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THE ROLE OF ANTIOXIDANTS IN HUMAN HEALTH MAINTENANCE: SMALL MOLECULES WITH INFINITE FUNCTIONS

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ABSTRACT

Oxygen is a necessary element that controls many metabolic reactions in human body. Increased amount of oxygen in the body leads to continuous chain of events resulting in the formation of deleterious toxic free radicals which are capable of quenching the macromolecules including proteins and nucleic acids. To counteract the toxicity of the free radicals, human body synthesizes both enzymatic and non-enzymatic antioxidants. Non-enzymatic antioxidants are small molecules, capable of preventing the oxidative damage incurred by free radicals. Antioxidants also play crucial role in the controlling the health problems such as heart diseases, cancer, aging etc. which occur mostly due to accumulation of free radicals. The present review focuses on the various roles of antioxidants in maintaining the human health.

INTRODUCTION: Oxygen is an essential element of the aerobic organisms for the production of energy. It is the key element in the human body, capable of combining with every other element leading to formation of essential components necessary for maintaining its regular metabolic activities. Oxygen regulates about 90% of the body function and plays a pivotal role in the respiration [1], gets absorbed by the blood stream in the lungs, transported to the cells and participates in complex processes of metabolic reactions involving enzymatic and non-enzymatic reactions with organic compounds catalyzed by ionizing radiations resulting in the formation of free radicals [2]. Free radicals have surplus free-floating electrons rather than having harmonized pairs and therefore unstable, but are highly reactive, moves freely through blood stream and in order to attain stability attacks nearby molecules including proteins, carbohydrates and nucleic acids damaging them by stealing their electrons through a process called oxidation. Types of free radicals include the hydroxyl radical ($OH^{\cdot}$), the superoxide radical ($O^{\cdot}_2$), the nitric oxide radical ($NO^{\cdot}$) and the lipid peroxyl radical ($LOO^{\cdot}$) [3]. External sources like air pollutants, industrial chemicals, cigarette smoke, alcohol, oxidized poly unsaturated fats and cooked food [4] also contribute to the formation of free radicals leading to irreparable damage to the several organs, causing malfunctions.

Diseases caused by free radical formation: The role of free radicals has been implicated in the development of at least 50 diseases. A few of them include arthritis, inflammatory diseases, kidney diseases, cataracts,

inflammatory bowel disease, colitis, lung dysfunction; pancreatitis; drug reactions, skin lesions, and aging. Free radicals are also associated with liver damage due to alcohol consumption and the development of emphysema due to cigarette smoking.

Aging is the prime mechanism oriented with the free radical accumulation in the humans as suggested in the Free Radical Theory of Aging [5]. A symptom of aging such as atherosclerosis is considered to be due to oxidation by free radicals. The primary site of free radical damage is the mitochondrial DNA. Damage to the mitochondrial DNA cannot be readily repaired and leads to the shutting down of mitochondria causing cell death and ageing [6]. Bombardment of free radicals with atoms of metals like mercury, lead, cadmium and even pesticides amplifies the production of free radicals several million times resulting in mitochondrial damage. Severe mitochondrial damage in the cells leads to apoptosis occurs due to a cascade initiated by Bcl-2 proteins on the surface of mitochondria. Destruction properties of free radicals will not limit only to the process of aging but also plethora of diseases via various metabolic activities

Accumulation of free radicals forms cataracts in the human eye. Scavenging of free radicals takes place in the eye, which gets hampered due to age-related insufficient production of antioxidant scavenging systems leading to the formation of an opaque spot on the eye lens causing blindness [7]. Myocytes are the source of free radical accumulation in the heart. Free radicals damage proteins and calcium pumps on the sarcoplasmic reticulum, resulting in the accumulation of calcium. High levels of calcium cause erratical contraction of the myocytes causing arrhythmia [8]. Spread of arrhythmia to other cells disrupts heart beat, causing severe complications.

Free radicals produced due to external sources especially radiation leads to cancer[9]. Most of the radiation energy is taken by the cells, which is absorbed by the water causing one of its oxygen-hydrogen covalent bonds to split and forms free radical. This free radical reacts with another molecule in microseconds of its generation attacks and injures the macro molecules of the cell such as DNA, disrupting its strands and causing mutations in its bases[10]. However, free radicals that are produced during combustion may last little longer in the lungs binding to other air pollutants leading to lung cancer [11].

Role of antioxidants in promoting Human Health: Antioxidants are the molecules, capable of limiting the macro molecule oxidation of free radicals by terminating the chain reactions, which are the main source of free radical formation in the cell. The critical role of antioxidants in ameliorating the free radicals have been elaborately studied, still it is not clear whether the production of free radicals is the consequence or the cause of a disease. Broadly antioxidants are classified into two types.

Enzymatic and non-enzymatic: The non-enzymatic antioxidants are again classified into hydrophilic and hydrophobic. Hydrophilic antioxidants can dissolve into blood and cytosol and react with free radicals. Hydrophobic antioxidants protect the cell membrane from lipid peroxidation, a mechanism by which free radicals degrade the membrane lipids [12].

The role of antioxidants in scavenging the deleterious effects of free radicals is complex, which depend on the interactions of various metabolites and enzyme systems having synergistic and interdependent effects on one another [13]. The performance level of antioxidants also depends on the concentration, reactive potentiality with the specific free radical, interaction and function with other antioxidant family members [14]. In this review, the critical roles of non-enzymatic and enzymatic antioxidants in improvising human health have been discussed;

β- Carotene: Also known as pro-vitamin A, having incredible antioxidant power by which it effectively protects the cells against multiple types of cancer, especially lung cancer. A part from this, □-carotene is particularly helpful against loss of vision and also protects the skin from an inherited skin disease erythropoietic protoporphyria, where the skin is sensitive to sunlight as a result of free radical damage and improvises immune response by increasing the T-helper cells [15]. Beta-carotene alone wipes out free radicals formed in the body especially singlet oxygen. With the help of Vitamin E, β-carotene completely

eliminates the oxygen species before it can turn into skin or lung cancer. Butternut squash, turnip greens, kale, beet greens, red peppers, tomatoes, collard greens, apricots, cantaloupe, peaches, prunes and sweet potato are the richest sources of β - carotene.

Pyridoxine and thiamine: Pyridoxine (vitamin B6) is a significant vitamin, which reduces the toxicity of the homocysteine by converting it to glutathione. The importance of this antioxidant is best exemplified in the depriving rats of dietary vitamin B6 severely compromises their antioxidant defenses, making them more sensitive to oxidative stress [16]. Supplementation with vitamin B6 reduced the oxidative stress levels by abnormally elevating the homocysteine levels in homocysteinemic rats as a consequence of depriving animals of folic acid [17].

The combination of creatine and pyridoxine will enhance the antioxidant status, hastens muscle recovery and accentuate muscle anabolism in the humans [18]. Pyridoxine represents yet another important possibility to reduce the harmful effects of oxidative stress. Thiamine (Vitamin B1) is getting prominence as a promising antioxidant in plants especially under abiotic stress conditions. In humans the deficiency of thiamine causes muscle cramps and Beriberi disease, which is due to the rapid activity of the free radicals in muscle cells. However, it is yet to be proved the exact role of thiamin in scavenging the toxicity of free radicals in humans.

Ascorbic acid: Ascorbic acid (Vitamin C) is the most potent antioxidant, which promotes the elimination of free radicals generated by the human body as well as the external sources. Critical role of ascorbic acid is concerned with regulating the activity of free radicals that alters skin health and leads to formation of skin spots, wrinkles, skin sagging, dryness, sunburn and even the development of melanomas or skin cancer. The action of free radicals on skin cells is one of the most important factors in skin aging. Being water soluble ascorbic acid can work both inside and outside the cells to combat free radical damages by donating the electrons to free radicals and neutralizing their reactivity [19]. Ascorbic acid also protects lungs, central nervous system from free radical damage and DNA from the attack of free radicals and mutagens which prevents harmful genetic alterations within the cells and protects lymphocytes from mutations to the chromosomes [20].

Interaction of ascorbic acid with folic acid (Vitamin B6) prevents free radical damage to the lipids reducing lipid peroxidation, blood pressure, cholesterol levels, help in thinning of blood and protects it against oxidation [21]. β- carotene and selenium are formed when ascorbate is added to α-tocopherol (vitamin E), helps in prevention of stroke [21] and in alleviation of pancreatitis, or an inflammation of the pancreas [22]. Regular consumption of ascorbic acid (1g/day) protects against Low Density Lipoprotein (LDL) cholesterol [23]. Vitamin C also prevents atherosclerosis by inhibiting the oxidative modification of LDLs and synthesis of collagen in the artery walls [24].

Vitamin E: Vitamin E is the collective name of a group of fat soluble compounds with distinctive antioxidant activities [25]. Vitamin E naturally exists in eight chemical forms (alpha-, beta-, gamma-, and delta-tocopherol and alpha-, beta-, gamma-, and delta-tocotrienol with varying levels of biological activities among which alpha (α- tocopherol is the only recognized form of vitamin E with sufficient concentrations in blood compared with other forms [26]. Vitamin E controls the production of ROS during the oxidation of fats by neutralizing the free radicals there by decreasing the intensity of the damage to the tissues and macromolecules (**Fig.1**).

Vitamin E prevents or delays the chronic diseases including Alzheimer's, macular degeneration, osteoarthritis, and prostate enlargement which are associated with the free radicals [27]. α- tocopherol serves as an effective anti-inflammatory agent and protects the skin from damage by sun rays and will reduce prostate cancer along with the considerable amounts of selenium and □-tocopherol[28]. Investigations are in progress to identify the critical mechanism of free radical inhibition by the □-tocopherol as well as other tocopherols. The roles of tocopherols in preventing the oxidative stress is depicted in **Table 1.**

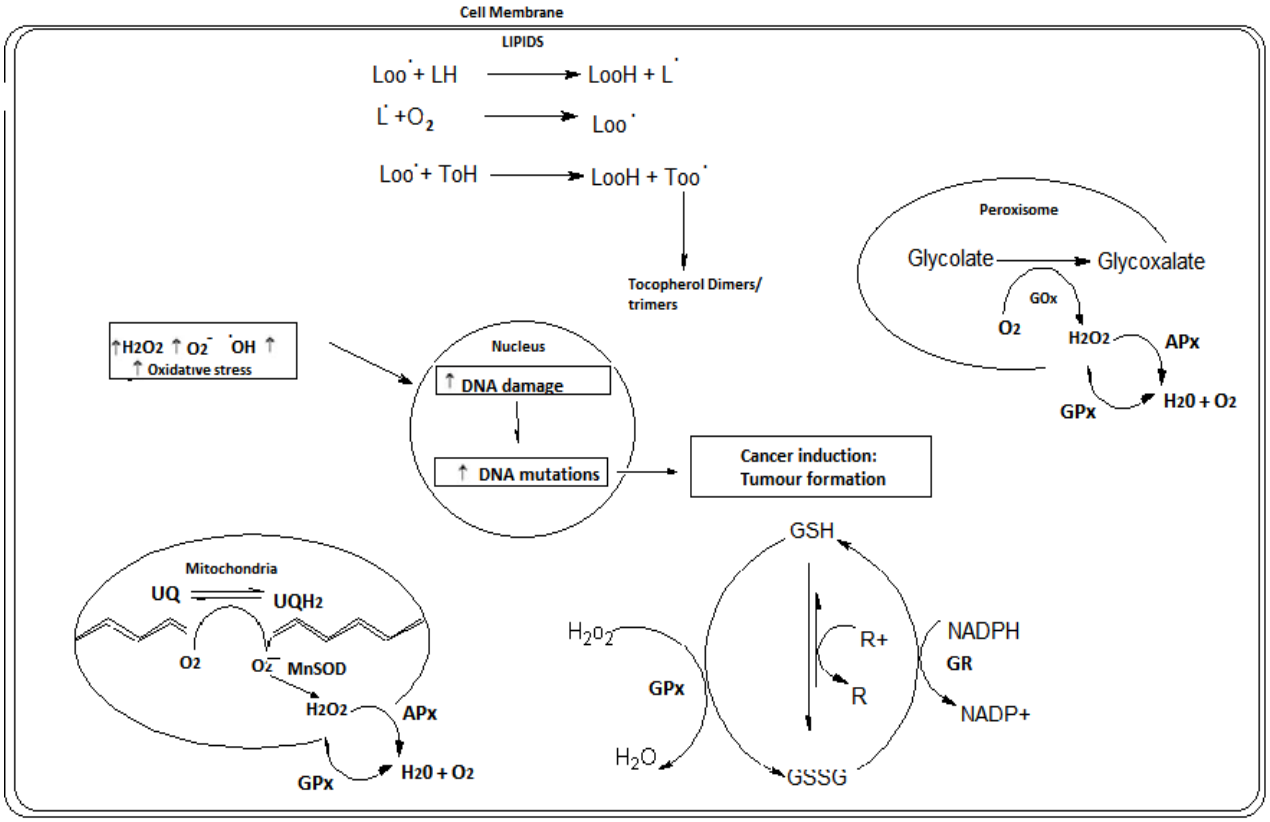

FIG. 1: MECHANISM OF ANTIOXIDATION IN DIFFERENT CELL ORGANELLE

TABLE 1: TYPES OF TOCPHEROLS AND THEIR ROLES CONTROLLING THE HUMAN HEALTH

Name	Source	Function
α-tocopherol	Nuts and sunflower seeds, almonds/almond butter, hazelnuts, and pine nuts	Significantly reduces the risk of developing bladder cancer
β-tocopherol	Beans, meat, peanut	Promotes fertility
γ –tocopherol	Black walnuts, sesame seeds, pecans, pistachios, English walnuts, flaxseed, and pumpkin seeds	Helps in protecting against colon and prostate cancers
Δ-tocopherol	Meat, milk, egg yolks, whole grain cereals, corn and cottonseed oil. It has many natural sources, including wheat germ, rice germ, maize and green leaves.	One of the most effective anti oxidants, important to the life of RBC& cell membranes

Glutathione: Glutathione is an effective antioxidant and important component of antioxidant network that protects our body against the effects of free radicals and aging. Glutathione is a small protein which is built with three amino acids cysteine, glutamic acid, glycine and generally exists in reduced (GSH) and oxidized states (GSSG). In reduced state, GSH can donate an electron to stabilize free radical. During this donation the GSH will become highly reactive and reacts with another GSH to form GSSG. GSSG will be reduced to GSH by the enzyme glutathione reductase [29] (**Fig. 1**).

The antioxidant property of glutathione includes detoxification from heavy metals, solvents, pesticides etc. and transforms them into a form which can be excreted along with urine. It is also involved in counteracting effects of free radicals in the body by oxidation. Dietary glutathione intake from fruit and raw vegetables has been associated with protection against some forms of cancer. Glutathione has also inhibited cancer in test tube and animal studies. In preliminary research, higher glutathione levels have also been associated with good health in older adults. Levels of free radicals were controlled by glutathione in the isolated *Colu-6* lung cells, which might help in reducing the growth of cancer cells [30].

Coenzyme Q10: Coenzyme Q10 is a natural compound found in the mitochondria of the cell involved in the manufacture of ATP, a major source of cell's energy which drives a number of biological processes including muscle contraction and the synthesis of proteins [31]. Coenzyme Q performs two major roles (i) carrier of electrons from respiratory complexes I and II to complex III, and (ii) anti-oxidant quenching of free radicals [32]. Coenzyme Q10 protects the body against free radical invasion and has been used both as preventative and treatment aid for many organ disorders [33]. Moreover, CoQ10 has been reported to enhance athletic performance in sports such as skiing and swimming [34].

7. α- lypoic acid: Alpha (α- lypoic acid is synthesized by almost all types of tissues. α- Lypoic acid is capable of solublilizing in both water and fats, hence can work throughout the body [35]. α- lypoic acid converts glucose into free energy. It is also involved in the regeneration of depleted antioxidants after free radical attack[36], lowering of blood sugar levels, reducing pain, burning, itching, tingling, and numbness in people who have nerve damage caused by diabetes called peripheral neuropathy [35].

Lutein and Lycopene: Lutein and lycopene belongs to the carotenoid family. Carotenoids are the powerful antioxidants that quench free radicals especially singlet oxygen, which is produced during exposure to ultraviolet light, which is the primary cause of skin aging[37]. The biological mechanisms for the protective effects of lycopene including modulation of functions and antioxidant properties are only partially known. Recent studies indicate that a regular intake of lutein is associated with a reduced risk of coronary heart disease and certain types of cancer [38].

Lycopene is found in red fruits. Lycopene controls lung, stomach and prostate cancers by activating special cancer preventive enzymes such as phase II detoxification enzymes, which remove harmful carcinogens [39]. Presence of lycopene in lung tissues protects lymphocytes from NO_2 damage [40].

Flavonoids: Flavonoids are phenolic substances which have been isolated from wide range of vascular plants. These compounds are ubiquitous in nature and are categorized, according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. So far, 8,000 flavonoids have been identified, many of which occur in fruits, vegetables and beverages. Flavonoids play a wide variety of roles in protecting human health as antiviral, anti- allergic, antiplatelet, anti- inflammatory, antitumor, antioxidants, antimicrobials, photo-receptors, visual attractors, feeding repellants, and for light screening [41].

However, most interest has been devoted to the antioxidant activity of flavonoids, due to their ability to reduce free radical formation and to scavenge free radicals. The capacity of flavonoids to act as antioxidants in vitro has been the subject of several studies in the past years, and important structure-activity relationships of the antioxidant activity have been established.

Selenium and Zinc: Selenium is a non-metal which exists in multiple oxidation states (+2, +4, +6). Within biological systems this element is a constituent of the amino acids that compromise proteins and an essential component of the glutathione peroxidase enzyme system with a significant function of protecting the cell from the oxidative stress and free radical formation. Selenium can be considered the "rate-limiting" substrate in the GSH-GSSG oxido-reduction [42] whose deficiency will affect the synthesis of peroxidase enzyme affecting the antioxidant protection by severely reducing the GSH-GSSG levels.

Zinc is the central component of more than 1,000 proteins including DNA-binding proteins with zinc fingers, copper/zinc superoxide dismutase (Cu/ZnSOD) and several proteins involved in DNA-damage repair such as p53, which is mutated in half of human tumors [43]. Insufficient zinc intake can impair antioxidant defenses and compromise DNA-repair mechanisms, making the cell highly susceptible to oxidative DNA damage.

Thus, deficits in zinc intake could have a significant impact on the development of cancer [44]. The list of various antioxidants in controlling human health has been displayed in **Fig.2** with their specific functions.

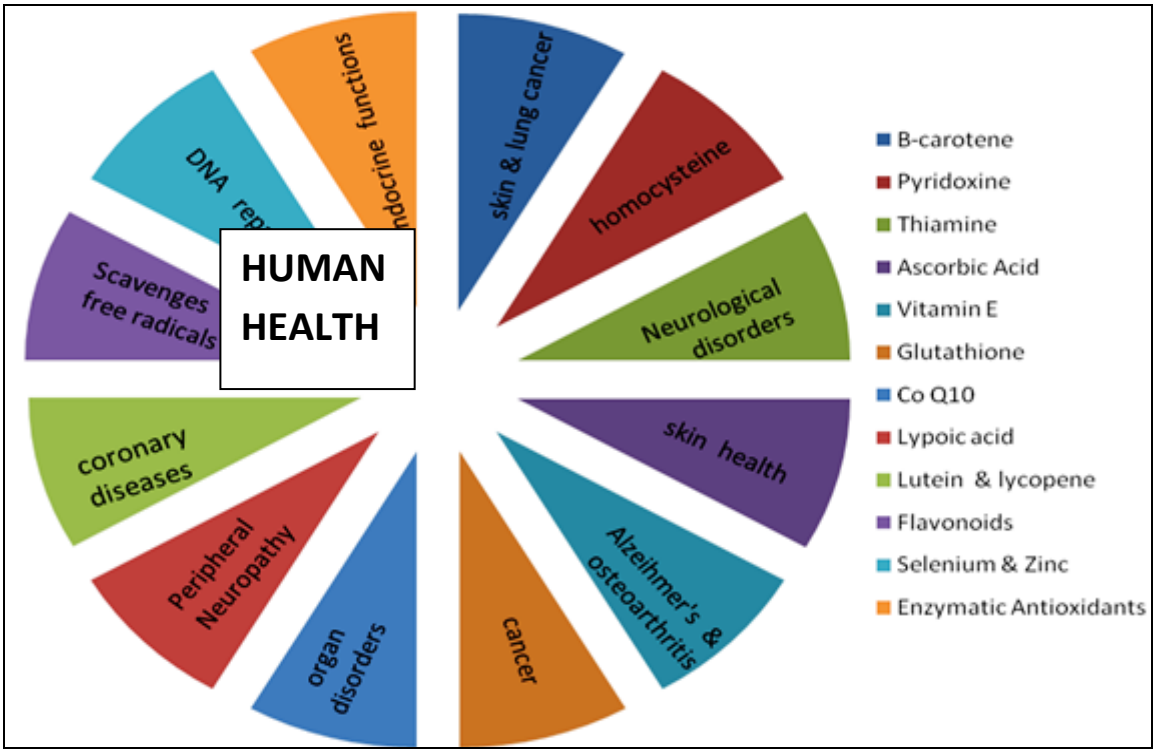

FIG. 2: ROLE OF NON-ENZYMATIC ANTIOXIDANTS IN DEFINING HUMAN HEALTH

Enzymatic antioxidants: Free radicals play a pivotal role in the cell growth, differentiation, progression and death. At low concentrations, free radicals provide certain benefits such as intracellular signaling and defense. Increased concentrations of free radicals enhances aging process as well as a number of human disease states, including cancer, ischemia, and failures in immunity and endocrine functions [45]. As a safeguard, several non-enzymatic (**Fig. 2**) and enzymatic (**Fig. 3**) antioxidant activities exist whose coordinated action will protect the cells from the lethal affects of the free radicals. When there is an increase in the free radical levels in the body, a defense system promotes the regulation and expression of these enzymes.

Superoxide dismutase (SOD) is a very important enzyme that functions as a cellular anti-oxidant. It is present as isoenzymes in different organelles as copper-zinc SOD in cytoplasm, as manganese SOD in mitochondria in order to maintain a low concentration of superoxide anion [3]. There is also an extracellular form of superoxide dismutase in plasma, lymph and synovial fluid that is different from the intracellular forms of the enzyme. The extracellular enzyme may function at cell surfaces. SOD catalyzes the dismutation of superoxide anion and the absence of this enzyme is lethal. The amount of superoxide dismutase is controlled by specific redox-sensitive genes in cells [46].

Catalase, a heme containing protein that catalyzes the reaction in which hydrogen peroxide is detoxified. Catalase is a cytoplasmic enzyme, usually found in peroxisomes of the cells and is expressed in all types of cells except erythrocytes as they do not contain the peroxisomes. Catalase provides a protective role that is similar to that of glutathione peroxidase because both are important means of removing hydrogen peroxide [47].

Both catalase and glutathione peroxidase are important in hydrogen peroxide detoxification. Glutathione peroxidase is a cytoplasmic and mitochondrial enzyme, important for detoxifying H_2O_2 in almost all the cells. It is a seleno protein, which contains a seleno-cysteine amino acid at the active site instead of a normal cysteine [48]. The selenium that replaces the normal sulfur in this amino acid has enhanced nucleophillic properties and ionizes more readily to release a proton. It is a much more effective catalyst in the reaction catalyzed by this enzyme.

The flavoprotein, glutathione reductase uses the reducing power for the pentose phosphate pathway (NADPH) to keep the glutathione pool in cell in a highly reduced state. Even when large amounts of hydrogen peroxide are present this enzyme is very effective at reducing the cellular glutathione pool. The net result of this cycle is to use NADPH to reduce hydrogen peroxide to water, a process that requires two electrons [49]. Other reductases can also catalyze reactions that reduce lipid peroxides, instead of hydrogen peroxide (**Fig.3**).

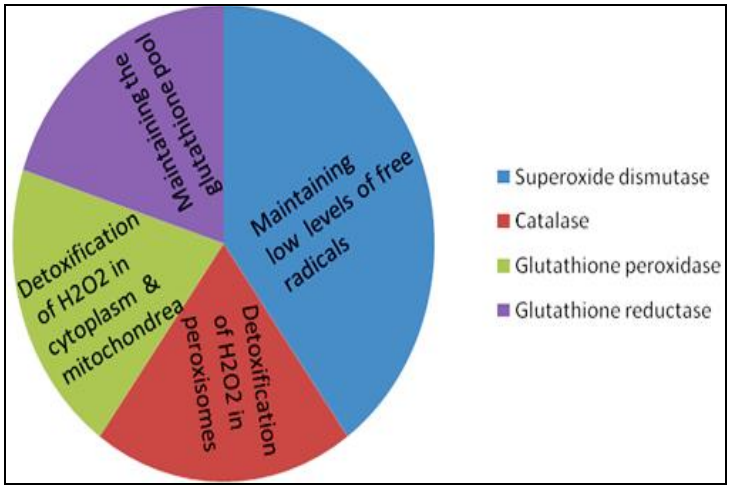

FIGURE 3: FUNCTIONS OF FOUR DIFFERENT ANTIOXIDANT ENZYMES IN DIFFERENT CELL ORGENELLE

CONCLUSIONS: Enzymatic and non-enzymatic antioxidants counteract the toxic effects of free radicals in human body, which is being supplemented in physiological, biochemical mechanisms. An appropriate balance of enzymatic and non-enzymatic antioxidant defense is necessary for withstanding the destruction caused by the active oxygen species and maintaining the human health. The present work complements similarity with other prokaryotic and eukaryotic systems where the possibility of over-expressing of the genes encoding these enzymes and understanding the critical roles of these mechanisms is much more. Such studies will open new doors in pharmacy and drug designing studies to identify new drug targets for successful amelioration of free radicals.

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