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REVIEW ARTICLE

The Role of Antioxidant to Prevent Free Radicals in The Body

Siti Thomas Zulaikhah
Department of Public Health Faculty of Medicine Sultan Agung Islamic University (UNISSULA) Semarang
Jl. Raya Kaligawe KM. 4 PO BOX 1054 Semarang Central Java Indonesia
Phone (+6224) 65833584, Fax (+6224) 6594366. Hp. 081390277161
*Correspondence: Email: thomaszulaikhah@gmail.com/sitithomas@unissula.ac.id

ABSTRAK
Antioksidan adalah senyawa yang mampu menangkal atau meredam dampak negatif oksidan dalam tubuh. Antioksidan bekerja dengan cara mendonorkan satu elektronnya kepada senyawa yang bersifat oksidan sehingga aktivitas senyawa oksidan tersebut dapat dihambat. Antioksidan dikelompokkan menjadi 2 yaitu antioksidan enzim dan non-enzim. Antioksidan enzim misalnya enzim superoksida dismutase (SOD), katalase (CAT), dan glutation peroksidase (GPx), Antioksidan non-enzim banyak ditemukan dalam sayuran dan buah-buahan, yang meliputi glutation tereduksi (GSH), vitamin C, E, β-karoten, flavonoid, isoflavon, flavon, antosianin, katekin, dan isokatekin, serta asam lipoat. Rendahnya antioksidan enzim dapat digunakan sebagai petanda tingginya kadar radikal bebas dalam tubuh. Review berikut bertujuan untuk memberikan gambaran tentang peran antioksidan dalam mencegah terbentuknya radikal bebas dalam tubuh.

Kata Kunci: Antioksidan, radikal bebas, superoksida dismutase (SOD), katalase (CAT), glutation peroksidase (GPx)

ABSTRACT
Antioxidants are compounds capable of counteracting or reducing the negative effects of oxidants in the body. Antioxidants work by donating an electron to an oxidant compound so that the activity of the oxidant compound can be inhibited. Antioxidants are grouped into two categories, namelu enzyme and non-enzyme antioxidants. Antioxidant enzymes such as superoxide dismutase (SOD) enzyme, catalase (CAT), and glutathione peroxidase (GPx), Non-enzyme antioxidants are found in vegetables and fruits, including reduced glutathione (GSH), vitamin C, E, β-Carotene, flavonoids, isoflavones, flavones, anthocyanins, catechins, and isocatechins, as well as lipoic acid. Low antioxidant enzymes can be used as a marker of high levels of free radicals in the body. The following review aims to provide an overview of the role of antioxidants in preventing the formation of free radicals in the body.

Keywords: *Antioxidants, free radicals, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)*

INTRODUCTION

Oxidative stress can occur as the amount of ROS in the body exceeds the amount of antioxidants, where the excess will attack the lipid, protein, and DNA components (Lobo, 2010; Halliwell and Gutterigde, 2015; Rani, 2015). Low levels of antioxidant enzymes can be used as a sign that free radical levels in the body are high. The harmful effect of free radicals in the body is the release of ROS (Durak, 2010), the emerging of ROS action is caused by the low antioxidant system so that its activity in preventing ROS becomes less effective. The low antioxidant defense system is due to the reduction of thiol groups in enzyme antioxidant proteins. These conditions led to a decline in the activity of antioxidant enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT), resulting in the depletion of the levels of reduced glutathione (GSH) and the build up of H_2O_2, which finally causes oxidative stress (Valko, 2007, Halliwell and Gutteridge, 2015).

Free radicals in biological systems of the body that most of the radicals derived from oxygen, and is known as ROS (Reactive oxygen species). Compounds in the form of radicals such as hydroxyl radicals (•OH) radicals peroksil (• OOH), and superoxide ion (O_2-•) and compound non-radicals such as singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hypochlorite ion (ClO-), in which free radicals are harmful, but can be very dangerous with the supporting factors of the environment (Halliwell and Gutteridge, 2015).

The high levels of ROS in the body can be seen in the low antioxidant activity of SOD, CAT, and GPx enzymes, as well as vitamin C, E, and β-carotene levels. In conditions where levels of endogenous antioxidants decrease, more exogenous antioxidants are needed in order to eliminate ROS (Winarsi, 2007; Astuti, 2008). Glutathione peroxidase (GPx) serves as a catalyst to decompose H_2O_2 into oxidized glutathione (GSSH) by using glutathione (GSH) as substrate (Murray, 2009). The following review aims to provide an overview of

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the role of antioxidants in preventing the formation of free radicals in the body.

Antioxidants

A. Classification of Antioxidants

1. Enzymatic Antioxidants

Enzymatic antioxidants are endogenous antioxidants, including superoxide dismutase (SOD) enzymes, catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR). These enzymes work by protecting tissues from oxidative damage caused by free radicals of oxygen such as superoxide anion (O_2•-), hydroxyl radical (OH•) radical peroxyl (ROO•) and hydrogen peroxide (H_2O_2) (Halliwell and Gutteridge, 2015).

a. Superoxide Dismutase (SOD)

Superoxide dismutase (SOD) was first isolated by Mann and Kleilin in 1938. This enzyme is known as a protein containing Cu, and is identified in various titles, such as erythrocuprin, indofenol oxidase, and tetrazolium oxidase (Winarsi, 2007). SOD enzyme functioning as a catalyst dismutase reaction of superoxide anions to hydrogen peroxide (H_2O_2) and oxygen (O_2) (Rani, 2015).

$$2\ O_2\bullet^- + {}_2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

This enzyme already exists in the body, but requires the help of mineral nutrients such as manganese (Mn), zinc (Zn), and copper (Cu) in order to work. SOD enzyme activity has an important role in the body's defense system, especially the activity of reactive oxygen compounds that can cause oxidative stress (Winarsi, 2007). Based on the presence of metal acting as a cofactor on the active side of the enzyme, the SOD enzyme can be grouped into 3, Fe-SOD, Cu/Zn-SOD, and Mn-SOD.

i. Fe-SOD

The Fe-SOD group is the first known SOD type. This is indicated by the presence of Fe as a metal cofactor in the active site, which is identified as the Fe in the form of dissolved Fe +++ in excessive amounts. Availability of O_2 in excessive amounts can cause mineral Fe oxidized, but if the availability of Fe ++ decrease, will increase the use of metal Mn +++. Isoenzyme Fe-SOD is found in prokaryotes and eukaryotes (Winarsi, 2007; Lobo, 2010).

ii. Cu/Zn-SOD

Cu/Zn-SOD is also known as SOD_1. This enzyme is a homodimer found in eukaryotic cytoplasm, peroxisomes, chloroplasts and prokaryotic periplasm. This enzyme plays an important role in the defense system against oxidants. One unit of Cu/Zn-SOD is defined as the number of enzymes necessary to inhibit 50% of pigalol autoimidation. In Cu/Zn-SOD, Cu minerals are important for the catalytic functioning of enzymes, whereas Zn is important for structural functions (Winarsi, 2007; Lobo, 2010).

iii. Mn-SOD

Mn-SOD is also known as SOD 2. Increased levels of O_2 environment will reduce the use of Fe ++ due to the increased availability of Mn +++. As a result, Mn-SOD becomes the second enzyme because the amount of Fe-SOD decreases. Mn-SOD is present in mitochondria and peroxisomes. Each Mn-SOD subunit requires only one metal atom. This enzyme can not function in the absence of Mn atoms on the active side. Mn-SOD works by attracting negatively charged radical superoxide (O_2•-) thus turned positive on the active side. Furthermore, the active metal gives one direct electrons to O_2• - thus reducing one molecule of O_2• - and one proton, to be converted into the form of H_2O_2 (Winarsi, 2007; Lobo, 2010).

b. Catalase (CAT)

Catalase is an enzyme-containing homotetramer ferriheme, with Fe as a cofactor that catalyzing hydrogen peroxide (H_2O_2) into water and oxygen (Lobo, 2010; Rani, 2015).

$$2H_2O_2 \xrightarrow{\text{CAT}} 2H_2O + O_2$$

Many researchers reported that catalase enzyme activity can be induced by antioxidant intake. The catalase activity increased when 23 healthy men were intervened with carotenoids derived from tomato juice 330 mL/day, 330 mL of carrot juice, and 10g of spinach flour for 2 weeks (Winarsi, 2007).

c. Glutathione Peroxidase (GPx)

Glutathione Peroxidase (GPx) is an antioxidant enzyme containing selenium (Se) on its active side. This enzyme works on reduced glutathione (GSH) and H_2O_2 to produce oxidized glutathione (GSSH) and H O (Halliwell and Gutteridge, 2015). This enzyme can also reduce lipid peroxidation

in cell membranes. The activity of glutathione peroxidase enzyme capable of reducing the 70% organic peroxidation and more than 90% H_2O_2 (Winarsi, 2007). In order for this enzyme to work, subtrates such as glutathione are always needed, which is the substrate of glutathione peroxidase enzyme (Lobo, 2010; Rani, 2015)

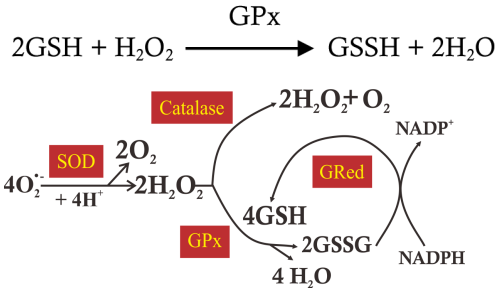

Figure 1. Role of SOD, catalase and GPx in eliminating ROS (Kunwar, 2011)

2. Non-Enzymatic Antioxidants

Non-enzymatic antioxidants are found in vegetables and fruits, which include reduced glutathione (GSH), vitamin C, E, β-carotene, flavonoids, isoflavones, flavones, antosionin, catechins, and isocatechins, and lipoic acid. These phytochemical compounds help protect cells from oxidative damage caused by free radicals (Rani, 2015). The study states that multivitamins and minerals can repair the type hypersensitivity response of the skin delayed type in the 59-85 year-old human (Winarsi, 2007).

Free Radicals

1. Definition

Free radical is a compound or molecule containing one or more unpaired electrons in its outer orbit. The presence of unpaired electrons causes the compound to be highly reactive looking for pairs, by attacking and binding to molecular electrons in the vicinity (Halliwell and Gutteridge, 2015; Rani, 2015; Murray *et al*, 2009)

In living cells, free radicals are formed on the plasma membrane, mitochondria, peroxisomes, endoplasmic reticulum and cytosol through enzymatic reactions that take place in the metabolic process. The body has a protective mechanism that neutralizes free radicals are formed, among others, with the enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). However, under certain circumstances, free radicals may exceed the body's defense system, this condition is referred to as oxidative stress (Halliwell and Gutteridge, 2015).

Damage to cell membranes by free radicals can occur by: (a) covalent bonding occurs between free radicals and membrane components, resulting in structural changes of the receptor function; (B) the oxidation of the thiol group On the membrane components by free radicals that cause the membrane transport process to be disturbed; (c) reaction containing membrane lipid peroxidation of PUFAs (polyunsaturated fatty acids).

Proteins, unsaturated fatty acids and lipoproteins, as well as elements of DNA including carbohydrates are the main targets of free radicals, but the most vulnerable are unsaturated fatty acids (Winarsi, 2007). Free radical attacks against surrounding molecules will lead to chain reactions, which then produce new radicals. The impact of free radical reactivity varies, ranging from cell or tissue damage, autoimmune diseases, degenerative diseases to cancer. High levels of free radicals in the body can be shown by high levels of malondialdehyde (MDA) in plasma and low antioxidant enzyme activity (Winarsi, 2007)

2. Free Radical Sources:

The sources of free radicals in the human body comes from endogenous and exogenous sources (Halliwell and Gutteridge, 2015; Rani, 2015).

a. Endogenous Source

Sources from the process of normal metabolism in the human body, from the body's metabolic process produces more than 90% oxygen through the process of oxidation of food to produce energy in the mitochondria known as the electron transport chain and will produce free radical superoxide anion (O_2-•), Free radicals are typically produced by white blood cells such as neutrophils used in host defense to destroy invading pathogens, the xanthine oxidation process (compounds found in most body tissues and fluids acting as enzymes involved in catalyzing the changes of hypoxanthine to xanthine and so on To the uric acid that produces hydrogen peroxide), reactions involving iron and other metals, excessive exercise with longer and more intensive exercises, more oxygen is consumed, for energy production absolute requires oxygen, but there is some oxygen that Will eventually form free radicals, inflammation, and ischemic.

There are several other free radical endogenous sources, namely:

i. Autoxidation

Autoxidation is the result of aerobic metabolic processes. Catecholamines,

hemoglobin, myoglobin, reduced cytochrome C, and thiols are molecules that undergo autoxidation. The autocidation of the above molecule results in a reduction of the radical oxygen and the formation of the oxygen reactive group. Superoxide is a radical initial formation. Ferrous ions may also lose its electrons through oxygen to make superoxide and Fe $^{3+\text{ ions}}$ through autoxidation process (Suryohudoyo, 2007).

ii. Enzymatic Oxidation

Type of the enzyme system capable of generating free radicals in a substantial number, including xanthine oxidase (activated in ischemiareperfusion), prostaglandin synthase, lipoxyangenase, aldehyde oxidase and amino acid oxidase. The enzyme myeloperoxidase neutrophil activation results, utilizing hydrogen peroxide to oxidize chloride ions into a strong oxidant hipochloric acid (Suryohudoyo, 2007).

iii. Respiratory burst

It is an illustration of the process by which phagocytic cells use large amounts of oxygen during phagocytosis. Approximately 70-90% of the use of oxygen can be accounted for in superoxide production. The phagocytic cells have membrane bound flavoproteins system cytochrome-b-245 NADPH oxidase. Cell membrane enzymes such as NADPH-oxidase come out in the inactive form. Exposure to immunoglobulin-bound bacteria, immune complexes, complement 5a, or leukotriene may activate the NADPH-oxidase enzyme. The respiratory burst activation initiated at the cell membrane to produce superoxide. Then H_2O is formed of superoxide by way dismutasi together the next generation of OH and HOCl by bacteria (Halliwell and Gutteridge, 2015).

b. Exogenous Source

Exogenous free radical sources include environmental pollution, drugs, radiation, cigarette smoke, ozone depletion, chemicals, toxins, alcohols, pathological microorganisms, anaesthetics and pesticides and solvents used for industry.

i. Drugs

There are several drugs that can increase the production of free radicals in the form of increased oxygen pressure. These materials react together when hyperoxide that can accelerate the rate of damage, such as antibiotics or bonded metal quinoid groups for activities (nitrofurantoin), cancer drugs such as bleomycin, anthracyclines (Adriamycin), and methotrexate, which have a pro-oxidant activity. Radicals may also be derived from phenylbutasone, some phenolic acids and aminosalicylic components of sulfasalacin may inactivate proteases and accelerate lipid peroxidation (Lobo, 2010)

ii. Radiation

Radiotherapy allows the occurrence of tissue damage caused by free radicals. Electromagnetic radiation (X rays, gamma rays) and particle radiation (electrons, photons, neutrons, alpha and beta particles) produce primary radicals by transferring their energy to cellular components such as water. Such primary radicals may undergo secondary reactions with oxygen decomposed or together with cellular fluid (Suryohudoyo, 2007).

iii. Cigarette smoke

Cigarettes contain oxidants 10^{7} molecules per rod. Free radicals from cigarette smoke cause peroxidation of unsaturated double fatty acids of cell membranes that strengthen oxidative stress during smoking. Exposure to oxidant chemicals in tobacco smoke is associated with decreased endogenous antioxidants, smoking can lead to low levels of antioxidants in plasma and wastage of vitamin C by up to 30% (Winarsi, 2007).

3. Types of Free Radicals

Free radicals in biological systems of the body that most of the radicals derived from oxygen, and is known as ROS (Reactive oxygen species). Compounds in the form of radicals such as hydroxyl radicals (• OH) radicals peroksil (• OOH), and superoxide ion (O_2 - •) and compound non-radicals such as singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hypochlorite ion (ClO -), in which free radicals are harmful, but can be very dangerous in the presence of environmental factors around the support.

ROS derived from oxygen (O_2), a compound that is required by all aerobic organisms including humans. Aerobic organisms require oxygen to produce ATP, a compound that is a source of energy for most living things, through oxidative phosphorylation occurring in the mitochondria. The process can simply be described as follows:

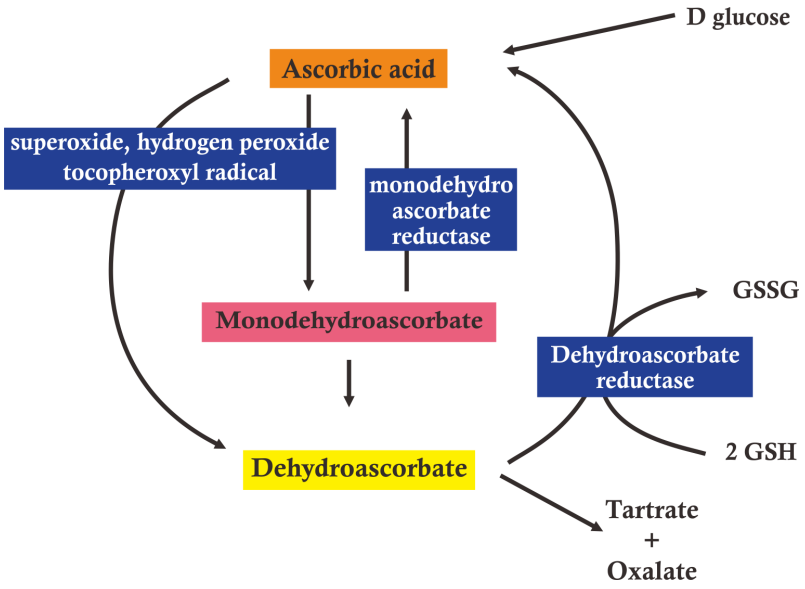

Figure 2. The mechanism of action of vitamin C as an antioxidant (Winarsi, 2007).

$$NADH + H^+ + O_2 \rightarrow NAD^+ + H_2O + energi$$
$$ADP + P + energi \rightarrow ATP$$

In the process above, reaction occurs in the reduction of O_2 to H_2O that can simply be written as follows:

$$O_2 + 4H^+ + 4e^- \rightarrow H_2O$$

From the equation above is easily seen that the reduction of oxygen to H_2O is a transfer of 4 (four) electrons (4 electron transfer) (Halliwell and Gutteridge, 2015). In certain circumstances the transfer of electrons is running less than perfect so that the reactive oxygen compounds are very dangerous, which will damage the cell if not muted. Such a thing happens in conditions called oxidative stress (Winarsi, 2007; Suryohudoyo, 2007)

DISCUSSION

1. Mechanism of action of vitamin C as an antioxidant

Vitamin C or L-ascorbic acid is a water-soluble antioxidant. This vitamin has a role as an electron donor, able to neutralize and reduce ROS so that cells are protected from free radical damage (Siswanto, 2013; Winarsi, 2007). ROS can be directly captured by ascorbate, either with or without enzyme catalyst. As an antioxidant, ascorbate can directly react with superoxide radicals, hydrogen peroxide, or tocopherol radicals to form monodehydroascorbic acid and or dehydroascorbic acid. The reduced form can be converted back to ascorbic acid by the enzyme monodehidroascorbat reductase and dehydroascorbate reductase, which is equivalent to NADPH or reduced glutathione (GSH). The ascorbate reaction with superoxide is physiologically similar to that of the SOD enzyme, namely:

$$2O_2^- + 2H^+ + ascorbic \rightarrow 2H_2O_2 + dehydroascorbate$$

Reaction with H_2O_2 catalyzed by the enzyme ascorbate peroxidase:

$$H_2O_2 + 2\ ascorbate \rightarrow 2H_2O + 2\ monodehydroascorbate$$

Indirect ascorbate can reduce its activity by turning tocopherol into reduced form. Ascorbate regenerates membrane antioxidant bonds such as α-tocopherol by capturing peroxyl and singlet oxygen. Vitamin C works synergistically with vitamin E. Vitamin E is oxidized by free radicals can react with vitamin C, after getting hydrogen ions from vitamin C then will turn into tocopherol (Winarsi, 2007).

Tocopheroxyl radical + ascorbic→tocopherol + monodehydroascorbate

Monodehydroascorbate spontaneously may undergo dismutation as follows:

2 Monodehydroascorbate →ascorbic + dehydroascorbate

Dehydroascorbate will break easily into tartaric and oxalate because this compound is unstable at pH above 6. To prevent this from happening, the dehydroacorbate reductase is rapidly reduced by dehydroascorbate reductase to ascorbate involving glutathione (GSH), such as the following reaction:

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2 GSH + dehydroascorbic → GSSH + ascorbate

The role of vitamin C works in synergy with vitamin E in inhibiting lipid peroxidation process. Vitamin E is oxidized by free radicals can react with vitamin C, after obtaining the hydrogen ion of vitamin C will be transformed into vitamin E. Vitamin E in the membrane to react with lipid radicals (LOO •) radicals form of vitamin E (vit. E •). Radical vitam E reacts with vitamin C to form free radicals vitamin C (vit. C •). Radical vitamin C (vit. C •) will regenerate into vitamin C, involving glutathione (GSH). GSH will be oxidized to oxidized glutathione (GSSH) by glutathione peroxidase (GPx) enzyme, GSSH will be reduced to GSH by glutathione reductant (GRed) enzyme by involving NADPH as an electron donor (Halliwell and Gutteridge, 2015; Rani, 2015). Supplementation of vitamin C and E may increase cytokine production. Administration of 1g of vitamin C and 400mg of vitamin E and combinations for 28 days increased the production of IL-1β by 1,8 times and TNF-α by 1.5 times (Winarsi, 2007).

2. The role of Mineral to the antioxidant enzyme superoxide dismutase (SOD)

   The effectiveness of SOD enzymes can be improved by the presence of mineral nutrients such as copper (Cu) and zinc (Zn) and manganese (Mn) (Winarsi, 2007). Mineral deficiency of Cu, Zn and Mn can decrease the activity of Cu-Zn SOD and Mn-SOD and may cause lipid peroxide (Halliwell and Gutteridge, 2015).

3. The role of selenium to the enzyme glutathione peroxidase (GPx).

   Selenium is an essential mineral that is essential for human health, plays an important role in protein synthesis and glutathione peroxidase (GPx) enzyme activity. Selenium in the enzyme glutathione peroxidase has a role as a catalyst in the breakdown of H_2O_2 which is formed in the body into compounds that are inactive so as not to damage the phospholipids are numerous in the cell membrane, this condition can prevent lipid peroxidation. Selenium deficiency can decrease GPx activity by up to 90% resulting in lipid peroxidation (Winarsi, 2007)

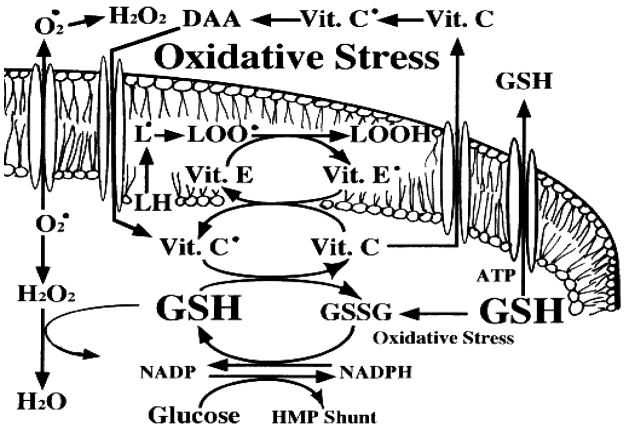

Figure 3. The role of vitamin C and E in inhibiting lipid peroxidation due to free radicals (Inoue M. 2001)

CONCLUSION

High levels of free radicals in the body can be shown by low antioxidants, when the amount of free radicals exceeds the amount of antioxidants in the body, the excess will attack the lipid, protein or DNA components causing oxidative stress. The status of antioxidants in the body can be observed in various parameters, for example: SOD, CAT, GPx, Vitamin C, vitamin E and others. SOD catalyzed dismutase reaction of superoxide anion radicals into H_2O_2, whereas CAT and GPx change the H_2O_2 to H_2O. Perfection of antioxidant enzyme system is fully played by three enzymes SOD, CAT, and GPx, but cellular antioxidants can not work individually without the support of secondary antioxidant intake from food. So

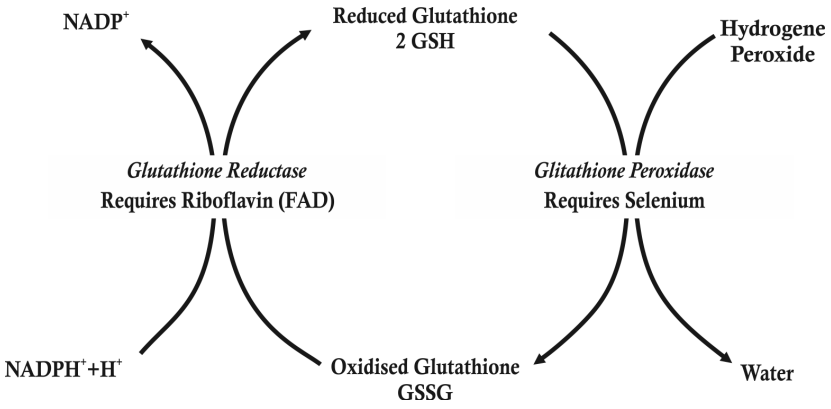

Figure 4. The role of selenium against the enzyme glutathione peroxidase (Fang, 2002)

it takes the consumption of foods rich in antioxidant components in sufficient quantities, in order to be able to induce the work of antioxidant enzymes in the body so as to maintain cellular antioxidants and suppress excessive cell damage due to free radicals.

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