45] Halliwell B. Biochemistry of oxidative stress. Biochemical Society Transactions 2007; 5:1147-1150.

[46] Finley JW, Kong AN, Hintze KJ, Jeffery EH, Ji LL, Lei XG. Antioxidants in foods: state of the science important to

the food industry. Journal of Agriculture and Food Chemistry 2011; 59:6837-6846.

[47] Lind J. Nutrition classics. A treatise of the scurvy by James Lind, MDCCLIII. Nutrition Reviews 1983, 41:155-157.

[48] Fletcher W. Rice and beri-beri: preliminary report on an experiment conducted at the Kuala Lampur lunatic asylum. Lancet 1907; 1:1776-1779.

[49] Wolf G. A historical note on the mode of administration of vitamin A for the cure of night blindness. The American Journal of Clinical Nutrition 1978; 31:290-292.

[50] Krinsky NI, Johnson EJ. Carotenoid actions and their relation to health and disease. Molecular Aspects of Medicine 2005; 6:459-516.

[51] Clevidence BA, Bieri JG. Association of carotenoids with human plasma lipoproteins. Methods in Enzymology 1993; 214:33-46.

[52] Jaganath IB, Crozier A. Overview of health promoting compounds in fruits and vegetables. In: phenolic compounds of plant origin and health: The biochemistry behind their nutritional and pharmacological value, edited by Chichester FC. United Kingdom: Wiley, 2009; 1-48.

[53] Spencer JP, Abd El Mohsen MM, Minihane AM, Mathers JC. Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. British Journal of Nutrition 2008; 99:12-22.

[54] Dalton DA. Antioxidant defenses of plants and fungi. In: Ahmad S (ed.) Oxidative Stress and Antioxidant Defenses in Biology. 1995 Chapman and Hall, New York, pp 298-355.

[55] Buettner GR. The pecking order of free radicals and antioxidants: lipid peroxidation, alpha- tocopherol, and ascorbate. Archives of Biochemistry and Biophysics 1993; 300:535-543.

[56] Baker RA, Milstien S, Katusic ZS. Effect of vitamin C on the availability of tetrahydrobiopterin in human endothelial cells. Journal of Cardiovascular Pharmacology 2001; 37:333-338.

[57] Patel KB, Stratford MR, Wardman P, Everett SA. Oxidation of tetrahydrobiopterin by biological radicals and scavenging of the trihydrobiopterin radical by ascorbate. Free Radical Biology and Medicine 2002; 32:203-211.

[58] Niki E, Traber MG. A history of vitamin E. Annals of Nutrition Metabolism. 2012; 61:207-212.

[59] Zingg JM. Vitamin E: An overview of major research directions. Molecular Aspects of Medicine 2007; 28:400-422.

[60] Burton GW, Joyce A, Ingold KU. Is vitamin E the only lipid-soluble, chain-breaking antioxidant in human blood plasma and erythrocyte membranes? Archives of Biochemistry and Biophysics 1983; 221:281-290.

[61] Sunde RA. Selenium. In: Ross AC, Caballero B, Cousins RJ, Tucker KL, Ziegler TR, eds. Modern Nutrition in Health and Disease. 11th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2012; 225-237.

[62] Terry EN, Diamond AM. Selenium. In: Erdman JW, Macdonald IA, Zeisel SH, eds. Present Knowledge in Nutrition. 10th ed. Washington, DC: Wiley-Blackwell; 2012; 568-587.

[63] Yu BP. Cellular defenses against damage from reactive oxygen species. Physiological Reviews 1994, 74:139-162.

[64] Dai P, Mao Y, Sun X, Li X, Muhammad I, Gu W, Zhang D, Zhou Y, Ni Z, Ma J, Huang S. Attenuation of oxidative stress-induced osteoblast apoptosis by curcumin is associated with preservation of mitochondrial functions and increased Akt-GSK3β signaling. Cellular Physiology and Biochemistry 2017; 2:661-677.

[65] Carr AC, Bozonet SM, Vissers MCM. A Randomised Cross-Over Pharmacokinetic Bioavailability Study of Synthetic versus Kiwifruit-Derived Vitamin C. Nutrients. 2013; 11: 4451-4461.

[70] Xue WZ, Yang QQ, Chen Y, Zou RX, Xing D, Xu Y, Liu YS, Wang HL. Kiwifruit alleviates learning and memory deficits induced by Pb through antioxidation and inhibition of microglia activation in vitro and in vivo. Oxidative Medicine and Cellular Longevity 2017; 2017:5645324-5645338.

[71] Ciacci C, Russo I, Bucci C, Iovino P, Pellegrini L, Giangrieco I, Tamburrini M, Ciardiello MA. The kiwi fruit peptide kissper displays anti-inflammatory and anti-oxidant effects in in-vitro and ex-vivo human intestinal models. Clinical and Experimental Immunology 2013; 3:476-484.

[72] Galati G, Sabzevari O, Wilson JX, O'Brien PJ. Prooxidant activity and cellular effects of the phenoxyl radicals of dietary flavonoids and other polyphenolics. Toxicology 2002; 177:91-104.

[73] Fang J, Lu J, Holmgren A. Thioredoxin reductase is irreversibly modified by curcumin: a novel molecular mechanism for its anticancer activity. Journal of Biological Chemistry 2005; 280:25284-25290.

74] Copley SD, Novak WR, Babbitt PC. Divergence of function in the thioredoxin fold suprafamily: evidence for evolution of peroxiredoxins from a thioredoxin-like ancestor. 2004; 43:13981-13995.