

# THE ROLE OF ANTIOXIDANTS AND OXIDATIVE STRESS IN NEURODEGENERATIVE DISEASES AND AGING

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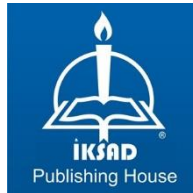
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## **CONTENTS**

### **EDITED BY**

### **PREFACE**

Assist. Prof. Dr. Tuba SEVİMOĞLU.....1

### **CHAPTER 1**

#### **RELATIONSHIP BETWEEN FREE RADICALS AND OXIDATIVE STRESS**

Assoc. Prof. Dr. Emel SERDAROĞLU KAŞIKÇI

Msc. Nazlı Melis MİSYAĞCI.....3

### **CHAPTER 2**

#### **ANTIOXIDANTS**

Prof. Dr. Sevil YÜCEL

Assoc. Prof. Dr. Vildan ENİSOĞLU ATALAY

Res. Assist. Yeşim AYIK.....33

### **CHAPTER 3**

#### **COMPUTATIONAL CHEMISTRY FOR ANTIOXIDANTS**

Prof. Dr. Sevil YÜCEL

Assoc. Prof. Dr. Vildan ENİSOĞLU ATALAY

Res. Assist. Yeşim AYIK.....65

### **CHAPTER 4**

#### **THE ASSOCIATION OF NEURODEGENERATIVE DISEASES WITH OXIDATIVE STRESS AND THE ROLE OF ANTIOXIDANTS**

Assist. Prof. Dr. Tuba SEVİMOĞLU

Mehveş KOKŞA.....87

### **CHAPTER 5**

#### **AGING**

Assist. Prof. Dr. Arzu TEMİZYÜREK

DVM Burcu ÇEVRELİ.....145



## **PREFACE**

Antioxidants have been a very popular topic in both print and visual media in recent years. Numerous scientific articles are published each year on these compounds and their effects. In addition to all these, the media and some communication channels state that many plant species have strong antioxidant properties and can be used in the treatment of diseases without their knowledge. The main reason for the writing of the book is to clarify the confusion to a certain extent and to constitute a resource especially on the neurodegenerative disorders and the free radical/antioxidant relationship.

The historical process of Computational Chemistry, which is basically the application of mathematical principles to solve chemical problems, dates back to the 1920s and getting more attention every day in parallel with the advances in technology. As a result, the number of computational chemistry programs, researchers interested in this subject and publications in this discipline is increasing day by day. There are many publications in the literature on the activities and functions of antioxidants molecules, which are seen as the key to a healthy life. Experimental measurement of antioxidant activity can be done with many different approaches. In this book, it is aimed to create a resource that explains the theoretical background of the calculation methods, how the antioxidant activity can be calculated theoretically and how the oxidation reaction mechanisms are modeled through equations. Our aim is to contribute to the theoretical identification of new candidate molecules with high antioxidant potential, to reduce the

number of experimental steps that will follow, and to reach new products much faster.

This book also explores the possibility of the usage of antioxidants in neurodegenerative illnesses such as AD, PD, and ALS. In these diseases oxidative insults may have the possibility of initiating various signal chains leading to apoptotic cell death. Recent findings have made it possible for several types of antioxidants to be effective by protecting cells from oxidative damage. However, further research is essential to fully explore the potential of these compounds in preventing the development of neurodegenerative disorders or providing treatment.

Aging is a wide field and it is connected with every subject. Contrary to the programmed nature of development, we tried to summarize in this book where we tried to find answers to the questions of whether aging is reversible, its relationship with cellular aging and how it is affected.

The overall aim of this book is to get out more information about free radicals formed in living organisms and their elimination by antioxidants. At the same time, this book will serve as a useful resource for undergraduate and postgraduate students.

## CHAPTER 1

### RELATIONSHIP BETWEEN FREE RADICALS AND OXIDATIVE STRESS

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## INTRODUCTION

Oxidative stress (OS) is a situation originated by the abnormal changing among oxidants as well as an antioxidant in a biological structure. The imbalance happens cause of the too much level of reactive oxygen species (ROS) either the inappropriate process of the antioxidant system [1]. The vital function of oxygen ( $O_2$ ) in the biological system is incontrovertible. The important cause is that it's suitable for the function of the cell as well as the life of whole living creatures. Even though  $O_2$  is important to sustain life and also takes part in cell signaling and for gene initiation, elongation, and termination as well as many other cellular functions. Furthermore, it has a destroying impact on biomolecules in the structure of free radical as well as reactive oxygen species which is also called ROS. Because of the univalent metabolic reduction situation, the antagonistic effect of  $O_2$  has appeared. This process is closely related to the formation of ROS.

In addition to ROS, nitric oxide (NO) is an important component of the process. It involves vital events in the system. It takes part in the regulation of the muscle cell, attachment of the leukocytes (WBC) which is also called adhesion, formation of the blood vessels, clustering of the platelets (PLT), blocking of the blood vessels, vascular strength as well as hemodynamics [2].  $NO\bullet$  in its free configuration is very catastrophic for biological molecules. Many chemical groups consisting of free radicals, including  $O_2$ . Hydroxyl radical ( $HO\bullet$ ), superoxide radical anion ( $\bullet O_2^-$ ), hydroperoxyl radical ( $HO_2\bullet$ ), and peroxy radicals ( $ROO\bullet$ ) are important examples for that

ROS represent  $O_2$  radicals and also non-radical which turned into radicals like  $H_2O_2$ ,  $^1O_2$ , and so on [3-6]. According to Halliwell's study, free radicals contain a single or more than one individual electron ( $e^-$ ) and having separately. [7,8].

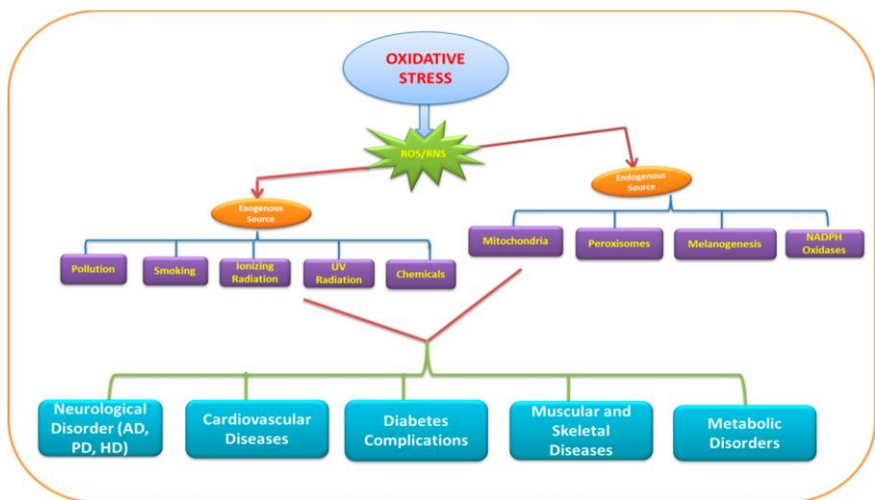
During the biologic transformation and also different biochemical processes, free radicals involve several important functions.  $O_2$  is the most critical as well as a significant component for whole creatures containing neurons which are essential components of the neurologic system played role in the composition of the tissue (cell, fibers, matrix, and also another important part of the tissue layers) when it is too much, it has destructive impacts on the system. As a result, it's needed for the utilization, as well as taking off the  $O_2$  and additional vital components, to be saved in the maximum protection along with controlled through the complicated process in the cell.

In the mitochondria,  $O_2$  takes part in important functions. For example the formation of the adenosine triphosphate (ATP) by phosphorylation of adenosine diphosphate (ADP), including the break up into glucose as well as the formation of the ATP in the mitochondrial environment. The mitochondrial DNA is responsible for completing the amino acid that is important for oxidative phosphorylation [9,10]. When there is a mutation in mitochondria obliquely, it's observed that protein formation became restricted.

$O_2$  and also different free radicals, made internally (Nicotinamide adenine dinucleotide phosphate-NADPH oxidase, mitochondria, and so on) and externally (chemicals, radiation, and so on), are continually

accrued inside of the cell [11].

The protection system structure in the pattern of antioxidant molecules as well as emerged to cope with the effect of the reactive O<sub>2</sub> group. Many different antioxidants, for example, glutathione, taurine(Thr) also known as 2-aminoethanesulfonic acid, creatine (Cr), zinc (Zn), vitamin E, C, and also A, and some polyphenols from some herbals for example extraction of the tea is formed against the ROS. The impact of the antioxidant is boost and assisted by some of the proteins. For example, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and so on[11–13].



**Figure 1.** Image belongs to internal as well as external components of ROS and RNS and its related diseases. PD stands for Parkinson’s disease, AD stands for Alzheimer’s disease and lasty HD is for Huntington’s disease [14].

## 1. Reactive Oxygen Species (ROS)

Since the mid-1970s, it's obvious that some studies entirely indicate free radicals. After that, it has been apparent for free radicals and nonradical substances, for example,  $\text{H}_2\text{O}_2$  or its also called hypochlorous acid ( $\text{HOCl}$ ), which has important functions and also very effective oxidizing components, take place in mechanism of the free radical reactions. When it considered whole agents, the accepted caption "ROS" was made known [15]. In addition, species including nitrogen, like nitric oxide ( $\text{NO}\cdot$ ) and peroxynitrite ( $\text{ONOO}^-$ ), were appeared to be vital biologic molecules as well as called RNS [16].

These perceptions prove that ROS were produced in the organic environment was also sustained beyond to cellular components of such class. Firstly, Jensen (1960) shown with the help of bovine heart and submitochondrial particle, mitochondrial hydrogen peroxide production and also antimycin-insensitive oxidation of NADH and succinate has been linked with hydrogen peroxide formation [17]. Later, it was shown that in the presence of oxygen, hydrogen peroxide was produced by animal cardiac system (pigeon heart) mitochondria with the succinate [18]. The finding that mitochondria have got superoxide dismutase (SOD), MnSOD [19] among with the consecutive detection of the mitochondrial production of superoxide radical anion [20] proved that hydrogen peroxide produced inside mitochondria appeared in distinction to dismutation of superoxide ( $\text{O}_2^{\cdot-}$ ) and the biological importance of mitochondrial  $\text{O}_2^{\cdot-}$  generation. Therefore, enormous studies have built up the background and consequences of a

mitochondrial generation of ROS. In addition, the finding by researchers that electron transfer along the inner mitochondrial membrane transports is associated with the formation of ROS has suggested mitochondrial involvement in many diseases whose treatment is complex and chronic, as well as in degenerative mechanisms associated with the cellular aging process.

Mitochondria are very important and valuable for the continuous production and circulation of ROS. While this is the case, this process does not constitute a single resource either for the organism or cellularly. Looking closely at the historical process, researchers in the 70s proved that the emergence of active oxygen species can occur as a by-product of other molecular mechanisms in other organelles, such as liver microsomes, which contain a rich enzyme process. To this should be added that NADPH oxidase catalyzes the oxidation of NADPH with oxygen, resulting in  $\text{NAD}^+$  and hydrogen peroxide [21]. Moreover, these research results became proof applied by scientists. It was stated prior with phenobarbital in animals' treatment, increased the rate of hydrogen peroxide generation [17] and that hydrogen peroxide generation was associated with the monooxygenation mechanism for xenobiotics since cytochrome P450 inhibitors affected production [22]. It is also a known fact that the microsome redox chain attached to NADPH forms hydrogen peroxide and superoxide radicals. In addition, the appearance of superoxide has a many effects. It is also important evidence that NADP-specific flavoprotein dehydrogenase (in other words NADPH-cytochrome c reductase) involves the autooxidation

mechanism and dissociation of the oxy complex of cytochrome P450 [17]. In addition, some organelles have a high level of oxidative activity. However, they contain peroxisomes and a very large proportion of catalase (CAT), which is responsible for its task of reducing oxygen to hydrogen peroxide, despite the oxidation of  $\text{RH}_2$ , which also acts as a substrate. The main task of catalase is to break down hydrogen peroxide to produce water [23]. In the early 1960s, Iyer et al. [24] discovered that bombardment of phagocyte respiration causes the formation of hydrogen peroxide. Later, in 1964, Rossi and Zatti [25] stated that an NADPH oxidase was primarily responsible for the respiratory burst.

Also, in 1973 Babior et al. [26] showed with their research that the first reaction product of respiratory burst oxidase was not  $\text{H}_2\text{O}_2$  but superoxide. The phagocyte NADPH oxidase does not release ROS as a by-product in the reaction. Instead, it is very important as it is the first clear example of a system that is presented as the main task of the enzyme mechanism system.

A multitude of soluble cellular elements containing molecular structures such as the group of thiols, hydroquinones, catecholamines, and flavins are designed to participate in redox reactions and also to generate ROS within a cell [27]. To these, it is necessary to analyze the cytosolic of a very different nature, which generates ROS through the catalytic reaction. Some researchers who are interested in xanthine oxidase (XOR) also explained that it is one of the proteins in ROS.

Researchers have been studying the structural and chemical properties and interactions of XOR which is one of the most important

flavoenzymes for many years [17]. One of the vital tasks of XOR is to catalyze the oxidation of hypoxanthine to xanthine and ultimately xanthine to uric acid using NADP<sup>+</sup> or oxygen in reactions as electron acceptors. Enzymes of organisms are found under optimal conditions, and xanthine dehydrogenase (XDH) is structurally dependent on NAD<sup>+</sup> in freshly prepared samples from organs. Most importantly, they show high xanthine/NAD<sup>+</sup> reductase activity even in the presence of oxygen [28]. In oxidatively degraded textural structures, XDH is converted to xanthine oxidase (XO) [28], which transports electrons to molecular oxygen that makes superoxide when displayed on its substrates due to proteolysis or oxidation of thiol groups of cysteine [29].

Several years later, it has been suggested that xanthine oxidase-derived oxidants induce degeneration of the capillaries, arterioles, and venules associated with reperfusion of the oxygen-deprived intestine [30]. As a result of these findings, the pathophysiological process continues in many complex organs and systems. [31].

## **2. Reactive Nitrogen Species (RNS)**

So far, only oxygen and oxygen-derived molecular structures have been focused on. In addition to all this, there are suitable radicals, nitric oxide, NO<sup>·</sup>, and also derivatives. First, Joseph Priestley discovered nitric oxide, a simple molecule consisting of only a single atom of oxygen and nitrogen. After this discovery, NO<sup>·</sup> was immediately recognized as an atmosphere-polluting molecule. Along with one of the other free radicals, nitrogen dioxide (NO<sub>2</sub><sup>·</sup>), it is presented as a whole



in a high-temperature combustion environment, such as that which occurs in the engines of cars. It is then oxidized with nitrogen dioxide, which creates ozone incredibly quickly with the oxygen present in the environment. Unlike nitric oxide, the toxicity of nitrogen dioxide is very destructive and significant, but rather limited. Nitrogen dioxide has a primary function in a photochemical smoke generation. The reason for this can be explained as follows; Ozone is primarily responsible for the formation of photochemical fumes, as it acts as the main intermediate to make many dangerous secondary pollutants such as nitric acid, nitrous acid, alkyl nitrate, and, in addition, peroxyacetyl nitrates[17].

NO $\cdot$  is a very important biological molecule that is involved in the mechanism of vasodilation. It is also related to researches on cyclic guanosine monophosphate (cGMP) and endothelial-derived relaxing factor (EDRF). Cyclic GMP was found to do its function as a second messenger in the late 1960s and early 1970s. At the same time, the presence of cellular, endogenously produced, cGMP was revealed by its isolation and identification from rabbit urine[17, 32]. This finding formed the basis of another study suggesting that cGMP is synthesized in a reaction catalyzed by a cyclase [33]. In the years that followed, phosphodiesterase specific and specific to cGMP was isolated and purified by partial extraction from a dog heart[34]. As a result of all these studies, the activity of guanylyl cyclase was finally explained [35]. However, steady-state levels of cGMP in tissue and cells were also found to be determined by the homeostasis of cGMP synthesis by

guanylyl cyclase and cGMP degradation by phosphodiesterases. The huge level of cGMP produced by guanylate cyclase in vascular smooth muscle permit blood vessels for relaxation and therefore rises blood flow.

In 1990, researchers whose Bretz and Snyder isolated and purified oxide nitric synthase (NOS), which is primarily responsible for NO synthesis from a rat cerebellum (NOS) [36]. As a result of these results, NOS is from the rat cerebellum and was defined as the neuronal NOS isoform (which is also nNOS or NOS-1). This important evidence of vitally expressed neuronal NOS was immediately confirmed by the identification of endothelium-derived NOS (eNOS or NOS-3), constitutively expressed [37]. Also, inducible NOS (iNOS or NOS-2), constitutively expressed [38]. In the 1970s, many researchers interested in this subject explained in more detail the chemical structure of peroxynitrite, which breaks down to form hydroxyl radical as well as nitrogen dioxide [39].

When  $\text{NO}\cdot$  was found in the years following the past, researchers were brainstorming on the hypotheses about superoxide's reactivity and biological effects. Therefore, the recognition of  $\text{NO}\cdot$  as a protective molecular agent, its ability and affinity to scavenge radical superoxide, and with it the result that it acts just like an antioxidant, is not very surprising [40,41]. In addition, according to Beckman et al.(1990) studies, a reaction product of superoxide and nitric oxide is peroxynitrite, which fallen down when adhered to form strong oxidants, hydroxyl radical as well as nitrogen dioxide [42].

The reaction of  $\text{NO}\cdot$  with oxygen occurs when there is the SOD in the environment biologically. This mechanism indicates that it is highly rapid to compete with the enzyme-catalyzed dis-mutation. Other than that, it converts the mild reductant into at least two effective oxidants. ONOOH homolysis to form  $\cdot\text{OH}$  leads to the assumption of biologically reliable and complete final steps of oxygen radical-induced molecular degeneration. This is more efficient and sufficient than the widely validated reaction of reduced iron with hydrogen peroxide. In summary, this process, which is also mentioned, is called the Fenton reaction as well as the iron-catalyzed Haber-Weiss reaction in the literature.

Moreover, the above-mentioned mechanism is an *in vitro* reaction that develops under optimal physiological conditions. It highlights the insignificant persistence of catalytic iron, especially in the free state, due to its specificity from its effective sequestration through proteins with many metal-binding properties. However, organisms with large iron loads have a greater proportion of freely available iron, such as in the conditions of iron overload called hemochromatosis, blood disorders like  $\beta$ -thalassemia, and hemodialysis. However, this has dangerous effects.

### **3. Biomarkers of Oxidative Stress and Mitochondria**

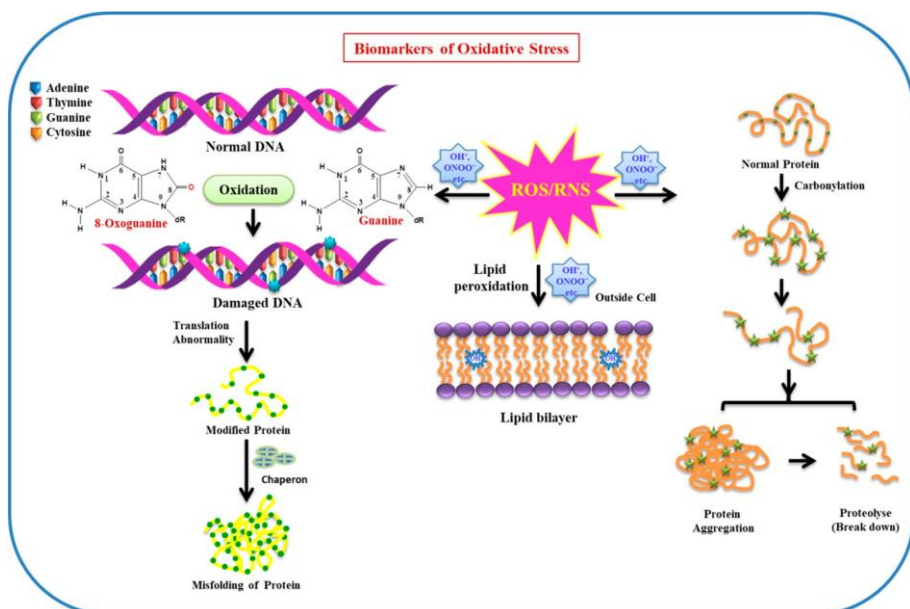
Because of the highest  $\text{O}_2$  consumption by the cells, and inadequate antioxidant protection mechanism along with a high content of polyunsaturated lipids, which tend to oxidation in neurons, OS has some destructive effects on some molecules. This is because the cells have a

high oxygen consumption and a non-optimal antioxidant defense process with a high polyunsaturated lipid-rich content, which tends to oxidize neurons. Some molecules, namely proteins, DNA/RNA, enzymes, and other biomolecules of this nature, have too many fragile and sensitive features against free radicals (ROS/RNS). However, given these changes and modifications, the situation can be reversed by being used as markers for OS. Functional lipids and proteins can be modified by ROS into many structures such as  $\text{OH}\cdot$  as well as  $\text{ONOO}^-$ . The heterocyclic bases of DNA/RNA tend to be oxidatively pure. In particular, guanine is purer against ROS counterattack. This causes it to regulate as 8-hydroxyguanine and 8-hydroxy-2-deoxyguanosine. Elevated levels of these modified bases are noticed in PD brains. In addition, it shows the participation of OH radicals as oxidative species. In addition, protein carbonylation and nitration are predominantly seen in AD brains [43,44].

Lipids are very important for brain function. It is one of the structures that make up the main structure of the cell membrane [45]. It is known for its ability to fight free radicals and encounter lipid peroxidation. Decreased membrane fluidity and increased membrane leakage are caused by lipid peroxidation. This makes it easy for these structures to enter the intracellular contents that cannot continuously cross the barrier, except for certain ion channels such as  $\text{K}^+$  or  $\text{Ca}^{2+}$ .

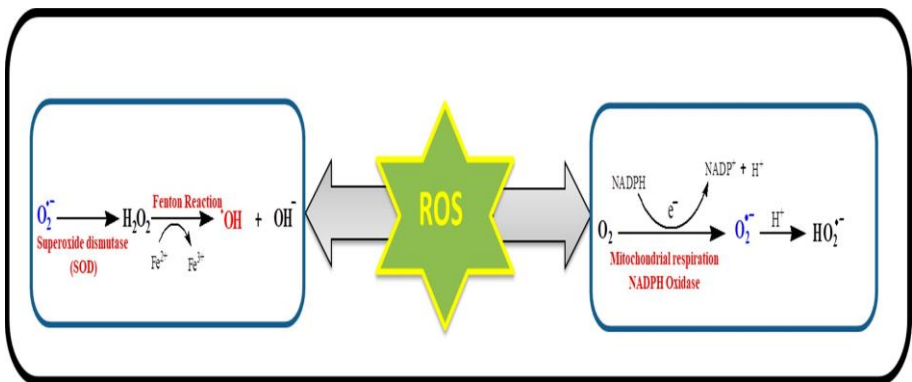
As a result, these structures cause the degeneration of membrane proteins, enzymes, or receptors[46]. The membrane lipid structure of neurons is very rich in polyunsaturated fatty acids (PUFA). Sidechains are particularly sensitive and fragile to ROS/RNS, which inevitably

causes oxidative stress. The degeneration of components has an important function as a biomarker for OS. The impact of ROS/RNS on molecules such as DNA, protein, and lipid, which are used as biomarkers of oxidative stress at the cellular level, is shown in Figure 2.



**Figure 2.** Some structures are used as biomarkers of oxidative stress at the cellular level of ROS/RNS, such as DNA, protein, lipid, etc. [14].

ATP is very important for many mechanisms that happen intracellularly, and also ATP is the energy unit of every mechanism. For example, it is indispensable for the functioning of the cell, its signaling, and all its other functions. Mitochondria, which are important organelles for ATP, produce ATP through the electron transport chain and oxidative phosphorylation mechanism. They are also involved in the production of molecules to deal with oxidative stress along with apoptosis as well as other respiratory functions in the cell. Enrichment of mitochondria by various redox enzymes and mitochondrial dysfunction is hypothesized to be responsible for ROS production in the cellular environment (Fig. 3) [47].



**Figure 3.** Some reactions for ROS production are shown in figure 3. Superoxide ( $O_2^{\bullet-}$ ) is facilitated by NADPH oxidase from oxygen in mitochondria or as a byproduct of the respiratory chain. Superoxide can be formed into hydrogen peroxide via superoxide dismutase. Hydrogen peroxide also causes the formation of hydroxyl radical and hydroxyl anions [14].

Molecules such as lipids, proteins, and DNA are used as biomarkers of oxidative degeneration of the aging process and some diseases such as Parkinson's disease, Alzheimer's disease, or Huntington's disease. It has been shown that ROS has the very important task of having a destructive effect on lipids. Evidence of this is that malondialdehyde (MDA) is involved in lipid peroxidation, protein carbonyls, and the oxidation of guanine in DNA to 8-oxo-deoxyguanosine[48,49]. Cardiolipin (CL) is an important phospholipid located in the inner membrane of mitochondria and is particularly associated with proteins of the electron transport chain. It is particularly necessary for adenine nucleotide translocase, which has important functions as an inner membrane transporter. The enrichment of cardiolipin in polyunsaturated fatty acids such as linoleic acid and its contiguous location in parts of ROS production in the mitochondrial electron transport chain makes it a vital target for ROS. CL is prone to oxidation, which causes mitochondrial electron transport chain disruption and results in the release of proapoptotic proteins [50, 51].

ROS have a detrimental effect on proteins and lipids directly, blocking bioenergetic function in mitochondria. Besides, it leads to a very deleterious and destructive effect on mitochondrial DNA in direct conjunction with promoter inactivation and downregulation of mitochondrial gene expression. As a result, in mitochondria with a longer half-life such as hydrogen peroxide, ROS formation is at an increased level, and lipid hydroperoxide or MDA, acrolein, etc. Active aldehydes like these run elsewhere but eventually end up in the

mitochondria. It can subsequently cause problems in mitochondria and eventually disrupt homeostasis by causing various health conditions.

#### **4. The Effect of Pro-oxidants on Oxidative Stress**

It has been discovered that many pro-oxidants, along with other external and internal factors, have a significant contribution to oxidative stress. Study results show that fruits and vegetables have a high source of polyphenols. In effect, they have a critical function as important antioxidants. Therefore, fruits and vegetables should be included in daily meals. However, it raises the idea that there may be other factors associated with chronic and metabolic diseases, neurodegenerative problems, antioxidants, as well as oxidative stress, which were later shown to be pro-oxidants [52]. In addition, pro-oxidants fall into any endobiotic or xenobiotic group that causes oxidative stress by generating ROS/RNS or block the functioning of the antioxidant system in cells or tissues. Pro-oxidants can be extracellular or intracellular. They also have some subgroups for example some external factors like pathogens, drugs, toxic substances, dietary components, etc and internal factors as anxiety, ion flux, climate, pollution, drug metabolites.

Some antioxidant flavonoids act by acting as pro-oxidant when certain transition metals are present. It is the flavonoid architecture that largely provides antioxidant properties and also the pro-oxidant property initiated by copper. Substitution of -OH in flavonoids causes flavonoids to act as antioxidants. Flavones and flavanones without -



OH groups help flavonoids to advance the spinal skeleton. These do not have antioxidant properties and pro-oxidant properties initiated by copper [52]. The pro-oxidant and antioxidant properties of the flavonoid myricetin were found with the help of variation in the Deoxyribose degradation assay by Chobot et al. It facilitates protection against oxidative diseases by neutralizing reactive metals that produce [54,55]. In addition, ascorbic acid has the property to act as an antioxidant and pro-oxidant in a dose-dependent effect. Conversely, ascorbic acid has a toxic effect due to its auto-oxidation, causing it to affect gene expression[56]. As a result, pro-oxidants, as well as antioxidants, have a very important role in oxidative stress and disease. Pro-oxidants are equally important for combat OS and neurodegeneration[57,58].

## **5. The Effect of Heavy Metals on Oxidative Stress**

The destructive effects of heavy metals on OS cover many systems. These systems are respiratory, cardiovascular, reproductive, renal, gastrointestinal systems and their main organs. Due to many factors, metal ions accumulate in the environment and also in humans, disrupting homeostasis [59]. Along with other xenobiotics such as pesticides, heavy metals have a detrimental and irreversible effect on the hematological and immune systems. The accumulation of heavy metals such as lead (Pb) and mercury (Hg) in the cell and the deficiency of primary metals such as selenium (Se) and zinc (Zn) cause oxidative stress. Consequently, the abnormality in the redox state of the cell causes detrimental effects on molecular structures such

as DNA, RNA, proteins, and lipids, and on vital organs of the gastrointestinal, excretory, and nervous systems [60].

Studies emphasize that Mercury (Hg) is not essential for any biological mechanisms, but its presence and accumulation cause harmful and vital effects in living creatures. Oxidative stress-induced by mercury causes cell membrane degeneration and oxidation of biomolecules. It also activates the production of hydrogen peroxide, lipid peroxidation of mitochondria membrane, and protein oxidation [61]. Hg has many dangerous neurotoxic properties. In other words, it affects cerebral mechanisms and abilities. Prolonged Hg exposure produces timidity, tremor, and pathophysiological symptoms in cognitive ability, as well as hearing and vision [62].

Lead (Pb) is a widely used metal, is very toxic to humans, especially due to the induction of oxidative stress. The harmful effect caused by Pb is correlated with the mode and duration of exposure, the disease state of the person, that is, the chronic and metabolic diseases of the person, and also the age [63]. Pb has a very high affinity for the -SH group in amino acids and metal cofactors. However, this causes a decrease in the activity of antioxidant enzymes, which play a role in many cellular mechanisms. It is also linked with increased oxidative stress and mitochondrial deterioration, causing degeneration in the essential organs such as the brain, liver, kidney [64].

In addition, prolonged exposure to arsenic (As) is known to cause cancer cell formation, the toxicity of cells and genes in humans. It is also a metalloid found to be toxic to all living creatures. As also has

the property to associate with the –SH group of glutathione, which causes the GSH from which hydrogen peroxide is produced to change to GSSG [65,66]. As inhibits glucose absorption in cells, causes gluconeogenesis and oxidation of fatty acids. It also leads to a dangerous effect on the Krebs cycle and, accordingly, leads to mitochondrial degeneration. Heavy metals are directly or indirectly responsible for the formation of ROS/RNS. As causes oxidative stress, which degrades mitochondria and molecules such as DNA, enzymes, and proteins, causing cell proliferation, cell differentiation, and also programmed cell death [65].

### **Conclusions and Outlook**

High oxidative stress is one of the potential common causes of many metabolic problems and neurodegenerative diseases. Generally, a stable balance between the presence of ROS and antioxidants is vital for the homeostasis of the cell. The production of ROS in the cell takes place in a different place, especially in the mitochondria. Antioxidants work to compensate for free radicals and thus act by attacking oxidative stress, preventing them from starting the chain reactions that lead to neurodegenerative diseases and premature aging. Under stable and optimal conditions, the presence of a natural antioxidant system plays a vital role in scavenging and eliminating ROS and acts by maintaining the typical cellular atmosphere. The initiation of oxidative stress makes ROS which has a destructive effect on enzymes and proteins leading to lipid peroxidation, protein misfolding and aggregation, DNA degeneration as well as mutations. The brain is the

most sensitive and fragile organ of the body. The aging process and lifestyles such as nutrition and exercise are other primary factors that play a vital role in the onset of many health problems. However, changing a person's physical and cognitive activity to be stable, having a balanced diet containing vitamins C and E, creating adequate antioxidants and for initial diagnosis can help treat neurodegenerative diseases. Existing research around the world welcomes new treatments and is working hard for anticipated therapeutic agents to control these neurodegenerative issues. Future studies will increase the knowledge on this subject and expand the translational effect of oxidative stress event both in biology and medicine.

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## CHAPTER 2

### ANTIOXIDANTS

**\* The chapter is translated from a master's thesis.**

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## **INTRODUCTION**

Antioxidants that have critical importance to maintain optimum health and welfare conditions are the first line of defense which organisms own to be protected from the damage of free radicals (Percival, 1998; Pietta, 2000). The quite complex and advanced antioxidant protection system can stabilize or deactivate free radicals before they attack cells (Percival, 1998; Sen & Chakraborty, 2011). In addition, antioxidants are also known as compounds that, when present in foods at low concentrations compared to an oxidizable substrate, can significantly delay or prevent oxidation of the substrate (Gutteridge, 1995; Shahidi, 2000). Thus, some antioxidants are among the main components that protect the quality of foods, especially by preventing the oxidation of lipids (Shahidi et al., 1992). Furthermore; it is also known that antioxidants have various biological properties such as antibacterial, antiviral, antimutagenic, antiallergic, anticarcinogenic, antimetastatic activity, anti-aging activity, anti-ulcer activity and ability to inhibit increase of blood pressure (Moure et al., 2001). However, low levels of antioxidants in the body increase the risk of numerous diseases, and insufficient intake of antioxidant-rich foods through diet doubles the risk of cancer (Sen & Chakraborty, 2011). Through these biological activities, antioxidants have attracted the attention of food manufacturers, biochemists and healthcare professionals for years. For instance, food manufacturers use antioxidants in their products to protect the nutritional value of many products and to prevent quality losses (Shahidi, 2000).



## 1. ANTIOXIDANTS

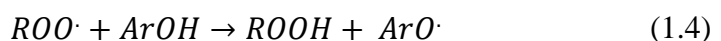
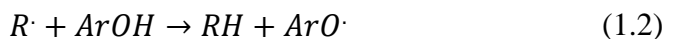
Antioxidants provides protection against free radicals in various ways such as preventing radical formation, intercepting the formed radicals, repairing oxidative damage caused by them, and eliminating damaged molecules created by radicals (Gutteridge, 1995). Antioxidants carry out their antioxidant activity by a wide range of ways such as removing the oxygen in or reducing the oxygen concentration of the environment, removing catalytic metal ions, removing essential reactive oxygen species (ROS, such as  $O_2\cdot$  and  $H_2O_2$ ), scavenging free radicals (such as  $\cdot OH$ ,  $RO\cdot$  and  $ROO\cdot$ ) that initiate damaging chain reactions, breaking a started chain or quenching and removing single oxygen (Aslankoç et al., 2020; Gutteridge, 1995). Sometimes antioxidants can be oxidized as a result of their activities then antioxidant radicals are formed. Since these formed radicals are less reactive, they play a rate limiting role in the initiation, propagation and termination of radical chain reactions (Wootton-Beard & Ryan, 2011).

Antioxidants can be classified as free radical terminators, metal ion chelators that can catalyze lipid oxidation, and oxygen scavengers that react with oxygen in closed systems, according to the above-mentioned types of activity (Shahidi et al., 1992). However, it is known that antioxidants are divided into two classes as primary or chain-breaking antioxidants and secondary or preventative antioxidants traditionally (Apak et al., 2007, 2016; Moure et al., 2001). For example, radical scavenging antioxidants such as vitamin C (ascorbic acid) and vitamin E ( $\alpha$ -tocopherol,  $\alpha$ -TH) act as primary antioxidants (Gutteridge, 1995;

Halliwell, 1996; Willcox et al., 2004). These type of antioxidants scavenge radicals to obstruct the initiation of the oxidation chain and prevent chain spread (Willcox et al., 2004). As an example;  $\alpha$ -TH, the most effective chain-breaking antioxidant that protects the fatty acids in the cell membrane from lipid peroxidation, inhibits lipid peroxidation by scavenging peroxy radicals ( $ROO\cdot$ ) in the chain reactions (Halliwell, 1996; Percival, 1998). The mentioned inhibition reaction is given by (1.1) below. The  $\alpha$ -tocopherol radical ( $\alpha T\cdot$ ) that formed as a result of this reaction is very slightly reactive and is much less effective than  $ROO\cdot$  in attacking fatty acid side chains (Halliwell, 1996).



Chain breaking mechanisms that for the breaking of oxidation chains of lipid radicals ( $R\cdot$ ,  $RO\cdot$  or  $ROO\cdot$ ) actualize in the way that converting antioxidants ( $ArOH$ ) into antioxidant radicals ( $ArO\cdot$ ) to protect lipid molecules (Apak et al., 2016). This is because primary antioxidants react with lipid radicals that own high energy and transform them into products that are more thermodynamically stable (Hamid et al., 2010; Shahidi et al., 1992). This situation can generally be demonstrated by the reactions given by (1.2) - (1.4) (Apak et al., 2007, 2016).

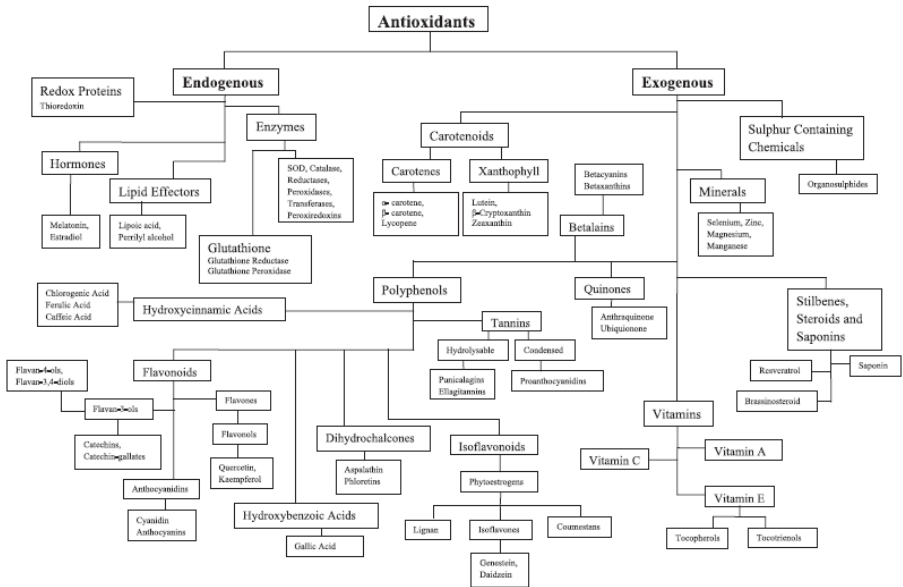


On the other hand; secondary antioxidants, act by delaying or preventing the oxidation of lipids, breaking down hydroperoxides and slowing the chain initiation rate of these compounds (Apak et al., 2016; Shahidi et al., 1992). For example, transition metal ion chelators show their antioxidant activity by preventing Fenton reactions that result in the formation of hydroxyl radicals ( $\cdot\text{OH}$ ) which can cause oxidative damage in biological macromolecules (Apak et al., 2007, 2016). In addition to these types, there are also antioxidant compounds that can act as both primary and secondary antioxidants (Moure et al., 2001).

## **2. CLASSIFICATION OF ANTIOXIDANTS**

Since molecules with antioxidant activity show a very heterogeneous distribution, antioxidants are a family of molecules that are difficult to classify according to their common structural properties (Vertuani et al., 2005). These molecules have been divided into many classes in line with their various characteristics from past to present. For example; as mentioned in Section 1, antioxidants can be classified as primary or secondary antioxidants according to their mechanism of action. In addition, antioxidants can be classified into various classes as natural or synthetic according to their origin, enzymatic or non-enzymatic according to their nature, hydrophilic or lipophilic according to their chemical properties further flavonoids or polyphenols according to their structure (Vertuani et al., 2005). On the other hand, antioxidant molecules can also be divided into two broad groups such as produced in the body (endogenous antioxidants) and taken through diet (exogenous antioxidants) (Duthie & Crozier, 2000; Okan et al., 2013;

Pietta, 2000; Wootton-Beard & Ryan, 2011). This classification of antioxidants is shown in Figure 1.

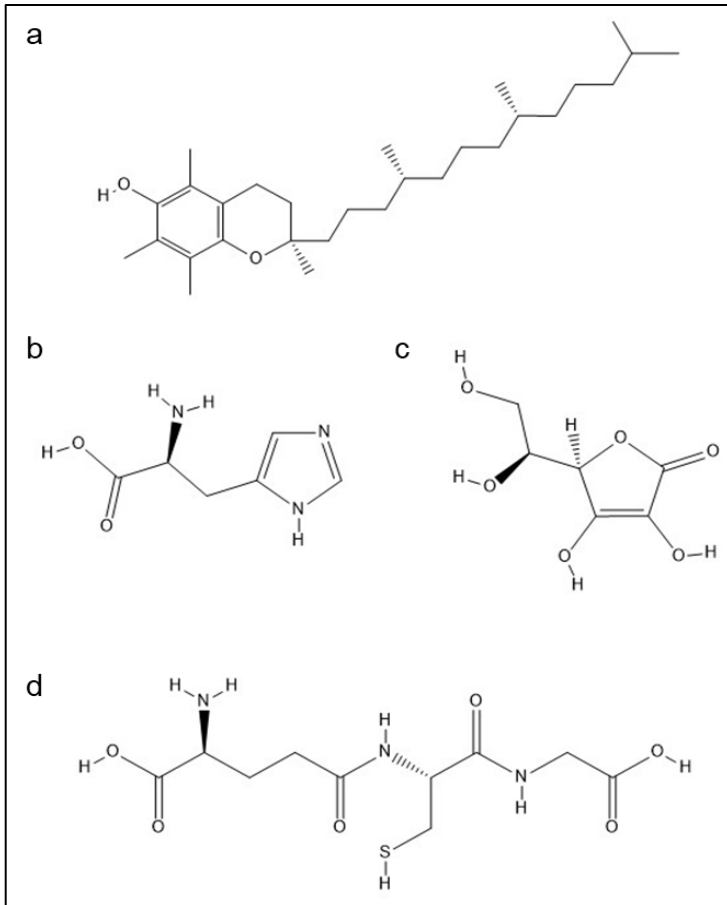


**Figure 1:** Classification of Antioxidants as Endogenous and Exogenous (Wootton-Beard & Ryan, 2011)

## 2.1. Natural Antioxidants

Naturally occurring antioxidant molecules that protect structures such as cells and tissues from effects of free radicals are present in organisms (Conner & Grisham, 1996; Moure et al., 2001). These molecules are classified as natural antioxidants and include both enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) or glutathione peroxidase (GSH-Px) as well as non-enzymatic antioxidants such as vitamins (E.g. vitamins A, E and C), amino acids (E.g. histidine), peptides (E.g. glutathione(GSH)), proteins (E.g. ferritin

and transferrin), assorted compounds from plant origin (E.g. saponins and xanthophylls) and minerals (E.g. zinc) (Figure 2) (Conner & Grisham, 1996; Moure et al., 2001; Okan et al., 2013; Pietta, 2000; Vertuani et al., 2005).



**Figure 2:** Several Natural Antioxidants (a:  $\alpha$ -TH, b.:Histidine, c: Vitamin C and d: GSH)

It is also known that in the protective effect of fruits and vegetables against various diseases a wide variety of natural antioxidants such as ascorbic acid,  $\alpha$ -TH,  $\beta$ -carotene or polyphenolic compounds have a role

(Moure et al., 2001). For example; ascorbic acid, a water-soluble natural antioxidant, can scavenge superoxide ( $O_2^-$ ) and  $\cdot OH$  radicals, while also regenerating  $\alpha$ -TH. As for  $\alpha$ -TH, a oil-soluble natural antioxidant, has protective effects against coronary heart diseases as it inhibits oxidation of low density lipoprotein (LDL) (Podsedek, 2007). In case  $\beta$ -carotene, another oil-soluble natural antioxidant, is considered the best quencher of singlet oxygen ( $^1O_2$ ) and provides antioxidant protection in lipid-rich tissues (Sen & Chakraborty, 2011).

Ergothioneine, a natural betaine, is an amino acid that can only be synthesized by fungi and mycobacteria. This amino acid known as a powerful compound that can scavenge various ROS, it reacts with peroxynitrite ( $ONOO^-$ ) or a derivative of it to prevent nitration of tyrosine and scavenge  $\cdot OH$  radicals at very high rate. Furthermore, it acts as an antioxidant as of the quencher of  $^1O_2$  (Chaudière & Ferrari-Iliou, 1999).

Divers peptides also can show antioxidant properties through the mechanisms by which other antioxidants carry out their activities. With the intention of obtain and be able to use these peptides as food antioxidants, various microorganisms and enzymes are used to break down many proteins since a long time. For example, proteases such as trypsin, alkalase, pepsin and papain break the peptide bonds in proteins, providing to the emergence of various peptides with antioxidant properties. However, different lactic acid bacteria such as *L. helveticus*, *L. rhamnosus*, *L. paracasei* and *L. casei* are used to induce the release of antioxidant peptides (Lorenzo et al., 2018). To give an example, in a

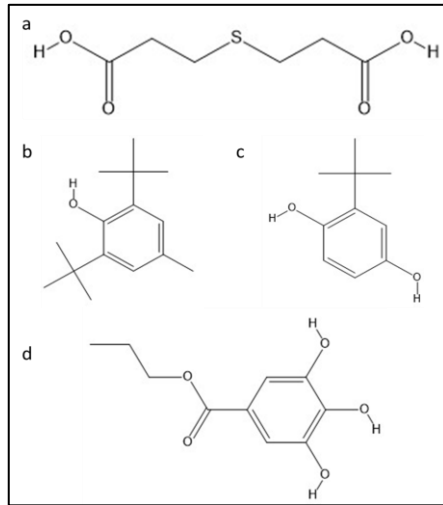
study conducted by Aguilar-Toalá et al., it has been stated that crude extracts rich in antioxidant peptides could be produced from fermented milk obtained by growing *Lactobacillus plantarum* strains in whole-fat bovine milk (Aguilar-Toalá et al., 2017).

GSH ( $\gamma$ -glutamyl cysteinyl glycine), a tripeptide found in structures such as mitochondria, nuclei and cytosol, synthesized in almost all eukaryotic cells; is the principal soluble non-enzymatic natural antioxidant in cell compartment. The tripeptide can directly remove  $^1\text{O}_2$  and OH, or detoxify  $\text{H}_2\text{O}_2$  and lipid peroxides by means of GSH-Px (Karabulut & Gülay, 2016; Masella et al., 2005; Sen & Chakraborty, 2011; Valko et al., 2006). Additionally, GSH also takes part in regeneration of some important antioxidants (Karabulut & Gülay, 2016; Sen & Chakraborty, 2011). For example, vitamins C and E are regulated by the tripeptide (Ferreira et al., 2009; Karabulut & Gülay, 2016; Sen & Chakraborty, 2011). In this process, the direct reduction of the tocopherol radical of vitamin E ( $\alpha\text{T}^\cdot$ ) or the indirect reduction of semidehydroascorbate to ascorbate takes place (Karabulut & Gülay, 2016; Sen & Chakraborty, 2011; Valko et al., 2006).

## **2.2. Synthetic Antioxidants**

Synthetic antioxidants which are synthesized by chemical methods, have been used for many years due to their high stability, cheapness and most importantly, their strong antioxidant activity (Deveci et al., 2016; Önenç & Açıkgöz, 2005). These antioxidants are generally known with their addition to foods as food preservatives to prevent lipid

peroxidation leading to quality losses and to extend the shelf life of foods (Çoban & Patır, 2010; Deveci et al., 2016; Sen & Chakraborty, 2011). Commonly used synthetic antioxidants in foods are as follows for this purpose; butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butyl hydroquinone (TBHQ) (Figure 3) (Moure et al., 2001; Öğüt, 2014; Shahidi, 2000). On the other hand, antioxidant supplements taken into the body through diet are also generally from synthetic origin. Some of these supplements do not able to have same effects with natural antioxidants in the body due to some processes performed during their production (Sen & Chakraborty, 2011).



**Figure 3:** Several Synthetic Antioxidants (a: TDPA b: BHT c: TBHQ d: PG)

Synthetic antioxidants also have different mechanism of activity like other antioxidants. For example; sulfides which act as glucose oxidase and ascorbyl palmitate scavengers, BHA, BHT, TBHQ and gallates which act as radical terminators, heavy metals such as iron and copper



that act as chelating agents are among the primary antioxidants. Synthetic antioxidants such as thiodipropionic acid (TDPA) (Figure 3) and dilauryl thio dipropionate are among the secondary antioxidants and generally break down hydroperoxides formed during the oxidation of lipids while providing in the formation of stable products (Sen & Chakraborty, 2011).

Synthetic antioxidants are compounds with generally phenolic structure and diverse grades of alkyl substituents (Kaur & Kapoor, 2001; Ögüt, 2014). While BHA and BHT compounds, which are monohydric phenolic antioxidants, are very well soluble in oils, they are insoluble in water. Besides, BHT, unlike BHA, suppresses the oxidation of animal fats more effectively than the oxidation of vegetable oils. In case, unlike BHT, BHA is more effective in protecting the color and flavor of essential oils, and is even the most effective compound among all food-approved antioxidants in this regard (Shahidi et al., 1992). BHA is also particularly effective in controlling the oxidation of short-chain fatty acids (such as the short-chain fatty acids in coconut and palm kernel oils which are used in a variety of cereal and confectionery products). In addition, BHA and BHT compounds, which have a synergistic effect when used in combination, are also used in packaging materials because they can pass into foods thanks to their volatile structure.

As for TBHQ, a diphenolic synthetic antioxidant, is sufficiently soluble in oils and is considered the best antioxidant compound that protects frying oils from oxidation. In addition, since TBHQ does not form a

complex with iron or copper, it does not discolor the processed products. On the other hand; PG, which is commercially produced by esterifying gallic acid with propyl alcohol, plays a successful role in stabilizing animal and vegetable oils. Although this antioxidant dissolves in water in small amounts, it shows amphiphilic properties and is very effective on dry vegetable oils (Shahidi et al., 1992).

It is known that commonly used synthetic antioxidants such as BHA, BHT, PG and ethoxyquin have beneficial roles in organisms such as radiological protection, preservation against acute toxicity of various chemicals, antimutagenic activity and antitumorogenic effect. However, as a result of the physical or chemical interaction of these antioxidants with harmful structures, negative effects such as radiosensitization, increase in toxicity of interacting chemicals, in activity of mutagens or in tumor yield may occur (Sen & Chakraborty, 2011).

As a result of many studies carried out by various researchers, it has been proven that synthetic antioxidants can show carcinogenic, teratogenic and toxic effects in living organisms as a result of high doses or long-term use (Çoban & Patır, 2010; Deveci et al., 2016; Kaur & Kapoor, 2001; Öğüt, 2014; Sen & Chakraborty, 2011). For example; it has been determined that BHA and BHT cause changes in enzyme activities and lipid levels and also the degradation products of these compounds cause toxic effects (Sen & Chakraborty, 2011). Furthermore, it has also been determined that synthetic antioxidants cause unpleasant tastes and odors in fried products and stimulate the

formation of cancerous cells (Önenç & Açıkgöz, 2005). Consequently, the use of these antioxidants, which can threaten human health and lead to the formation of chronic diseases, has been restricted or banned in various countries (Kaur & Kapoor, 2001; Öğüt, 2014; Önenç & Açıkgöz, 2005; Sen & Chakraborty, 2011). Therefore the interest in natural antioxidants has increased and this provided their importance understood (Kaur & Kapoor, 2001).

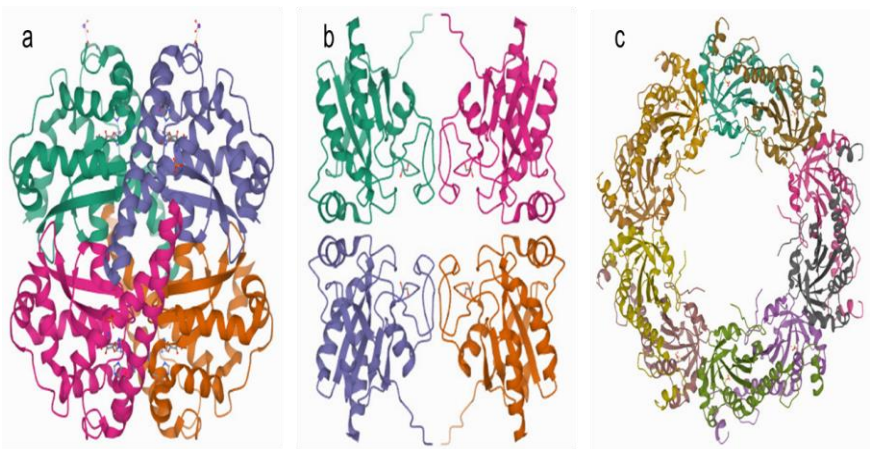
### **2.3. Endogenous Antioxidants**

Antioxidants that passivate free radicals by neutralization and protect the body from the harmful effects of these compounds by providing redox (oxidant/antioxidant) balance in the body, thus acting as a barrier against free radicals, are also divided into broad subgroups as endogenous and exogenous (Karabulut & Gülay, 2016; Sen & Chakraborty, 2011). The endogenous/exogenous antioxidant defense system maintains or regenerates the redox balance in the body to prevent lipid peroxidation processes that have the potential to affect membrane fluidity and damage membrane proteins (Radomska-Lesniewska et al., 2017).

Compounds in the group of endogenous antioxidants refer to enzymatic and non-enzymatic antioxidants produced in the body (Aydemir & Karadağ Sarı, 2009; Karabulut & Gülay, 2016; Pietta, 2000). For example; enzymes such as SOD, CAT, glutathione reductase (GR), GSH-Px and peroxiredoxin I-VI (Prx I-VI) are enzymatic endogenous antioxidants (Aalen, 1999; Huang et al., 2005; Moure et al., 2001; Pietta, 2000; Radomska-Lesniewska et al., 2017). Furthermore;

melatonin, bilirubin, albumin, histidine, GSH, uric acid,  $\alpha$ -lipoic acid, coenzyme Q10 (ubiquinone, vitamin Q10, CoQ10), ceruloplasmin, ferritin and transferrin are among the non-enzymatic endogenous antioxidants (Aydemir & Karadağ Sarı, 2009; Karabulut & Gülay, 2016; Pham-Huy et al., 2008; Pietta, 2000).

Enzymatic endogenous antioxidants generate a barrier against free radicals in the cell (Descalzo & Sancho, 2008). These antioxidants work by whether breaking down and eliminating free radicals (Nimse & Pal, 2015). 3D images of some enzymatic endogenous antioxidants taken from the Protein Data Bank (PDB) are shown in Figure 4.

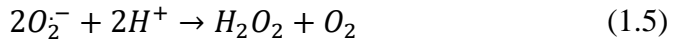


**Figure 4:** 3D Images of Some Enzymatic Endogenous Antioxidants (a: Natural Human Manganese-SOD, PDB Code-5VF9; b: Human GSH-Px 5, PDB Code-2I3Y and c: Human Prx-IV, PDB Code-3TKS)

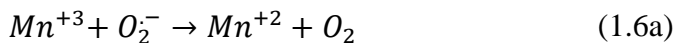
Enzymatic endogenous antioxidant structures, convert harmful oxidative products through mechanized reactions to first other reactive species such as  $H_2O_2$  and then to water, in the presence of cofactors such as copper (Cu), iron (Fe), zinc (Zn) and manganese (Mn) (Nimse & Pal, 2015). In this way, antioxidant enzymes play an important role

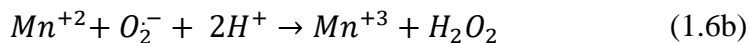
in preventing lipid peroxidation, protecting the structures and functions of biological membranes by reducing lipid hydroperoxide and  $H_2O_2$  levels in cells (Koruk et al., 2004; Nimse & Pal, 2015). However; the *in vivo* activities of these compounds, can be affected by various factors such as cell damage, stress, inflammatory reactions and cancer formation (Descalzo & Sancho, 2008).

SOD enzyme, which is the first line of defense against ROS, is an enzymatic endogenous antioxidant that catalyzes  $O_2^-$  to  $H_2O_2$  and  $O_2$  in the presence of various metal ion cofactors (Aydemir & Karadağ Sarı, 2009; Karabulut & Gülay, 2016; Nimse & Pal, 2015; Pham-Huy et al., 2008). The reaction that catalyzed by SOD is given by (1.5).



There are three forms of SOD in humans; Mn SOD, Cu/Zn SOD and extracellular SOD (EC SOD) (Karabulut & Gülay, 2016; Sen & Chakraborty, 2011). The Cu and Zn containing form of this enzyme is found in the cytosol, while the Mn containing form is found in the mitochondria (Aslankoç et al., 2020; Karabulut & Gülay, 2016). Mn SOD prevents the negative effects of  $O_2^-$  produced by the electron transport system by converting these radicals into molecular oxygen ( $O_2$ ) and  $H_2O_2$  through cyclic reduction and oxidation by means of the Mn transition metal in its active zone (Azadmanesh et al., 2017). This reaction mediated by the SOD enzyme containing Mn takes place with the following reaction steps, (1.6a) and (1.6b).





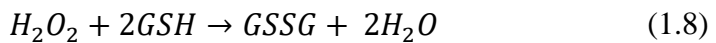
On the other hand; EC SOD, the only antioxidant that can enzymatically inactivate  $O_2^-$  at the extracellular level, is found in the extracellular matrix of tissues (Gao et al., 2008). This enzyme is known to have a considerable role in protection from various lung diseases (Gao et al., 2008; Karabulut & Gülay, 2016).

CAT, a metalloenzyme, is the most effective enzyme commonly found in cells (Koca & Karadeniz, 2003; Sen & Chakraborty, 2011). This endogenous antioxidant is in the form of a tetramer composed of four polypeptide chains and contains four porphyrin heme groups (Sen & Chakraborty, 2011). These groups allow the enzyme to react with  $H_2O_2$  and thus CAT can catalyze the reaction in which  $H_2O_2$  has converted to  $H_2O$  and  $O_2$  (Bast et al., 1991; Nimse & Pal, 2015; Sen & Chakraborty, 2011). Each CAT enzyme has the potential to decompose millions of  $H_2O_2$  molecules into  $H_2O$  and  $O_2$  in one second (Aslankoç et al., 2020; Sen & Chakraborty, 2011). The reaction that catalyzed by CAT is given by (1.7).

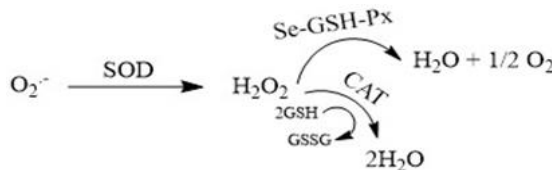


The GSH-Px enzyme family, which is found in the cytoplasm and almost every human tissue extracellularly, protects cells against oxidative damage caused by peroxides by reducing various organic and inorganic hydroperoxides (Bast et al., 1991; Masella et al., 2005; Sen & Chakraborty, 2011). While the selenium (Se) independent GSH-Px, since it does not contain Se element in its active site, protects cells

against ROS by accepting mostly organic hydroperoxides (ROOH, for example; lipid hydroperoxides or DNA hydroperoxides) as substrates then reducing them; the selenium-dependent GSH-Px (Se-GSH-Px), which contains Se in its active site, protects cells by preventing the formation of OH<sup>-</sup> from H<sub>2</sub>O<sub>2</sub> as well as organic hydroperoxides (Bast et al., 1991; Karabulut & Gülay, 2016; Sen & Chakraborty, 2011). Se-GSH-Px achieves its protective effect against H<sub>2</sub>O<sub>2</sub> by catalyzing the reaction in which H<sub>2</sub>O<sub>2</sub> is reduced to H<sub>2</sub>O along with GSH oxidation by using GSH tripeptide as the source of electron (Sen & Chakraborty, 2011). As a result of this reaction, glutathione disulfide (GSSG) compound, which is the oxidized form of GSH peptide, is also formed as well as H<sub>2</sub>O (Karabulut & Gülay, 2016; Koca & Karadeniz, 2003). The mentioned reaction is represented by (1.8) below.

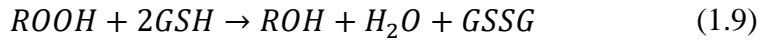


In the light of this information, it is understood that SOD, CAT and Se-GSH-Px enzymes show a synergistic effect in scavenging O<sub>2</sub><sup>-</sup> radicals (Nimse & Pal, 2015). The situation can be outlined as stated in Figure 2.5.



**Figure 5:** Synergistic Relationship Between SOD, CAT and Se-GSH-Px Enzymes  
The Se independent GSH-Px also uses GSH as the source of electron while catalyzing reactions in which organic hydroperoxides are reduced

(Bast et al., 1991; Pham-Huy et al., 2008). Thus, GSSG formation is observed also in this case. The reaction is indicated by (1.9).



Prxs are a large family of sulfhydryl-dependent peroxidases that have been first identified in yeasts and then found to be present in a variety of organisms, from bacteria to humans (Aalen, 1999; Rhee, 2016; Zhu et al., 2012). Members of the endogenous antioxidant family protect cellular structures from oxidative damage by catalyzing the reduction of  $H_2O_2$ , organic hydroperoxides and  $ONOO^-$  (under appropriate conditions) (Rhee, 2016; Zhu et al., 2012). In addition, it has been determined in various experimental studies that the important enzyme family exhibits also protective properties against neuropathophysiological processes (Zhu et al., 2012). Accordingly, it is clearly understood that antioxidant enzymes play a significant role in preventing the damage that free radicals cause or can lead, and maintaining the health conditions of organisms.

Melatonin (N-acetyl-5-methoxy-tryptamine), a hormone that is mostly produced in the pineal gland and secreted into the circulation, shows a wide distribution by reaching almost all organelles of the cells by means of lipophilic structure (Aydemir & Karadağ Sarı, 2009; Karabulut & Gülay, 2016). In addition, it can easily cross cell membranes and even the blood-brain barrier (Hamid et al., 2010).

Melatonin protects entire macromolecules and nuclear structures except protein and lipid structures from oxidative damage in all intracellular compartments (Karabulut & Gülay, 2016). However, it provides



widespread protection in organisms as a powerful non-enzymatic endogenous antioxidant, both directly by scavenging free radicals and as a result of its indirect protection activities (Hamid et al., 2010; Karabulut & Gülay, 2016). For example; it is known that the hormone acts directly by scavenging reactive species such as  $\cdot\text{OH}$ ,  $\text{H}_2\text{O}_2$ ,  $^1\text{O}_2$ ,  $\text{NO}\cdot$ ,  $\text{ONOO}^-$  and  $\text{ONOOH}$ , and indirectly by stimulating endogenous antioxidant enzymes such as SOD, CAT, GSH-Px and GR (Aydemir & Karadağ Sarı, 2009; Karabulut & Gülay, 2016). In addition, it can also show its antioxidant activity indirectly by increasing the GSH level, suppressing various prooxidative enzymes, strengthening cellular membranes and increasing the efficiency of the electron transport system (Karabulut & Gülay, 2016).

Bilirubin, the main pigment of saffron, is also an endogenous non-enzymatic antioxidant like melatonin (Halliwell & Gutteridge, 1990). Bilirubin is actually the final product that is formed as a result of the catabolism of the heme proteins in erythrocytes and can show cytotoxic properties (Karabulut & Gülay, 2016; Sen & Chakraborty, 2011). However, at micromolar levels, it has been found that it can effectively scavenge peroxy radicals and protect linoleic acid that bound to albumin from oxidative damage caused by ROO, thus showing antioxidant properties (Aydemir & Karadağ Sarı, 2009; Halliwell & Gutteridge, 1990; Sen & Chakraborty, 2011).

CoQ10, a vitamin-like benzoquinone compound, is an endogenous antioxidant found in lipoproteins and almost all cell membranes (Karabulut & Gülay, 2016). The compound is a powerful lipophilic

antioxidant, cofactor and coenzyme involved in energy production period in mitochondria and in processes where vitamins such as A, C and E are regenerated (Houston, 2005). The antioxidant shows its antioxidant activity by suppressing the oxidation of lipids, membrane phospholipids, DNA and various proteins or by scavenging free radicals (Houston, 2005; Karabulut & Gülay, 2016). The reduced form of this compound, ubiquinol (CoQH<sub>2</sub>), neutralizes oxidants by donating electrons and exhibits a very strong antioxidant activity. In conclusion, CoQ10 provides effective protection against toxic reactive species such as H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub><sup>-</sup> (Karabulut & Gülay, 2016).

$\alpha$ -lipoic acid, another endogenous antioxidant also produced naturally in the body, is classified as "thiol" or "biothiol" because it contains sulfur (S) in case (Eken, 2015; Percival, 1998; Sen ve Chakraborty, 2011). Whereas  $\alpha$ -lipoic acid is involved in the Krebs cycle, it also plays a role in vital processes such as energy production at the cellular level (Eken, 2015; Percival, 1998). The antioxidant and its reduced form, dihydrolipoic acid (DHLA), are called "universal antioxidants" because they can quench free radicals by showing both lipophilic and hydrophilic properties and they are unique in this field.  $\alpha$ -lipoic acid exerts a protective effect on other antioxidants by helping to rearrange the levels of antioxidants such as GSH, cysteine, vitamins C and E in tissues and blood (Houston, 2005; Karaca, 2015).

On the other hand, there are also two important endogenous antioxidant proteins responsible for extracellular antioxidant activity in the body. One of these antioxidants is ceruloplasmin that contains Cu, while the

other is transferrin, a Fe-binding protein (Gutteridge, 1986). The both plasma proteins are known as important proteins synthesized in a wide variety of tissues in the body, including brain tissues (Karabulut & Gülay, 2016).

Although transferrin is mainly found in blood serum, it is a protein that can also be found in low concentrations in other body fluids and regulates Fe homeostasis. Ceruloplasmin, on the other hand, is an important glycoprotein that carries about 95% of the Cu in the bloodstream (Altamura et al., 2009; Karabulut & Gülay, 2016). The main function of the protein is to create water without causing the formation of ROS, to reduce the molecular oxygen level, and to oxidize ferrous ions ( $\text{Fe}^{+2}$ ) to the form of ferric ions ( $\text{Fe}^{+3}$ ) which have less toxic properties through its ferroxidase enzyme activity (Altamura et al., 2009; Halliwell & Gutteridge, 1990; Pacht & Davis, 1988; Sen & Chakraborty, 2011). The formed  $\text{Fe}^{+3}$  ions are then transported to the cells by tightly binding to transferrin, and thus the level of free iron ions in the environment that can cause Fenton reactions decreases (Altamura et al., 2009; Aydemir & Karadağ Sarı, 2009; Pacht & Davis, 1988). Thus, the reaction of free  $\text{Fe}^{+3}$  ions with hydroperoxides prevents the formation of  $\cdot\text{OH}$  radicals, which are known to have the most toxic properties among oxygen metabolites (Altamura et al., 2009). Furthermore, lipid peroxidation is also prevented by the collaboration of these two proteins because of the fact that free  $\text{Fe}^{+3}$  ions can catalyze lipid peroxidation processes (Altamura et al., 2009; Halliwell & Gutteridge, 1990; Pacht & Davis, 1988). According to the protective effect, it can clearly be evident that these serum proteins possess

antioxidant activity (Altamura et al., 2009; Halliwell & Gutteridge, 1990).

#### **2.4. Exogenous Antioxidants**

In addition to the endogenous antioxidants mentioned in Section 2.2.3, antioxidant-containing foods or antioxidant supplements which taken from exogenous sources are also of great importance for the protection of organisms from free radicals. In this context, antioxidants, which are taken from external sources without being produced in the body and called exogenous antioxidants, help protection of organisms from free radical damage, while also helping to preserve food quality and extend the shelf life of foods by preventing oxidative spoilage of them (Ferreira et al., 2009; Moure et al., 2001). There is also epidemiological evidence that exogenous antioxidants found in fruits and vegetables can affect the development of various diseases and may have a role in the prevention of these diseases (Huang et al., 2005; Wootton-Beard & Ryan, 2011). In addition to fruits and vegetables, other food products such as grains, teas, legumes and nuts are also known to contain many exogenous antioxidants (Kaur & Kapoor, 2001; Sen & Chakraborty, 2011). Moreover, it is known that various plants, marine plants and seafood that feed on the marine plants are also good sources of antioxidants (Eken, 2015; Kaur ve Kapoor, 2001). Exogenous antioxidants can protect the body by suppressing free radicals or reactions caused by them and preventing lipid peroxidation, as well as by strengthening the endogenous antioxidant defense system of the body (Benzie, 2003; Ferreira et al., 2009; Sen & Chakraborty, 2011). In

this manner, they can show their antioxidant activity with a protective or therapeutic effect (Benzie, 2003; Ferreira et al., 2009; Sen & Chakraborty, 2011). Thereby, increasing the intake of exogenous antioxidants is an effective option to create an optimal antioxidant defense in the body (Benzie, 2003).

## **CONCLUSION**

Antioxidants are definitely behind several crucial processes that occur in the living systems with their essential roles and also broad diversity as mentioned in this chapter. That compounds take part in protection or treatment of several both basic and complex diseases, preservation of some food contents and thus food quality. In consequence of their extraordinary defense performance, it is clear that the family is one of the fundamental components of the living systems. But generally, the members of the family come into prominence with their defense performance on free radicals. Diverse antioxidant compounds that are in subclasses of the family show their performance by different ways when they encounter with free radicals. Hereby, it is important to pay attention on each type of antioxidant compound in line with their multifunctional performance, manner of work etc. Using antioxidants as drug candidates for several diseases, to develop functional foods, to strengthen performance and improve functions of various cosmetics etc. have been the subjects of crucial studies for years and number of these kind of studies is increasing day by day.

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## CHAPTER 3

### COMPUTATIONAL CHEMISTRY FOR ANTIOXIDANTS

**\* The chapter is translated from a master's thesis.**

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## **INTRODUCTION**

In line with the developments in the fields of hardware and software, there has been an expressive increase in the power of calculations performed on computers in recent years (Galano & Alvarez-Idaboy, 2013). Accordingly, the accuracy and reliability of studies based on quantum mechanics are gradually increasing (Galano et al., 2016). With these rapid developments observed in microprocessor technology, the number of scientists who also perform theoretical calculations in their scientific studies continues to increase significantly, by means of the availability of advanced electronic structure programs (Feller, 1996). As a result of the increase in studies based on quantum mechanics, it has been possible to solve unsolved questions related to difficult chemistry problems involving processes such as solid state physics, various biochemical reactions, high temperature superconductivity and transition metal catalysis (Galano et al., 2016; McArdle et al., 2020). In addition, it is thought that by using quantum systems as simulation platforms, it will also be possible to find answers to unsolvable classical problems in physics and materials sciences along with chemistry. Basis of the idea is the important contributions of classical simulations to rationalizing experimental results, testing physical models, and understanding system properties (McArdle et al., 2020).

With the computational chemistry methods developed in line with quantum mechanics, it is possible to determine various chemical reactivity indexes, electronic and physicochemical properties of compounds without experiments (Günay et al., 2011; Morales et al.,



2012). This state is crucial since it is not possible to determine various information such as dipole moment, ionization potential, heat of formation, electron charge, molecular structure and bond lengths with a single experimental method (Günay et al., 2011). In a such way, computational chemistry methods also provide an opportunity to understanding of various chemical processes at the molecular level (Galano et al., 2016).

Computational chemistry, which is widely used in the pharmaceutical industry to explore the interactions of various biomolecules with potential drugs, as well is used in materials science to investigate the properties of many solids such as plastics. In addition, methods of computational chemistry are also used in order to examine many important reactions in various fields (Lewars, 2016). It is also possible to detect and design many new compounds of scientific and industrial importance with high-precision quantum simulations performed through developing computational chemistry methods (McArdle et al., 2020). Computational chemistry, which is developing at a very fast pace, has many advantages such as being specific, being faster and environmentally safe compared to experimental studies, and providing a wide variety of reliable data at little cost (Galano et al., 2016; Galano & Alvarez-Idaboy, 2013; Lewars, 2016; McArdle et al., 2020; Morales et al., 2012).

## **1. HISTORICAL DEVELOPMENT OF THE COMPUTATIONAL CHEMISTRY**

The history of computational chemistry, which was developed based on quantum mechanics, back to the 1920s. After 1927, ideas about the applications of the newly developed quantum theory to unsolvable problems in the field of chemistry, to researches on chemical bonds, to molecular structures and spectra have been begun to emerge (Lipkowitz & Boyd, 2002). Also in 1929, Arthur Haas has been first used the word "Quantum Chemistry (Quantenchemie)" in his presentations at the Chemical-Physical Society (Lipkowitz & Boyd, 2002).

Hans GA Hellmann, who lived in Hannover, Germany and was very close to the development center of this new field, has an important place in the history of computational chemistry due to his scientific achievements that enabled the development of quantum chemistry. In the mid-1950s, Hermann Hartmann, living in Frankfurt, have been pioneered the introduction of theoretical chemistry to the chemistry community almost until the mid-1960s, through his textbook as titled "Chemical Bond Theory (Theorie der chemischen Bindung)". By virtue of the international exchange of ideas that started in these years, the application of quantum mechanics to unsolvable problems in the field of chemistry have been begun to be considered in the 1960s. In 1961, a central computer that open to all German universities have been established in Germany in order to carry out studies based on quantum mechanics. Furthermore, in the second half of the 1960s, training in the field of computational chemistry has been begun with the addition of a

few topics related to theoretical chemistry to the education programs in order to teach some theoretical concepts to chemistry students at universities. In this direction, the theoretical chemistry program, which was the only program in the branch of chemistry among the 14 priority programs accepted by the German Research Foundation in 1966, was one of the most successful priority programs and has been attracted a lot of attention (Lipkowitz & Boyd, 2002).

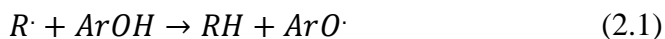
By the mid-1970s, researchers conducting experimental studies noticed the great progress in theoretical studies; some of these researchers have been tended to cooperate with researchers conducting theoretical studies while others have been defied with theorists. The field of computational chemistry, which developed quite in the 1980s, has been attracted the attention of many young students because it provides a combination of computer use and chemical research. In these years, international use of computer programs in molecular modeling studies has been become possible. By the middle of these years, molecular mechanics (MM), molecular modeling, molecular dynamics (MD), and some ab initio applications have been become important mediators in the examination of quantitative structure-activity relationships (QSAR) in industry (Lipkowitz & Boyd, 2002). Computational chemistry, which developed very rapidly after those years, is accepted as an interdisciplinary field nowadays (McArdle et al., 2020). In addition, it is also known that subjects that cannot be explained by experimental studies in many fields are easily and cost-effectively clarified with computational chemistry studies and reliable results are obtained

(Galano & Alvarez-Idaboy, 2013; Günay et al., 2011; Morales et al., 2012). As quantum hardware develops today, it is even claimed that studies based on quantum mechanics will be more accurate than experimental studies in the future (McArdle et al., 2020).

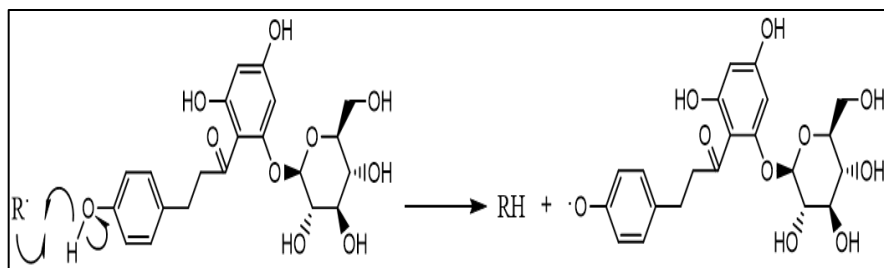
## **2. THE RELATIONSHIP BETWEEN MECHANISMS OF ACTION OF ANTIOXIDANTS AND COMPUTATIONAL CHEMISTRY**

### **2.1. Hydrogen Atom Transfer (HAT) Mechanism**

The Hydrogen Atom Transfer (HAT) Mechanism, which has an importance in chemistry in general, keeps going with the transfer of an hydrogen (–H) atom from the compounds with antioxidant activity (ArOH) to a free radical by attack of the free radical, then proceeds with inactivation of the free radical and radicalization of the ArOH compound (in the form of ArO·) (Leopoldini et al., 2011; Rimarčík et al., 2010; Xue et al., 2012, 2014). Since the radical compound formed as a result of the HAT reaction can be stabilized by various factors, it is less reactive than the reacting free radical (Leopoldini et al., 2011). The reaction is indicated by equation 2.1.



The HAT reaction of phlorizin, one of the phenolic compounds that has antioxidant activity, with any free radical is shown in Figure 1 as an example. Phlorizin will be the model compound in the chapter.



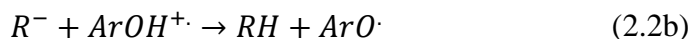
**Figure 1:** HAT Reaction of Phlorizin with a Free Radical

The antioxidant activities of compounds is related to the energetic parameter, bond dissociation enthalpy (BDE), which is the indicator of the suitability of relative O-H, N-H or S-H bonds etc. to transfer  $-H$  atom in the HAT mechanism (Lu et al., 2014; Rimarčík et al., 2010). The low BDE value indicates that the related bond is weak and thus the  $-H$  atom transfer between the antioxidant compound and the free radical occurs rapidly (Leopoldini et al., 2011; Wright et al., 2001). As a result, compounds with low BDE value have higher antioxidant activities (Xue et al., 2012, 2013, 2014; Zheng et al., 2017).

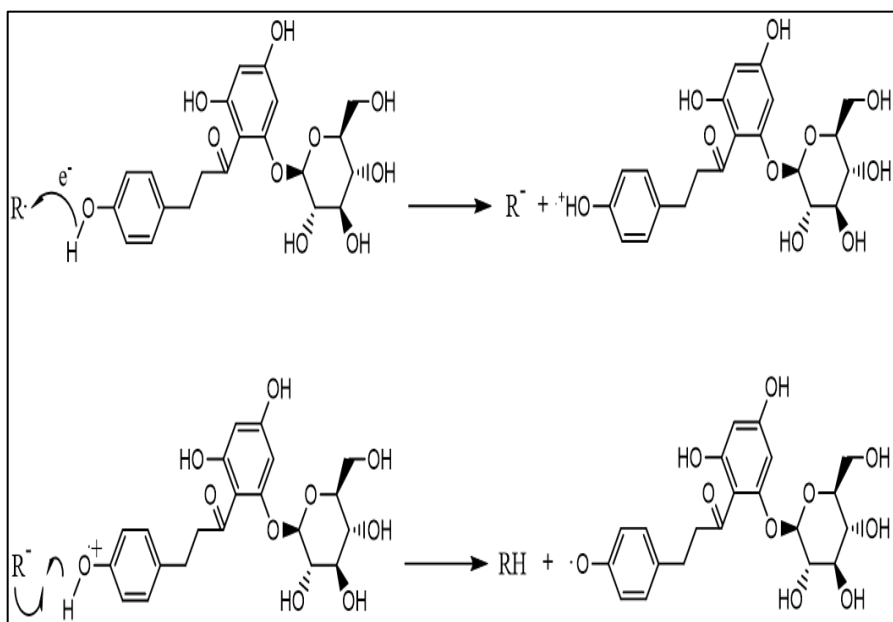
## 2.2. Single Electron Transfer – Proton Transfer (SET-PT) Mechanism

The Single Electron Transfer - Proton Transfer (SET-PT) Mechanism, which is one of the reaction mechanisms that results in the inactivation of free radicals by compounds with antioxidant activity, proceeds with a proton transfer from the radical cation ( $ArOH^+$ ) that formed as a result of the removal of an electron from an antioxidant compound ( $ArOH$ ) by the free radical, to the anionized free radical (Xue et al., 2012, 2014; Zheng et al., 2017). As a more general statement, first of all as a result of electron transfer from an antioxidant compound, the formation of a

radical cation of this compound occurs, and then deprotonation from the formed radicalic cation is observed (Rimarčík et al., 2010; Wright et al., 2001). Since the radical cation formed in the first step of the SET-PT mechanism is stable, it is less reactive than the initial free radical and therefore does not react with other molecules (Leopoldini et al., 2011; Xue et al., 2012). The steps of the mentioned SET-PT mechanism are indicated by equations (2.2a) and (2.2b).



The SET-PT mechanism of the model compound with any free radical is shown in Figure 2 as an example.

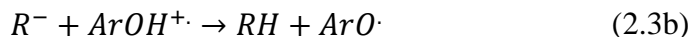


**Figure 2:** SET-PT Mechanism of Phlorizin with a Free Radical

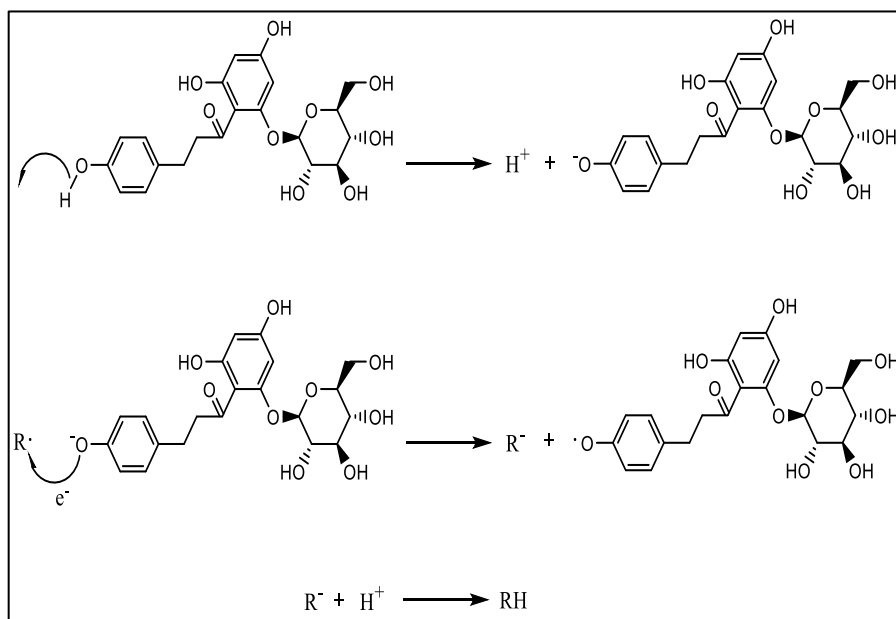
In the SET-PT mechanism, the antioxidant activities of the compounds are related to the ionization potential (IP), which is an indicator of their electron donating abilities, and the proton dissociation enthalpy (PDE), which expresses the acidity of the radical cation formed in the second step of the mechanism (Rimarčík et al., 2010; Stepanić et al., 2013; Xue et al., 2012, 2014; Zheng et al., 2017). It is understood that the lower IP value of a compound under investigation, the easier electron loss will occur from this compound and the easier this compound will react with free radicals (Leopoldini et al., 2011; Lu et al., 2014). As a result, compounds with lower IP and PDE values have higher antioxidant activity (Xue et al., 2013, 2014; Zheng et al., 2017).

### **2.3. Sequential Proton Loss Electron Transfer Mechanism**

The Sequential Proton Loss Electron Transfer (SPLET) Mechanism is another reaction mechanism associated with antioxidant activity (Zheng et al., 2017). This reaction mechanism takes place in 3 steps. In the first step, proton loss is observed from a compound with antioxidant activity and the compound comes to the anion form. In the second step, electron transfer from the anionic antioxidant compound to the free radical takes place associated with the reaction of a free radical with the antioxidant that in the anionic form, thus the free radical comes to the anionic form. As for in the last step, the free radical, which becomes anionic in the second step, combines with the proton, which separated from the antioxidant compound in the first step, and thus the free radical becomes inactive (Enisoğlu Atalay & Ayık, 2019). These steps of the SPLET mechanism are indicated by equations (2.3a) - (2.3c).



The steps of the SPLET mechanism that the model compound will perform with any free radical are shown in Figure 3 as an example.



**Figure 3:** SPLET Mechanism of Phlorizin with a Free Radical

In the SPLET mechanism, the proton affinity (PA) of the antioxidant in the anionic form, which is the reaction enthalpy of the first step, and the electron transfer enthalpy (ETE), which is the reaction enthalpy of the second step, are related with the antioxidant activities of the compounds (Lu et al., 2014; Rimarčík et al., 2010; Xue et al., 2014; Zheng et al., 2017). It is known that compounds with lower PA and ETE values have higher antioxidant activities (Zheng et al., 2017).



### 3. SOME PARAMETERS USED IN ANTIOXIDANT ACTIVITY ANALYSIS BY COMPUTATIONAL CHEMISTRY

#### 3.1. Molecular Descriptors

Molecular descriptors, which are closely associated with molecular structures, are based on various theories such as quantum chemistry, information theory, organic chemistry, and graph theory. Molecular descriptors have been used for many years to model many different properties of chemical structures in a wide variety of scientific fields such as analytical chemistry, toxicology, physical chemistry, pharmaceutical and environmental chemistry. For example, some molecular descriptors, processed by various methods provided by fields such as statistics, chemometry and chemoinformatics, are used to identify structural features or new candidate drug molecules responsible for various biological activities.

Molecular descriptors, which are obtained rapidly by converting the chemical information naturally encoded in the symbolic chemical forms of the compounds into numerical form as a result of logical and mathematical operations, are also very useful in better understanding and interpreting the experimental results (Todeschini & Consonni, 2009).

Various molecular descriptors obtained by computational chemistry methods are also used in the interpretation of antioxidant activities of compounds. For instance, ionization potential (IP), electron affinity (EA), electronegativity ( $\chi$ ), chemical hardness ( $\eta$ ) and softness (S), chemical potential ( $\mu$ ), and electrophilicity ( $\omega$ ) are various molecular

descriptors that are used in many studies to determine and compare the antioxidant activities of diverse compounds. Electron affinity, one of these molecular descriptors, is defined as the ability of a molecule to definitely accept an electron from a donor (Parr et al., 1999). Electronegativity, on the other hand, is quantitatively defined as the tendency of an atom in a compound to attract electrons in a chemical bond (Iczkowski & Margrave, 1961; Sadasivam & Kumaresan, 2011). Electrophilicity index, which is another descriptor of the ability of compounds to attract electrons, also measures the tendency of a compound to absorb electrons (Parr et al., 1999; Sadasivam & Kumaresan, 2011). Although the electrophilicity index is related to electron affinity, it is not exactly the same. The reason is that the electron affinity reflects the ability of a compound to accept only one electron from its surroundings, while the electrophilicity index reflects electron flows between a donor and acceptor, which may be more or less than one (Parr et al., 1999). For compounds, the low levels of these three molecular descriptors indicate that the compounds can donate electrons during the oxidation-antioxidant action process, while the high levels indicate that the compounds are highly related to electrons and their ability to donate electrons is low.

As for the ionization potential, which is another molecular descriptor, expresses the minimum energy required to remove an electron from a compound (Günay et al., 2011). Accordingly, it is easier to separate electrons from compounds with low ionization potential and these compounds have higher antioxidant activity (Mohajeri & Asemani, 2009; Santos et al., 2020). Chemical hardness, on the other hand, refers

to the resistance to charge transfer within the compounds (Günay et al., 2011; Sadasivam & Kumaresan, 2011). Compounds with low chemical hardness values are highly polarizable, whereas compounds with high hardness values cannot be easily polarized since there is little or no internal charge transfer (Günay et al., 2011; Pearson, 1988). This molecular descriptor is mostly used in the fields of acid-base chemistry and inorganic chemistry (Lipkowitz & Boyd, 2002).

### **3.2. Molecular Electrostatic Potential**

Electrostatic potential is a three-dimensional local property that can be evaluated at any or all points in the universe of a system (Murray & Politzer, 2011). Electrostatic potential, a measure of charge distribution, is a chemical reactivity guide that provides useful information for interpreting and predicting the reactive behavior of many different chemical systems in electrophilic and nucleophilic reactions, biological processes and research involving interactions with hydrogen bonding (Lewars, 2016; Politzer & Murray, 2004). For instance, the Molecular Electrostatic Potential Map (MEP), which is created so as to show the electrostatic potential in the outer regions of a compound, guides the understanding of how that compound will exhibit reactive behavior against reactants, especially in non-covalent interactions (Murray & Politzer, 2011).

The reactivity information of the compounds is understood by the color codes on the MEPs (Uğurlu, 2019). The red regions on the MEPs indicate the electron-rich, negatively charged electronegative regions in that compound while the blue colored regions indicate the electron-poor

positively charged regions (Günay et al., 2011; Uğurlu, 2019; Wang et al., 2017). The color scale proceeds from the most electronegative regions to the most electropositive regions in the direction of electron concentration in MEPs as red-orange-yellow-green-blue. The red regions in a compound represent the regions that can form chemical bonds with various reactants by donating electrons while the blue regions represent the regions that can react by gaining electrons from the reactants (Khamees et al., 2019). Therefore, MEPs are very convenient in observing the regions that will show antioxidant activity in compounds.

### **3.3. Molecular Orbital Energy**

By visualizing molecular orbitals, as in MEPs, the positions of the regions where the highest energy electrons are concentrated, that is, the Highest Occupied Molecular Orbitals (HOMOs) and the Lowest Unoccupied Molecular Orbitals (LUMOs) can be determined (Lewars, 2016). These molecular orbitals are very important since they are the basic orbitals of the compounds that participate in chemical reactions (Günay et al., 2011). For example, while the  $E_{\text{HOMO}}$  value of a compound expresses the electron donating ability of the compound, it is related with the ionization potential of the compound (Günay et al., 2011; Lipkowitz & Boyd, 2002; Sadasivam & Kumaresan, 2011). The  $E_{\text{LUMO}}$  value of a compound is related to the electron affinity of the compound and expresses its ability to acquire electrons (Günay et al., 2011; Lipkowitz & Boyd, 2002). The gap between the  $E_{\text{HOMO}}$  and  $E_{\text{LUMO}}$  values of the compounds ( $\Delta E = E_{\text{LUMO}} - E_{\text{HOMO}}$ ) is related to their

chemical stability (Günay et al., 2011). A low  $\Delta E$  value of a compound indicates that the compound is unstable, and the lower this value, the easier the compound can react and donate electrons (Günay et al., 2011). Therefore, compounds with antioxidant activity have low  $\Delta E$  values, and antioxidant candidates should also have low  $\Delta E$  values.

## CONCLUSION

Computational chemistry is a developing area in line with developments in the computer science since it depends on the quantum mechanics. Quantum mechanics have been had a crucial role in solvation of many problems in chemistry for years. Nowadays, studies with computational chemistry methods are in demand due to different factors such as low cost, quickness, safeness, high-precision etc. Furthermore, increasing studies also induce increase of the accuracy and reliability of the methods. With these methods it is possible to investigate chemical processes at the molecular level, know diverse chemical, electronic and physicochemical properties of compounds by kinds of computations.

In light of the above-mentioned advantages, computational chemistry is quite helpful for studies on antioxidant activities. Modeling of reaction mechanisms related to antioxidant activity and calculation of some parameters used in antioxidant activity analysis is also possible through computational chemistry, and therefore, the interpretation of antioxidant activities of compounds can be done without doing experiments.

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**CHAPTER 4**

**THE ASSOCIATION OF NEURODEGENERATIVE DISEASES  
WITH OXIDATIVE STRESS AND THE ROLE OF  
ANTIOXIDANTS**

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## **Introduction**

Neurodegenerative diseases like Parkinson's disease (PD), Alzheimer's disease (AD), and Amyotrophic Lateral Sclerosis (ALS) are clinically important motor neuron diseases. According to many experimental studies in this field, the risk factors include advanced age, genetic disorders, antioxidant enzyme abnormalities, autoimmunity, cytoskeletal unusualness, oxidative stress, lack of some minerals, toxicity of metabolism, hypertension and other vascular disorders. These disorders are classified according to their clinical manifestations. The most common ones are extrapyramidal-pyramidal movement disorders and cognitive or behavioral disorders. Most patients have complex clinical features whereas few patients have uncomplicated syndromes (Dugger & Dickson, 2017).

The insufficiency of the antioxidant protective defending system exposes the central nervous system (CNS) to oxidative damage. Therefore, factors such as high utilization rate of oxygen, polyunsaturated fatty acid contents that are highly oxidizable and metal ions, which are redox-active transition, can cause oxidative impairment in the brain. Diseases that are more common in old age, for instance AD, PD, and ALS may be incorporated with oxidative stress which is a significant age-related factor that makes the brain susceptible to neurodegenerative diseases (Casetta et al., 2005).

## **1. Effects of Oxidative Stress and Antioxidant Defense Mechanism on Several Neurodegenerative Diseases**

Antioxidant defense mechanisms are insufficient against excess free radicals and products. The oversupply of free radicals produced, or the insufficiency of the antioxidant defense mechanism leads to this imbalance. Free radicals are molecules that contain non-pairing electrons, commonly mentioned as reactive oxygen species (ROS). ROS can co-action with biological compositions like nucleic acids, proteins, lipids, and change the structures and functions of these biomolecules. Oxidative stress which refers to the gathering of oxidized products, can create some changes in cells, such as extension of bases from DNA oxidation, extension of iso-prostanes and aldehydes, which are products of lipid peroxidation in cells, and extension of protein carbonyls, which are products of protein oxidation (Markesbery, 1999). Lipid peroxidation is an important determinant of oxidative stress, and unsaturated lipids are more susceptible to oxidative change.

Free radicals attack the double bond of unsaturated fatty acids like arachidonic acid and linoleic acid. The purpose of this assault, called lipid peroxidation, is to create highly reactive lipid peroxy radicals, creating a chain reaction that initiates other assaults. The products of the chain reaction are cleavage products including acrolein, malondialdehyde, 4-hydroxy-2,3-nonenal (HNE), and F2-isoprostanes. These products are effective in the formation of neurodegenerative illnesses. For instance, high HNE levels were determined in PD and AD

brain tissues, while increased HNE was determined in the cerebrospinal fluid (CSF) of ALS cases (Arimon et al., 2015).

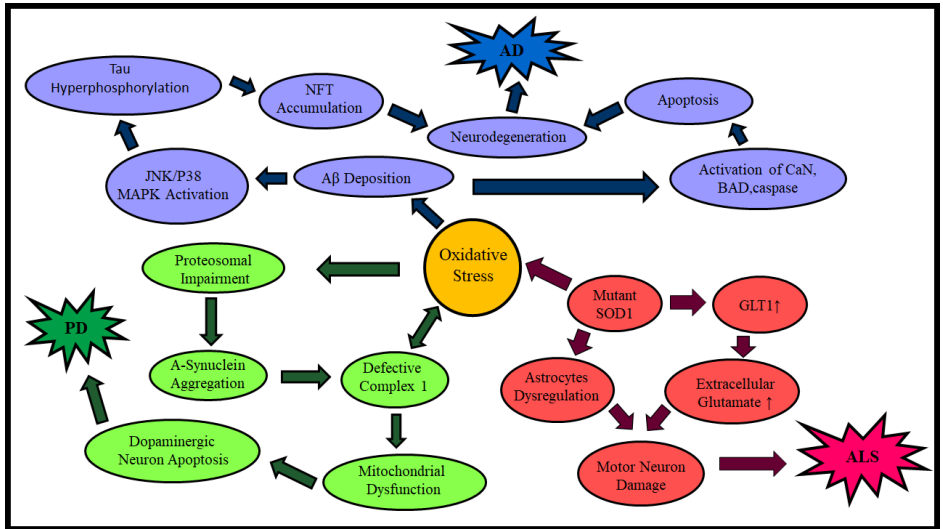


Figure 1: Key role of Oxidative stress in the development of neurodegenerative diseases

Antioxidants are responsible for preventing the detriment caused by oxidative stress to proteins, neuronal DNA and lipids. In more detail, antioxidants have neuroprotective effects through various biochemical events like interactions with transient metals, nonviolence of free radicals, regulation of activation of different types of enzymes, and intracellular signaling pathways and gene expression. (E. Obrenovich et al., 2012) Antioxidants include enzymes such as retinoic acid, glutathione, CAT, ascorbic acid, lipoic acid, glutathione peroxidase, SOD, tocopherol, and phytonutrients such as carotenoids and flavonoids.



### **1.1. Alzheimer's Disease and Oxidative Stress**

AD is one of the most challenging neurodegenerative diseases that generally involve a social impairment, accompanied by both emotional and behavioural abnormalities, resulting in memory loss, language, and some other cognitive functions. The main reason why AD occurs is unknown; nevertheless, it has been linked to several genetic disorders. ROS are present in the pathophysiology of the disease, along with disruptions in calcium-dependent potassium channels and basal apoptotic activity in the development of AD (Halliwell & Gutteridge, 1985).

During metabolic activities, oxidative damage in aging occurs because of cumulative damage to macromolecules caused by the long-term pro-oxidant presence. Protein oxidation, nucleic acid oxidation, advanced glycation end products, lipid peroxidation, and some other biochemical markers describe the oxidative stress that AD brain is exposed to (Floyd & Hensley, 2002). Figure 1 shows the oxidative stress and other mechanisms that cause AD.

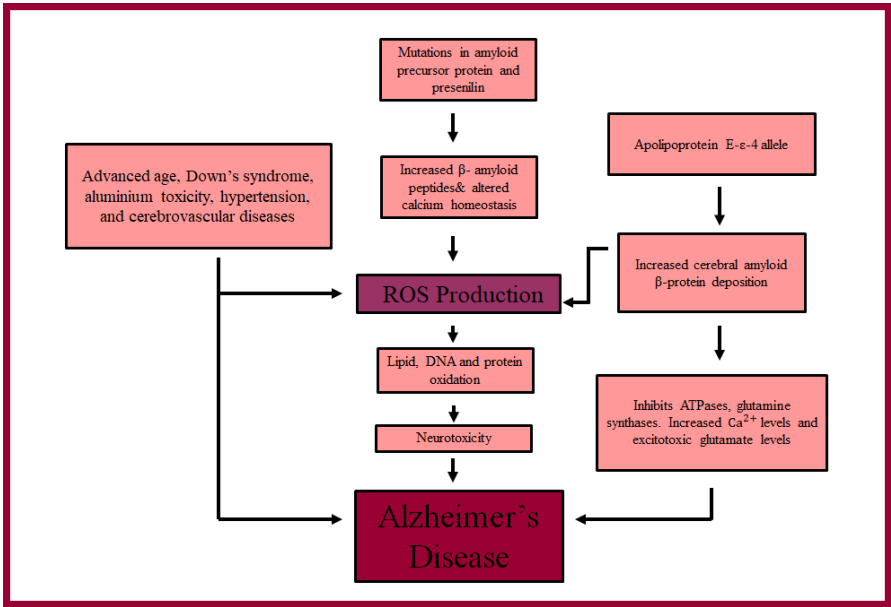


Figure 2: Oxidative Stress and other mechanisms that cause AD.

### 1.1.1. Lipid Peroxidation

Lipid peroxidation is quite common in AD brains. Substances that react with thiobarbituric acid take up more specifically in the piriform cortex and hippocampus in almost every part of the AD brain. When the brains and cerebrospinal fluid (CSF) of AD patients were examined in proportion to age-matched controls, it was observed that the polyunsaturated fatty acids' the product levels of reaction of free radical oxidation are ascended. These products are isoprostans and neuroprostanes. As predicted, the reduction in polyunsaturated fatty acids for instance arachidonic acid and docosahexaenoic acids are primarily companions of lipid peroxidation (Markesbery & Carney, 1999).

The formation of highly reactive aldehydes, for instance, acrolein and 4-hydroxynonenal (HNE) is a result of lipid peroxidation. Because of their high cytotoxicity levels, these alkenes cause deterioration and demise in cultured neurons in hippocampus. Protein structures must be changed to achieve a covalent bonding through the Michael reaction. This effort inactivates enzymes with functional proteins such as Na / K-ATPase, alpha-chetoglutarate dehydrogenase and glutamate transporter Glt-1, disrupting calcium balance and reducing brain metabolism. Increasing levels of protein bound and free HNE have been declared in CSF of people with AD. Due to the ascent in protein carbonyl content, the decrease in creatine kinase and glutamine synthetase enzyme activities, especially in the frontal lobe and occipital of patients with Ad, has left question marks on these enzymes' oxidative inactivation (C. D. Smith et al., 1991).

Oxidative alterations in glutamine synthetase can increase creatine kinase enzyme activity, which can impair energy metabolism, as well as increasing excitotoxicity. HNE protein extensions were observed in the NFT together with amyloid plaques as covalently cross-linked. The oxidative alterations of A $\beta$  and Tau proteins, which are the end products of the advanced glycation reaction (AGE), has a great effect on AD development. Tau and A $\beta$  antigens found in NFTs are located together. The presence of hemeoxygenase-1, which is an antioxidant enzyme in NFTs, and nitro tyrosine, a marker of strong radical peroxy nitrite, add to the role of oxidation damage in NFT formation (M. A. Smith et al., 1995).

### **1.1.2. A $\beta$ Deposition**

A $\beta$  accumulation has an important effect on AD pathogenesis. It is created by a proteolytic process of the amyloid precursor protein (APP), which is a membrane protein (Butterfield et al., 2001). Hypothesis of amyloid cascade, which demonstrates the role of high-dose A $\beta$  production and high levels of A $\beta$  accumulation in neurodegeneration, was driven by genetic evidence suggested by familial AD cases. Other genetic causes such as the missense mutations found in presenil 1 and 2 can be suggested as well. The cause for the excessive accumulation of AB42, which is more fibrillogenic than AB40 in AB plates, can be attributed to the mutations of APP and presenile (Gupta et al., 2010).

Some effects of A $\beta$  accumulation, such as the inflammatory response triggered by A $\beta$ , produce the formation of oxidative damage and free radicals of lipids and proteins. Free radicals cause damage in A $\beta$  toxicity. This damage is suppressed with some antioxidants such as free radical scavengers, the spin-trap compound PBN (alpha-Phenyl-N-tert-butyl Nitron), lazaroids, Vitamin E, and curcumin. In non-genetic AD cases, there is not enough information about how the mechanism that causes A $\beta$  accumulation works. Due to the split of APP, it becomes A $\beta$  production. Abnormal interactions of high levels of copper, iron, and zinc metal ions in A $\beta$  deposits result in A $\beta$  precipitation and accumulation. The function of the zinc ion here is to be responsible for the precipitation of A $\beta$  into amyloid aggregates that are resistant to the protease enzyme. In addition to zinc metal, Copper and iron also form A $\beta$  accumulation under certain specific conditions. Hypermetallation of

A $\beta$  provides catalysis of hydrogen peroxide synthesis, which contributes to oxidative toxicity. Cu and Fe are the main resources of free radical production in the brain. A $\beta$  and APP can bind Cu (II) strongly in vitro, also can abate it to Cu (I). In a region rich in N-terminal cysteine, such as growth factor, the APP-Cu binding domain (CuBD) stands (Rossjohn et al., 1999). APP is part of the multigene family derived from the Cu-metallized human brain, which contains paralogous amyloid precursor-like proteins such as APLP1 and APLP2. These proteins oxidize the low-density lipoprotein (LDL) of full-length APP ectodomain and induce neuronal cell killing in vitro (White et al., 2002). LDL is important for its role in the combination of membrane cholesterol and lipoproteins in AD and can induce APP Cu neurotoxicity through Cu (I) mediated oxidation. Histidine remnants H147, H149 and H151 affect APP-mediated Cu toxicity. The ability of non-mammalian APP orthologs to potentiate Cu-mediated toxicity thanks to its well-preserved central histidine site highlights the significance of the origin histidine site in APP Cu toxicity. Replacement of histidine remnants H151 and H147 for lysosine and tyrosine in APP CuBD, respectively, increase the protective properties of these peptides in their phenotype. In addition, the effect that would drastically alter the oxidative potential of APP are inhibitors at regions 147 and 151 of APP targeted to histidine residues. Cu reduction was reduced in histidine-alanine substituted mutant APP 147-151 peptides (Ruiz et al., 1999). APLP1, eAPP, APPL and APL-1 genes all produced non-toxic preservative activities in the entity of Cu. This is because each has different residues within the histidine domain. The high protection

created against Cu toxicity APL-1CuBD is proof for A $\beta$  (1-42) related to ROS production in in vitro and in vivo for oxidation reaction of protein (Yatin & Link, n.d.).

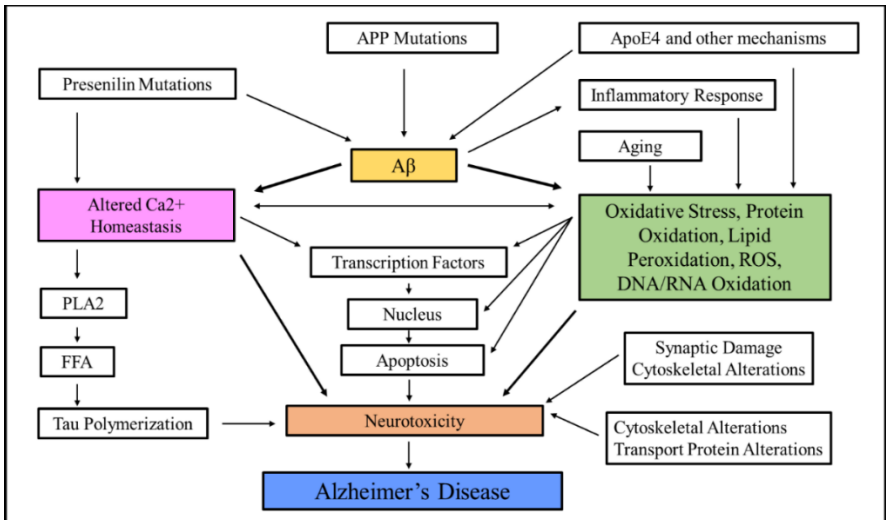


Figure 3: The Role of A $\beta$  Deposition in Alzheimer's disease

### 1.1.3. ApoE Gene

In the A $\beta$  (1-42) model, which has a high amount of increased protein oxidation and provides expression of animals, methionine cause cysteine to mutate. This event leads to lipid peroxidation and cell death thus causing AD with membrane dysfunction of different systems (Yatin & Link, n.d.). The Apolipoprotein E (ApoE) gene, which is identified by AD and found in brain plaques and neurofibrillary tangles of normal brains, is transferred as one of three alleles, mainly  $\epsilon$ 3,  $\epsilon$ 2, and  $\epsilon$ 4. The  $\epsilon$ 4 allele is expressed as the "gene dose" due to its potential to develop AD in older adults (Namba & Ikeda, 1991). On the other hand, it has been stated that the  $\epsilon$ 1 and  $\epsilon$ 2 alleles have a protective effect

against the risk of AD development. The compactness of the  $\epsilon 4$  of the ApoE gene is higher in AD, which is genetic, sporadic and appears subsequently (Strittmatter et al., 1993). The depredation caused by oxidative stress in the AD brain's frontal cortex is considerably greater. This damage is caused by the ApoE genotype and ROS. The level of lipid peroxidation is higher in tissue samples with homozygous AD taken for the  $\epsilon 4$  allele of the ApoE gene compared to  $\epsilon 3 / \epsilon 3$  cases and tests. Deficient antioxidant protection contributes to  $\beta$ -amyloidosis in the  $\epsilon 4$  allele. Protein carbonyl formation caused by oxidative stress is higher in vascular smooth muscle samples collected from the human brain with the ApoE genotype  $\epsilon 4 / \epsilon 4$  compared to  $3\epsilon / \epsilon 3$  and  $\epsilon 3 / \epsilon 4$  (Mazur-Kolecka et al., 2002).

Considering studies on knock-out mice, the ApoE gene, a high-density lipoprotein component secreted by astrocytes, which is part of the central nervous system, has been found to cause accumulation of amyloid beta protein at the cerebral level. ApoE causes neuronal death due to the absorption of this gene by neurons and the lysosomal gathering of  $A\beta$  protein and APP moieties which is amyloidogenic (Tomiya et al., 1999).

## **1.2. Alzheimer's Disease and Antioxidants**

The pathology of Alzheimer's disease includes the formation of extracellular senile plaques, neuronal and synaptic loss, and intracellular neuro-biliary nodes in the brain. Oxidative stress is effective in the adaptation of multiple cells signaling pathways that subscribe to nascence of lesions, although oxidative stress constitutes

the early stage of AD. Antioxidant cures are generally successful in preclinical studies. Some types of antioxidants contribute to the breakdown of intracellular and extracellular superoxide radicals, which are by-products of ordinary cells, thanks to their action as the intracellular second messenger of microglia before the radicals' damage cells. Considering the pathogenesis and mechanisms related to oxidative damage underlying AD, the existing network between varied antioxidants, and the correlation between pro-oxidant and antioxidant factors, potential antioxidant therapeutic methods in the future of neurodegenerative diseases can be gained (Butterfield et al., 2008).

### **1.2.1. Vitamin E and Vitamin C**

Vitamin E ( $\alpha$ -tocopherol) and Vitamin C (ascorbic acid) are widely used antioxidants in disease treatments. These supplements delayed functional impairment in patients with moderately advanced AD (Sano et al., 1997). In the light of these data, it may be possible to reduce the risk of AD if vitamin C along with large doses of vitamin E is given to AD patients. In immature samples of cerebellar granule cells, induction of apoptosis and nuclear fragmentation, DNA laddering, and chromatin condensation occurs when neuronal cells are exposed to S-nitroso glutathione (GSNO), NO donor, or sodium nitroprusside (SNP). In addition, the pretreatment provided by free radical scavengers such as L-ascorbic acid potassium salt (EPC-K1), which is an integration of E and C vitamins, alleviated mitochondrial dysfunction with NO-induced oxidative stress, as well as protecting cells from apoptosis. Oxidative stress due to superoxide/peroxynitrite may cause NO-related neuronal



damage and cause induced with NO neurotoxicity by breaking down the ROS and extra products of the EPC-K1 antioxidant. Due to its molecular structure, EPC-K1 has strong scavenging effects on both hydrophilic and hydrophobic radical groups. EPC-K1, besides being a highly effective inhibitor on lipid peroxidation, is a powerful scavenger on lipid radicals; at the same time, grade of scavenging on hydroxyl and alkyl radicals is plausible. Therefore, the cleaning performance of superoxide radicals in the rat brain is high. EPC-K1 can react with hydroxyl radicals and linoleic acid radicals (Wei et al., 1999).

Some studies have shown benefits for the prevention of AD when taken long-term in consolidation of vitamin C and vitamin E. It was thought that negative epidemiological studies were insufficient to detect the effect of vitamin C and vitamin E supplementation on cognitive impairment, and if there is a benefit, it would not mean much clinically because the number of negative studies in this area is relatively less than positive ones(Boothby & Doering, 2005).

### **1.2.2. Coenzyme Q10**

Coenzyme Q10 (CoQ10), which is involved in ATP synthesis and is in the mitochondria's inner membrane, is a component of the electron transport chain and is also known as ubiquinone. Since CoQ10 is a free radical scavenger, it has protective antioxidant activity in mitochondrial lipid membranes against ROS created by oxidative phosphorylation (Linnane et al., 2007). In one of the scientific studies conducted, it has been incorporated with the increased risk of developing dementia, considering the amount of cholesterol and the low serum CoQ10 ratio.

On the other hand, when these rates are examined in some studies, no big difference was found in CoQ10 levels between control group patients, patients with vascular dementia and AD patients. Even more, these trials demonstrated no difference in CoQ10 levels between clients with vascular dementia and ordinary clients. CoQ10 activity has been found to improve behavioral and cognitive activity in Alzheimer's transgenic mice, although it has no impression CSF biomarkers incorporated with A $\beta$  and tauopathy, on AD clients (Elipenahli et al., 2012).

### **1.2.3. Green Tea Polyphenols**

Green tea polyphenols are vigorous antioxidants against NO, lipid peroxidation and hydroxyl radicals. They contain flavonoids such as catechin and its derivatives. The basic ingredient of these bioactive chemicals is epigallocatechin-3-gallate (EGCG), which constitutes only 10% of the dry weight. Then comes epicatechin (EC), epigallocatechin (EGC), and epicatechin-3-gallate (ECG). When they are listed according to their antioxidant potential, this order is as EGCG>ECG>EGC>EC (Morris et al., 2002; Moyers & Kumar, 2004). The groups in the ring structures of the compounds in question, such as the group catechol on the B ring the group of hydroxyls on the A collet, and the gallate part on the C ring determine the scavenging potential of these substances on free radicals. Simultaneously, the hydroxyl groups in catechin have significant efficacy in determining the free radical scavenging. The antioxidant potential ranking is formed accordingly.

In neurodegenerative diseases, the use of green tea extract has been observed to reduce A $\beta$  generation and gathering rate when APP and A $\beta$  rate is overexpressed in mice and APP and A $\beta$  rate is overexpressed in neuronal cell cultures through exaggerated enzymatic functions of beta-secretase. EGCG, which is the most important flavonoid variety and obtained from green quarters, has prevented neuronal cell death in AD due to decreasing A $\beta$  aggregation, increasing beta & gamma sequetase activity thus weakening the amyloidogenic pathway (Lee et al., 2009). According to a study that used PC12 cells, EGCG extract obtained from grape skin had a protective effect against A $\beta$ -induced neurotoxicity. On the other hand, epicatechin and catechins obtained from grape seeds have an inhibitory effect on tau accumulation. Thanks to the interaction of some polyphenols with some metals like copper, a mechanism is activated that reduces A $\beta$  aggregation in neurons. The interaction of AB aggregates with copper increases ROS production (Adlard & Bush, 2006).

#### **1.2.4. Curcumin**

Curcumin, which has been used as a food ingredient and herbal medicine for centuries in Asia, is a non-flavonoid polyphenol type and is derived from a medicinal herb *Curcuma longa* Linn. Curcumin can neutralize reactive nitrogen species along with reactive oxygen species and protect the brain from oxidative damage. In recent studies, it has been reported that curcumin has anti-amyloidogenic effects as well as its powerful anti-oxidative and anti-purulent features. The usage of curcumin and reduced oxidized proteins significantly reduced

interleukin-1b, an elevated pro-purulent cytokine in the brain of AD transgenic mouse models. With low-dose curcumin treatment, the astrocytic marker GFAP was reduced, and with soluble/insoluble AB, plaque burden was also significantly reduced. However, there was no diminished change in APP grades in the membrane fraction. Despite these properties that make curcumin an up-and-coming agent in the suppression and cure of AD, no clinical trials have so far has been brought forward, as no definitive data have been obtained. Curcumin inhibits AB aggregation, which attaches forthrightly to amyloid, inhibition of fibril and oligomer constitution. Data from a study using aged animals showed low-dose inhibition and suppression of amyloid oligomer and fibril formation through the antioxidant, anti-inflammatory and anti-amyloid activities of curcumin in two animal models, supporting future clinical trials for the prevention and even treatment of AD (F. Yang et al., 2005).

### **1.2.5. Vitamin A and Carotenoids**

Vitamin A and carotenoids possess anti-oxidative protection and opposition to accumulation in experimental models. However, there are no clinical studies with sufficient data regarding the utilization of vitamin A and carotenoids in the remedy of NDD. levels of plasma and serum in Vitamin A,  $\alpha$ -carotene,  $\beta$ -carotene, lycopene and lutein in AD clients have been proclaimed in several studies, whereas alternative investigations have shown the collision results in plasma lutein,  $\alpha$ , $\beta$ -carotene, and lycopene grades in AD (Chang et al., 2018). High plasma B-carotene concentration strengthens the memory of elderly people,

while lycopene and lutein levels have also been consolidated with a diminished jeopardy of AD. According to animal experiments, incompetence of vitamin A collocates beta region APP degradation enzyme 1, regulated A $\beta$  production and nascency of neurotic plaque and thus intensify retention shortage in double transgenic APP-PS1 mice. Later recuperative proportion of vitamin A supplementation improved memory impairment caused by vitamin A deficiency (Zeng et al., 2017).

Vitamin A is known to lead inhibitory efficacy on the oligomerization of A $\beta$ , and decreased A $\beta$  accumulation and tau phosphorylation in the brain of APP-PS1 transgenic mice was observed after eight weeks of intraperitoneal treatment with vitamin A. In addition, in APP-PS1 mice treated with vitamin A, abated microglia and astrocytes' function, weakened neuronal degeneration, and improvements in learning and retention abilities were observed (Ding et al., 2008). Carotenoids have protective activity on lipid membranes through free radical capture and oxidation intervention. Treatment with carotenoids highly potent anti-oxidative bustle, showed several types of cognitive functions in healthy group of people whose level of forgetfulness gradually increased with advancing age, according to a randomized, double-blind, placebo-controlled study. However, multiple carotenoid supplements did not improve cognitive function in patients with advanced AD. Similarly, oral supplementation with lutein/zeaxanthin did not have a relatively significant influence on cognitive mechanism in double-blind randomized clinical trials (Nolan et al., 2015).

### **1.2.6. Lycopene**

Lycopene is a type of carotenoid with no pro-vitamin A activity,  $\beta$ -carotene's acyclic isomer, and a type of phytochemical antioxidant found in tomatoes, tomato products, and some other vegetables and fruits. Lycopene is found in tropical fruits like pink grapefruit, watermelon, apricot, and pink guavas as well as tomato and tomato products. It is one of the most powerful types of antioxidants, and its singular oxygen extinguishing ability is multiple and multiple of  $\beta$ -carotene and  $\alpha$ -tocopherol. According to some recent reports, lycopene has been shown to be effective in reducing  $A\beta$ -induced damage and improving cognitive function (Wang et al., 2019).

In the treatment with lycopene, the effect of tyrosine phosphorylation of insulin receptor substrate-1 to reduce IGF-I stimulation and the DNA lashing aptitude of the AP-1 transcription structure has been determined. These odors were consonant with the increment in membrane-associated IGF-binding proteins, thereby explaining that IGF-I signaling was suppressed by lycopene. Based on these data, advancement through the cell life revolution expedited by IGF-I was diluted by lycopene supplementation. However, no apoptotic or necrotic cell demise was observed due to this effect. In the light of epidemiological observations, there is considerable evidence to support the role of lycopene in the prevention of chronic diseases. However, only a few clinical dietary supplement studies have gone into the literature. According to some scientists, *in vivo* lipid oxidation increased by 25% and serum lycopene loss of 50% was observed with

diet without taking lycopene for fourteen days in ordinary human subjects (Rao & Agarwal, 1998). Lycopene is decreased in vascular dementia. Patients with AD have some extent of impairment in antioxidant equilibrium with supplementation of oxidative stress antioxidants like lycopene. One of the studies shows that in addition to the influence of lycopene on the attenuation of A $\beta$ -induced oxidative stress and apoptosis, lycopene also contributes to  $\beta$ -secretase (BACE) expression (Fang et al., 2020). Other studies on mice receiving lycopene supplements show that lycopene concentrations in the CNS are lower than most other tissues. In addition to the fact that NDD is a major health problem, little has been learned about lycopene's role in relieving pain against NDD (Clinton, 1998). Explorations have disclosed that subjects with AD or vascular dementia have a degree of discomfort that can lead to accelerated oxidative stress. The impressions of some antioxidants, such as lycopene, on neurons and oxidative stress are controversial.

### **1.2.7. Docosahexaenoic Acid and Eicosapentaenoic Acid**

Epidemiological studies have shown that excessive use of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are polyunsaturated fatty acid structures that are responsible for regulating oxidative metabolism and mitochondrial biogenesis in the brain, are associated with an increased risk of AD cultivation (Kitajka et al., 2002). Some other studies have shown that DHA was able to downregulate A $\beta$ -42 induced inflammation in human microglia. However, according to a double-blind randomized control study, DHA

had no slowing effect on the rate of cognitive and functional decline in AD patients (Quinn et al., 2010).

### **1.2.8. Myricetin and Quercetin**

Flavonoids are a group of polyphenolic complexes in the phytonutrient antioxidants category. The role of flavonoids is to be chelators of transition elements and scavengers of free radicals, and their structures are similar to that of Vitamin E. The absorption capacity of phenolic compounds such as flavonoids has not been fully explained. However, several flavonoids, such as quercetin found in red onion, can be absorbed in humans, and this absorption amount is directly proportional to the amount of glucose (Hollman et al., 1996). Polyphenols obtained from herbal remedies and nutrients such as forest fruits, spinach, tea, capias, garlic have protective effects against neurological disorders. Quercetin and other flavonoids such as catechin could inhibit neurodegenerative events such as chronic inflammation or cerebral skeleton due to the oxidative stress caused by NO production in cultured cell features such as fibroblasts and HT 22 cells. Quercetin and catechin attenuate the killing of hippocampus cells and intracellular ROS gathering, These data are presented based on experiments accomplished in cultured cells of the hippocampus, the region of the brain most susceptible to oxidative stress and highly effective in ischemia with AD (Furuta et al., 1995).

Ingredients of ginkgo biloba leaves are flavonoid glycosides and ginkgolides. They are utilized as a powerful lipid peroxidation inhibitor and an important type of free radical scavenger. Extraction of G.biloba



(EGB) has therapeutic efficacy against neurological disarrangements such as age-related dementia, AD, and sluggishness of the brain (Tan et al., 2015). The antioxidative efficacy of components such as myricetin and quercetin obtained from EGB and other flavonoids on rat cerebral neurons were investigated and it was explained that these components have the flair to advocate neurons as oxygen radical scavengers (Oyama et al., 1994). Myricetin and quercetin's antioxidative influence is related to the dose used since it produces semi-maximal inhibition at certain concentrations. The usage of EGB to cure cognitive deterioration in AD has been tried, and it has been accomplished that there is a significant effect, albeit small, on objective surveys of cognitive activity in AD when given a certain dose of EGB for a certain period. In addition to the evidence showing that EGB administration slows AD progression, it is concluded that the use of myricetin and quercetin in elderly humans and animals increase antioxidative activity, improving the cognitive performance in the brain and the Ca<sup>2+</sup> +  $\beta$ -induced increases in the oxidative metabolism of neurons (Kanowski et al., 1996; Kleijnen & Knipschild, 1992).

In one of the studies, the relationship of EGB components with lipid peroxidation and the relationship of these components with the activities of SOD and CAT, which are antioxidant enzyme types in the SN, hippocampus, and striatum of rats, were examined. As a result of the study, an increase in SOD and CAT enzymatic activities and a decrease in lipid peroxidation were spied upon in the substantia nigra (SN), hippocampus, and striatum (Bridi et al., 2001). Other studies have

represented the EGB effects, which affects AD, on hippocampal primary cultured cells against toxicity caused by A $\beta$  derived proteins such as A $\beta$ -25-35, A $\beta$ -40, and A $\beta$ -42. EGB protected hippocampal cells from toxicity caused by H<sub>2</sub>O<sub>2</sub>, involved in regulating A $\beta$  toxicity. In addition, this protection is maximized for neurons in hippocampus at the highest *G. biloba* concentration (Bastianetto et al., 2000).

### **1.2.9. Selegiline**

In Alzheimer's patients, selegiline, which can act as an antioxidant by reducing neuronal damage in the brain and inhibiting oxidative deamination, is a monoamine oxidase inhibitor and has been associated with increasing the lifespan of animals. It has been observed that short-term selegiline trials on AD patients made a significant contribution, albeit a small one, to the elimination of cognitive disorders. (Cai & Yang, 2020).

### **1.3. Parkinson's Disease and Oxidative Stress**

PD is a neurodegenerative disease expressed by progressive dopaminergic neuron decrement in the SN and dopamine (DA) incompetence in the striatum. Although the root lead to PD is not known exactly, dopaminergic neuronal degeneration due to oxidative damage is an important reason for PD. Treatment of the disease mainly includes DA substitution and adjuvant medical treatments that alleviate many motor symptoms (Ebadi et al., 1996). Due to aging, the number of dopaminergic neurons gradually decrease since they are particularly susceptible to oxidative damage. Free radicals, especially the most dominant types such as superoxide and hydroxyl, are products of

cellular metabolism. Some molecules are not free radicals but tend to form free radicals thanks to some functional chemical interactions. Examples of these are peroxynitrite and hydrogen peroxide (Jenner & Olanow, 1996). For this reason, the hydroxyl radicals called ROS are constituted by the Fenton reaction of H<sub>2</sub>O<sub>2</sub> in the existence of degraded metal, or by the breakdown of peroxynitrite, which is produced by the reaction of NO and superoxide. If the production of these biochemical molecules exceed the level at which the cells can defend themselves against these molecules, oxidative changes can be observed within the cell (Simonian & Coyle, 1996).

ROS could oxidize important biomolecules (like DNA, proteins and membrane lipids) and induce apoptosis or necrosis. While apoptosis creates nuclear changes such as chromatin condensation and margination, DNA fragmentation, cell shrinkage, and membrane swelling necrosis causes events such as disruption of plasma membrane entirety, constitution of large vacuoles and cell swelling. There is significant proof that neuronal loss of life in PD is principally due to the presence of apoptotic mechanisms. It has been revealed that there is a relationship between necrosis or apoptosis induction and ROS production and the pathogenesis of neurodegenerative disorders (Leonidas et al., 1997).

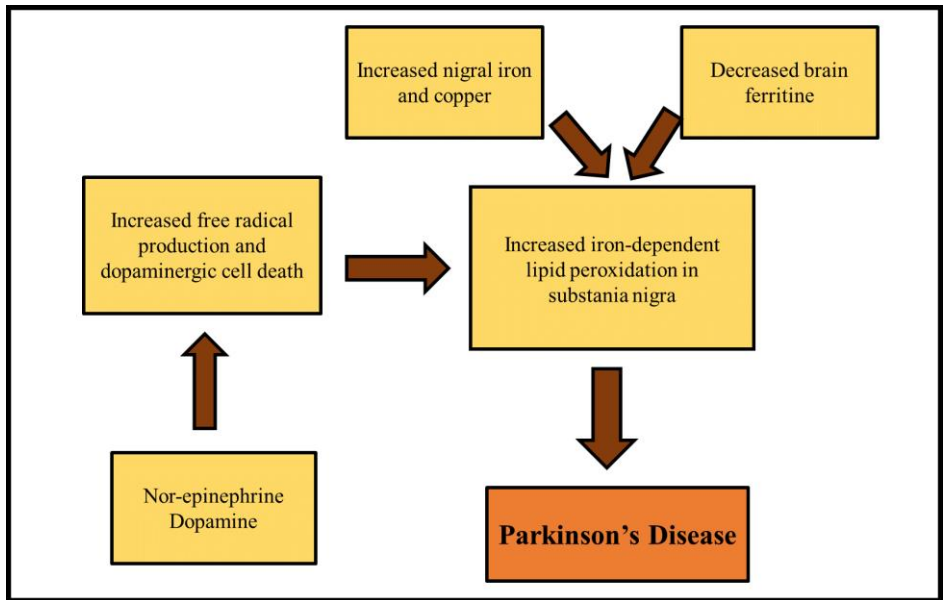


Figure 4: Oxidative Damage in Parkinson's Disease

### 1.3.1. NF- $\kappa$ B Activation

When the pathogenesis of PD is examined, there is also an indirect mechanism involving 4-HNE as an effect other than ROS damage. In addition, levels of 8-hydroxy-2'-deoxyguanosine, that is a DNA oxidation marker, and nitrotyrosine, that is a protein oxidation marker, are also quite high (Zhang et al., 1999). The proliferation of nuclear factor-kappa B (NF- $\kappa$ B), a transcription factor, is another indicator of oxidative stress. This proliferation enables the ROS to act as a secondary messenger aiding intracellular responses such as NF- $\kappa$ B activation. In addition, activated NF- $\kappa$ B is responsible for affecting the expression of several genes such as the SOD-2 gene. Additionally, The task of NF- $\kappa$ B is to incite the expression of inhibitors of apoptosis proteins such as calbindin and Bcl-2. At the neuronal level, NF- $\kappa$ B

activation occurs through different intercellular signals such as cytokines, neurotrophic factors, and neurotransmitters. The fact that these agents activate NF- $\kappa$ B is cited as the cause of oxidative stress. ROS proliferation is present in some cell types to respond to agents responsible for activation of NF- $\kappa$ B. Antioxidants can oppose any stimulant that activates NF- $\kappa$ B. In some cell types, ROS proliferation is associated in reception to agents for activation of NF- $\kappa$ B. Antioxidants can block all the stimuli that activate NF- $\kappa$ B (Flohé et al., 1997; Schreck et al., 1992).

NF- $\kappa$ B activation is used to stop cell death in various culture patterns. Since this activation can transmit intra-neuronal anti cell death signals. Finding high levels of NF- $\kappa$ B in dopaminergic neurons of PD patients suggested that NF- $\kappa$ B may have protective effects for PD. Instead of using antioxidant enzymes to gain the apoptotic effect exerted by NF- $\kappa$ B, it may be preferable to transcribe genes encoding calcium regulatory proteins and trophic factors. Besides these, another domain of NF- $\kappa$ B activation is the provision of DA-induced apoptosis in PC12 cells (Panet et al., 2001).

### **1.3.2. Accumulation of Neuromelanin**

One of the most prominent features of neurons in the SN is neuromelanin accumulation due to aging. High probability of presence of neuromelanin-containing cells disappears in PD cases. The content of neuromelanin is not clearly defined, but it contains most dopamine redox chemistry products. Neuromelanin, which is responsible for the accumulation of metal ions, especially iron, is a brown pigment

(Wakamatsu et al., 2003). Synthetic melanin is manufactured by incubating dopamine with  $\text{Cu}^{2+}$  and  $\text{Fe}^{3+}$  metals. Besides being an important neurotransmitter, dopamine is a good metal reducer because it is a catechol. The primary task of dopamine is to be responsible for the coordination of metals like  $\text{Fe}^{3+}$  and  $\text{Cu}^{2+}$ . In the situation of this metal oxidation then contribute to the production of  $\text{H}_2\text{O}_2$ . The main purpose of neuromelanin is unknown, but for high metal loads, melanin act as prooxidants. Another task is that it is an iron storage molecule. Neuromelanin isolated from human SN has low and high intimacy  $\text{Fe}^{3+}$  binding regions and iron binding to neuromelanin has a redox active effect (Smythies, 1996).

Oxidative stress associated with PD may have been caused by a disorder in the regulation of neuromelanin-iron biochemistry (Faucheux et al., 2003).  $\alpha$ -synuclein's main task is to regulate the DA activity. The A53T mutation incorporated with hereditary PD disrupts vesicular preservation of DA and causes DA accumulation, which causes ROS formation through its interaction with iron with increasing age. These mutations in  $\alpha$ -synuclein caused the change of dihydropteridine reductase expression, which is responsible for the regulation of dopamine synthesis (Baptista et al., 2003). Immunoprecipitation experiments revealed that the  $\alpha$ -synuclein molecule combines with the human DA neurotransmitter to form complexes, thereby inhibiting dopamine uptake of the transporter and that  $\alpha$ -synuclein is responsible for the regulation of dopamine manufacturing by attenuating tyrosine hydroxylase (Wersinger et al., 2003). The relevance between  $\alpha$ -

synuclein and redox chemistry incorporated with iron-bound dopamine/neuromelanin has also been demonstrated by a study showing that it coincides with  $\alpha$ -synuclein accumulation in lipofuscin and neuromelanin deposits. The defects in the  $\alpha$ -synuclein-modulated DA balance are due to the pathogenicity of the mutant  $\alpha$ -synuclein being dependent on DA (Xu et al., 2002).

#### **1.4. Parkinson's Disease and Antioxidants**

The nature of neurotoxins that cause degeneration in DA neurons in PD is not fully known, nevertheless oxidative stress is one of the mediating risks factors that can initiate or induce degeneration of DA neurons. Antioxidant treatments can prohibit or diminish the progression of this disease. Supplements with more than one antioxidant in appropriate doses may be required since the types of free radicals are so diverse. Different types of antioxidants have different ability to quench free radicals. Due to the high level of oxidative stress in the brains of PD patients, appropriate antioxidant supplements are among the important options available for the prohibition of PD in high-risk populations. Several antioxidants are used in traditional experimental studies to prevent PD. These studies have shown that antioxidants are not appropriate for the maximum effectiveness of antioxidant cure due to the diverse effects of antioxidants, various environments at cellular and organ levels, and the diverse nature of free radicals. Using more than one antioxidant is more beneficial for PD prevention trials. Almost all types of antioxidants can function as free radicals when oxidized. Therefore, using a single antioxidant in any clinical trial is quite

insufficient to cure or prevent the disease (Prasad et al., 1999). Many types of antioxidant studies have been conducted to improve or prevent PD.

#### **1.4.1. Vitamin E and Vitamin C**

Clinical studies and animal experiments performed show nigral degeneration and the preliminary or development of PD in case of Vitamin E deficiency (Dexter et al., 1994). In mice injected with N-methyl-1,2,3,6 tetrahydropyridine (MPTP), pre-treatment with high-dose vitamin E supplementation prevented glutathione loss, and even partially preserved nigrostriatal neurons (Perry et al., 1987). Although growing data from studies using in vitro animal models suggest a potential role for vitamin E and vitamin C supplementation in the prevention of PD, nutritional supplementation intervention in humans' remains controversial. Results from studies investigating the relationship between PD development and vitamin E and vitamin C supplementation are unclear, and randomized controlled studies among patients with pre-existing PD are insufficient to fully state that Vitamin E and Vitamin C supplementation has a positive effect on PD. Vitamin E and Vitamin C interact directly with ROS and are effective in ending oxidative chain reactions and are involved in signal transduction to deduct damage that promotes PD development. These antioxidative micronutrients are also involved in compensator genes that control the improvement and staying alive of dopaminergic neurons. (Park & Ellis, 2020).



#### **1.4.2. Coenzyme Q10**

Unlike PD patients, the amount of oxidized CoQ10 in platelets and plasma was much higher than in the control group patients. One of the early studies showed that functional decline was slowed in early PD patients treated with CoQ10. Another study reported that CoQ10 treatment showed a mild symptomatic benefit. CoQ10 has neuroprotective effects in multiple in vitro and animal models of neuronal toxicity. In one of the studies, it was observed that CoQ10 supplementation reduced the loss of dopamine and dopaminergic axons in the striatum in MPTP-induced PD mice. Based on recent studies, CoQ10 activity has no effect on any disease modification on PD (Zhu et al., 2017).

#### **1.4.3. Curcumin**

Studies on  $\alpha$ -synuclein in Parkinson's disease have revealed the anti-fibril properties of Curcumin. Several studies have shown that curcumin may also augment its effects on neuronal preservation for AD and PD by increasing Nrf2 expression. (C. Yang et al., 2009). Curcumin is responsible for increasing tyrosine hydroxylase and dopamine levels by deterring the expression of glial fibrillary acidic protein (GFAP) and iNOS proteins, thus conserving dopaminergic neurons from MPTP-induced neuronal detriment. The anti-apoptotic characteristics of curcumin have been confirmed thanks to its ability to conserve dopaminergic neurons by preventing the reduction of mitochondrial membrane potential (MMP). Another mission of curcumin is to conserve neurons from oxidative detriment. Preclusion of this damage

can be in two ways; By re-activating the mitochondrial membrane potential, thereby increasing the regulation of Cu-Zn superoxide dismutase and deterring intracellular ROS production. Studies using the transgenic PD model of *Drosophila* have shown that curcumin diminishes the levels of lipid peroxidation and protein carbonyl aggregation in the brain. (Bhat et al., 2019).

#### **1.4.4. Vitamin A and Carotenoids**

Neuroprotective effects of Vitamin A and carotenoids against MPTP-induced neurotoxicity were identified in the mouse model induced by MPTP, a neurotoxin that induces PD, thanks to pre-treatment with  $\beta$  carotene or lutein. Vitamin A deficient rats have been observed to develop atrophy, muscle weakness, and significant losses in spinal motor neurons, particularly in the hind limbs. A significant reduction in PD risk in individuals consuming foods containing carotenoids and  $\beta$ -carotene crosschecked to the control group was observed (Miyake et al., 2011). Studies on the heart and liver have revealed that vitamin A has antioxidant properties, but at the same time, these study data do not fully prove whether vitamin A has an antioxidative or pro-oxidative effect on neurodegeneration of oxidative dopaminergic neurons. (Anaïs Marie et al., 2021). Although epidemiological trials in Parkinson's positively affect the risk degradation of carotenoids and Vitamin A, therefore, the need to investigate the act of Vitamin A supplementation on oxidative stress in PD residuals.

#### **1.4.5. Docosahexaenoic Acid and Eicosapentaenoic Acid**

DHA has some specific effects for neuronal protection in animal models for PD. An alternative study showed a significant depreciation in dyskinesia related levodopa in animals treated with DHA. According to the study data investigating the neuroprotective effects of two DHA substructures (RvD1 and RvD2) in Pd models, it was revealed that RvD1 frustrates the expression of some proinflammatory mediators. Another DHA substructure, RvD2, frustrated dopaminergic neuron detriment and microglial dysfunction by preventing NF- $\kappa$ B activation (Chamani et al., 2020).

#### **1.4.6. Lycopene**

In a study in mice in which Lycopene supplementation was administered for seven days, lycopene was shown to be a suppressive factor of MPTP-induced striatal dopamine demolition. With this data, it was concluded that by feeding lycopene, dopaminergic neurons can be protected against stimuli that may cause PD. These results provide hope for future investigation of the prophylactic and therapeutic effects of lycopene on PD. With this seven-day treatment, it was also observed that the increase in MPTP-induced neurotoxicity in apoptotic markers such as bax and caspase in the mouse striatum was diminished, and it was concluded that lycopene supplementation could mediate by diminishing neuronal apoptosis. However, it is still not known exactly how lycopene obstruct apoptosis. Therefore, more research data are needed to establish a relationship between the anti-apoptotic and anti-oxidative properties of lycopene (Prema et al., 2015).

### **1.5. Amyotrophic Lateral Sclerosis Disease and Oxidative Stress**

Amyotrophic Lateral Sclerosis (ALS), also known as Charcot or Lou Gehrig's disease, is a neurodegenerative disease defined as motor neuron loss in the cerebral cortex, brainstem, and spinal cord. In individuals with ALS symptoms, muscle loss, stroke and death are ineluctable in a few years. The name ALS comes from muscle atrophy (amyotrophic) and muscle scarring (sclerosis). Symptoms in ALS patients are speech disturbance, difficulty in breathing and swallowing, progressive paralysis, and dysphagia. The main death in ALS is due to respiratory failure. Motor neuron loss in the pathology of the disease occurs due to inclusions in motor neurons that remain intact, glial activation, abnormal mitochondria and neurofilament clusters. There is only one licensed drug for ALS that extends lifespan up to 3 months, and that is Riluzole. The purpose of use is that this drug is an agent that provides glutamate signaling in ALS (Niedzielska et al., 2015). Although the causations of ALS disease are not fully known, the progressive operations of the disease are diverse and complex in terms of neurodegeneration. The disease can be assorted into two types, sporadic (SALS), or familial (FALS) depending on whether it contains any genetic factors in ALS. The SALS type typically occurs later in individuals' over middle age. It is not known why SALS started, so it is unclear whether environmental factors or genetic events have an effect. The situation is different in FALS. Alterations in SOD1 caused 20% of the cases (Liu et al., 2017).

As confirmation that oxidative stress is effective in ALS disease, the presence of mutations in copper/zinc superoxide dismutase 1 (SOD1), an

oxidative damage marker and an antioxidant enzyme, can be shown as the triggering effect of the mechanism that causes motor neuron death. Other genes that undergo mutations that cause adult-onset ALS and are sparser than SOD1 mutations are sarcoma fused/translated in liposarcoma (FUS/TLS), TAR DNA binding protein-43 (TDP-43), angiogenin, charged multivesicular body protein -2B (CHMP2B) and vesicle-associated membrane protein B (VAPB) (Greenway, 2006).

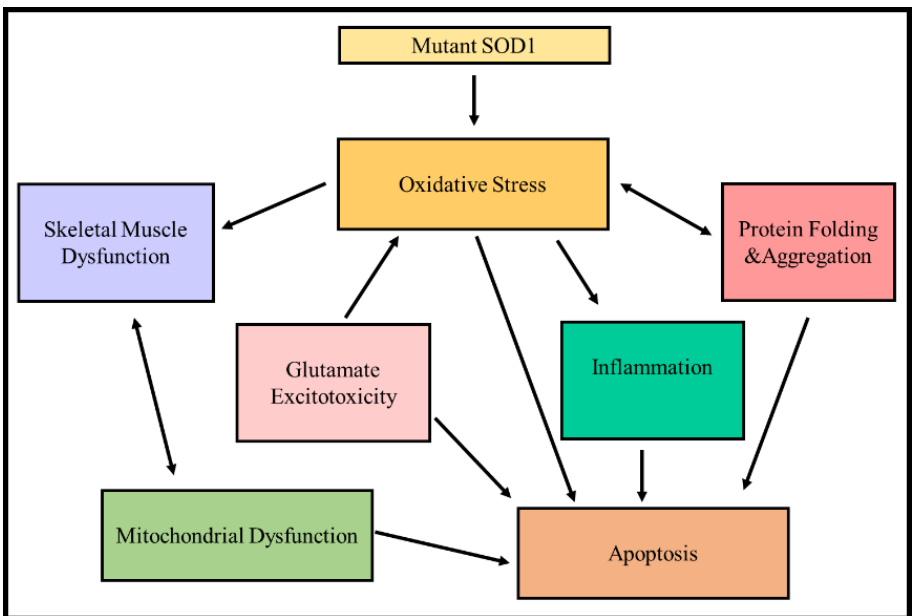


Figure 5: Relationship Between Oxidative Stress and Amyotrophic Lateral Sclerosis

### 1.5.1. SOD1 Mutations

SOD1 mutations are among the causes that lead to ALS, along with oxidative stress. Based on the altered dismutase activity, there are several mechanisms involved for the SOD1 mutation: (1) The mechanism that

increases production of superoxide that can react with NO to spawn peroxynitrite with the loss of dismutase function,

(2) The negative mechanism by which the SOD1 function expressed by the normal allele of the mutated SOD1 protein is inhibited,

(3) SOD1 activity mechanism to increase levels of hydrogen peroxide and hydroxy radical (Rosen, 1993). According to the research data that emerged later, the idea that the SOD1 mechanism is not that simple has been put forward.

In addition to the presence of mutations that reduce dismutase activity, mutations that preserve this activity have also been identified (Borchelt et al., 1994). However, studies have shown that SOD1 knockout mice do not improve ALS, and even increased/decreased wild-type SOD1 levels have no effect on ALS. For this reason, the decrement of SOD1 activity is insufficient to bring about disease development and altered SOD1 is toxic due to a different gain of function. Altered SOD1 contributes to the new toxic function by catalyzing various abnormal oxidation reactions. Thusly, it was assumed that the mutant SOD1 had a more extensive structure compared to the wild-type protein, though it caused the addition of non-superoxide substrates to the active site and react with the zinc and copper ions in its content.

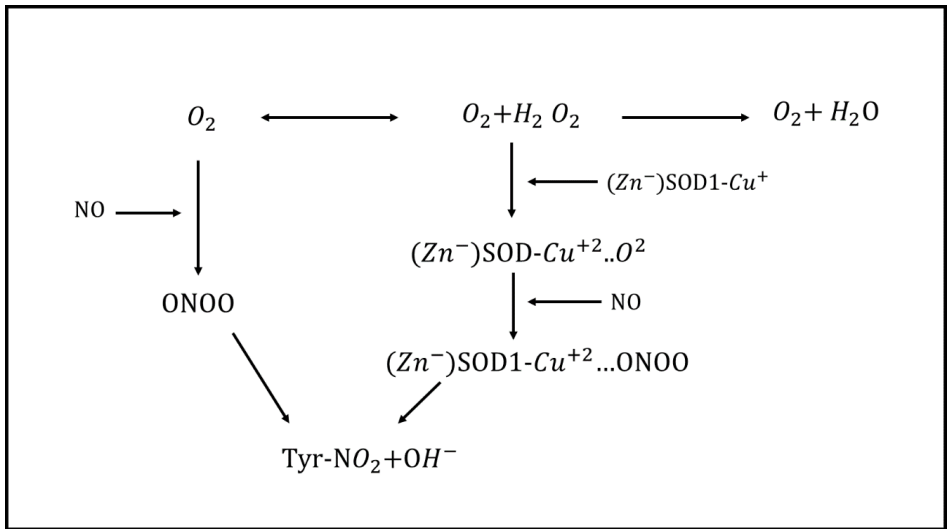


Figure 6: Summary of SOD Activity

The active role of SOD1 in these two-stage redox reactions is to reduce the copper ion and to re-oxidize it and to decompose the superoxide radicals into hydrogen peroxide and oxygen. In addition, another task of SOD1 is to provide catalysis in the inverse of the normal dismutase activity or to act as a peroxidase in the use of hydrogen peroxide, which is used as a substrate in the production of hydroxyl radicals thanks to Fenton reaction (Sankarapandi & Zweier, 1999). SOD1A4V and SOD1G93A mutant genes that cause ALS have high peroxidase efficiency. However, considering the benefits of peroxidase activity, no difference was detected between mutant SOD1 and wild type SOD1. Zinc-deficient SOD1 authorizes reducing agents such as glutathione and ascorbate to elicit snappy with the oxidized  $Cu^{2+}$  ion in the active region. When looking at the structure of the SOD1 variants associated with ALS, it appears that they are bound with zinc. The diminished SOD1- $Cu^+$  couple could produce superoxide in contrast to the normal dismutase reaction. In this way, it

reacts with NO in the active site to form peroxynitrite and thus tyrosine nitration takes place (Crow et al., 1997).

In animal experiments, SOD1G93A was observed to accelerate the development of ALS in mice, while zinc supplementation was observed to slow death relatively. In addition, if the peroxynitrite molecule had a share in the progression of the disease, the NO level reduction used in the production of peroxynitrite would have a different course of the disease. It appeared that the deletion of inducible or neuronal NO synthase had no efficiency on the enhancement of disease in mutated SOD 1 mice. Although it has been proven that reactions catalyzed by the alternative SOD1 molecule do not always occur, since these reactions do not have Cu ions found in active sites, they cannot be the main source of oxidative stress produced by the SOD1 mutant (Bruijn et al., 2004).

### **1.6. Amyotrophic Lateral Sclerosis and Antioxidants**

ALS disease has a special place among neurodegenerative disorders due to oxidative stress in its pathology. Point mutations in Cu/Zn-SOD, one of the main enzymatic antioxidant defenses of eukaryotic cells, have been described in some familial cases of the disease. Numbers of patients use dietary antioxidants on the instructions of their doctors. However, several reviews of randomized or semi-randomized controlled trials have been conducted to be able to say something clear about antioxidant therapy for ALS disease. (Orrell et al., 2007). To have a certain effect on subjects, the pharmacological dose that will penetrate the CNS must be determined well because the diffusion barrier called the blood brain barrier (BBB) restricts shipping of compositions. Although it is not fully explained whether the



mechanism of BBB is impaired in ALS clients, there are animal experiments performed on it. Exit of a nontoxic fragment of tetanus toxin which is a peptide and Evans blue dye which is protein-bound small molecule from the CNS was observed in the SOD1G93A mouse model. Whether antioxidants work in the treatment of ALS is a matter of debate. Although the agents have shown effective results in animal models, a conclusion on their effectiveness on ALS disease could not be drawn, and for this reason there is ongoing research on antioxidants for this disease (Ay et al., 2008).

#### **1.6.1. Vitamin E and Vitamin C**

Vitamin E is the most powerful antioxidant among the scavengers of reactive nitrogen and oxygen species, and therefore its effects on ALS disease have been extensively studied. Vitamin E supplementation was tested in mutated SOD1 transgenic mice and although it delayed clinical disease onset and slowed disease progression, it did not prolong survival. Almost the same results have been acquired in studies on humans. According to some clinical trials of vitamin E in the cure of ALS, Vitamin E may not prolong the life expectancy of ALS patients, but it has a decreasing effect on the risk of exacerbation and progression of the disease (J. Majima et al., 2011).

#### **1.6.2. Coenzyme Q10**

CoQ10 is a powerful antioxidant, found in the structure of lipid and mitochondria membrane surfaces. It is found in mitochondria because it is the mitochondrial electron transport system's co-factor. It takes electrons emanating from complex I and complex II systems and transports them to

the complex III system. It has been found that CoQ10 administration is clinically beneficial for clients with mitochondrial disarrangements. According to controls in ALS clients, grades of serum CoQ10 has not been altered but increases in survival activity were detected in SOD1G93A transgenic mice fed CoQ10 before the onset of clinical indications after several feedings (Li et al., 2005). CoQ10 has been shown to be well digested at certain doses in ALS clients. However, a phase II randomized, placebo-controlled, double-blind clinical study used a different dose of CoQ10 in ALS clients. However, the difference between the groups were insignificant, and the Phase III study was halted. In these double-blind randomized control experiments, CoQ10 activity did not have a slowing or attenuating effect on ALS patients (Kaufmann et al., 2009).

### **1.6.3. Selegiline**

Selegiline hydrochloride also known as L-diphenyl is a discriminating inhibitor of monoamine oxidase B. It also has antioxidative features due to increased SOD and CAT levels in some brain regions (Takahata et al., 2006). Though selegiline is easily aspirated from the intestinal villi and can rapidly enter the CNS, an advantageous efficacy of selegiline in clients with ALS has not been found yet. The doses used for these supplementation studies are adequate to inhibit the level of monoamine oxidase B, but these doses may not be adequate to induce the selegiline's antioxidative activity in mice. Therefore, it has not been clearly explained whether selegiline has therapeutic benefit for ALS patients (Orrell et al., 2007).

#### **1.6.4. N-acetylcysteine**

Glutathione is an important ROS scavenger. Exhausted glutathione repository can be filled with the oral supplement execution of N-acetyl-L-cysteine (NAC). Studies have shown that NAC supplementation significantly prolongs survival and improves motor performance in 4–5-week-old SOD1G93A mice. However, in a randomized, double-blind, clinical trial, ALS clients self-tried NAC supplementation approximately 1 year and found no substantial improvement in survival or disease progression based on these trial data (Louvers et al., 2015).

#### **1.6.5. Docosahexaenoic Acid and Eicosapentaenoic Acid**

In an observational research study, it was proclaimed that a use of polyunsaturated fatty acids could reduce the jeopardy of improving ALS, but the existence of another mouse model study in which it aggravated the condition in ALS and accelerated disease progression made this situation contradictory. In consequences, more experimental evidence is needed before the appropriateness of using unsaturated fatty acids in the treatment of ALS is acceptable (Fitzgerald et al., 2014; Yip et al., 2013).

## **2. CONCLUSION**

Free radicals can be produced in living organisms and contribute to the formation of ROS. NO and OONO are the strongest of the ROS produced free radicals and cause protein, lipid, and nucleic acid degradation, causing inactivation of enzyme activities, altered ion balance, and genetic modifications, which can lead to apoptotic or necrotic cell demise. ROS formation is a significant factor in the emergence of neurodegenerative

diseases. With aging, neuronal cells in the partial regions of the brain can be subjected to ROS attack subsequently apoptotic cell death may occur. In neurodegenerative illnesses such as AD, PD, and ALS, the illness can progressively worsen due to neural network malfunction. Regardless of the mechanism by which it occurs, oxidative insults can initiate various signal chains that lead to apoptotic cell death. The agents that can protect against these oxidative attacks are natural antioxidants and defense enzyme systems. Recent findings have made it possible for several types of antioxidants to be effective by protecting cells from oxidative damage. However, further research is essential to fully explore the potential of these compounds in preventing the development of neurodegenerative disorders or providing treatment.

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## CHAPTER 5

### AGING

Assist. Prof. Dr. Arzu TEMİZYÜREK<sup>1</sup>

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Aging can be manifested by loss of physiological integrity, which destroys functionality and increases susceptibility to death. Following questions are waiting to be answered (López-Otín et al.,2013).

What are the physiological origins of aging-damage, the compensatory mechanisms of age-related processes, the relation between compensation and damage, and the external intervention possibilities to delay aging. In terms of determining the molecular features of aging and the distinguishing features that are thought to have an impact on the course are given as hallmarks as follows (Gems D., Partridge L.,2013).

## **1.GENOMIC INCONSTANCY**

The occurrence of genetic destruction throughout life is a common feature of aging (Moskalev AA.,2012).

Extrinsic factors are threats that constantly affect the integrity and stability of DNA (ROS). (Hoeijmakers JH.,2009).

A wide variety of factors ranging from point mutations to telomere shortening cause genetic lesions. To keep these lesions to a minimum, organisms have developed a complex DNA repair mechanism. Apart from these, in DNA defects, errors in nuclear structure called laminopathies causes instability and premature aging syndromes (Worman HJ.,2012).



### **1.1.Nuclear DNA**

Somatic mutations appear to accumulate in elderly individuals and cells from model organisms chromosomal aneuploides and duplication variations have also been considered as related DNA damage forms (Moskalev AA., and Faggioli F et al.,2012).

These DNA changes mentioned above can have an impact on specific genes and the resulting transcriptional pathways. This is particularly important in DNA damage. Tissue regeneration is limited in terms of affecting the functional ability of stem cells (Jones DL, Rando TA., 2011).

### **1.2.Mitochondrial DNA**

Other factors also have an effect on aging. Especially changes on mitochondrial DNA (Park CB., Larsson NG.,2011).

In terms of somatic mutations associated with aging, mtDNA has been considered as a main target. Insufficient protective histones, oxidative microenvironment of mitochondria, and insufficient mtDNA repair mechanism have made mtDNA a target. (Linnane et al., 1989)

The causal significance of mtDNA mutations due to heteroplasmy is still a matter of debate. However, according to single-cell tests analysis, mutational burden becomes important for individual senescent cells and may become homoplasmy, where the mutant genome dominates over the normal (Khrapko et al.,1999).

The view that replication errors occurring in the early period of life are more effective in older mtDNA mutations than oxidative damage has created an opinion contrary to previous expectations. These mutations can cause dysfunction in different tissues (Ameur et al.,2011).

### **1.3. Nuclear Architecture**

Genome instability is important in the aging process. This includes defects in the lamina as well as genomic damage affecting nuclear or mtDNA (Dechat et al.,2008).

Nuclear lamins mainly form components of the nuclear lamina. They act as scaffolds to bind chromatin and protein complexes that direct genomic stability (Gonzalez-Suarez et al.,2009).

Demonstrating the structural and dynamical properties of the nuclear lamina to accelerate aging has brought this structure into focus. Changes in the lamina and production of progerin have also been shown to occur during a person's aging process (Ragnauth et al.,2010).

In vitro studies indicate that telomere dysfunction is effective in progerin production in normal human fibroblasts, during normal aging. This suggests that there may be a close relationship between telomere regulation and progerin expression (Cao et al.,2011). In type A lamins, both changes in type A lamins and B1 levels decrease with aging. This indicates the use of the process as a biomarker (Freund et al.,2012).

## 2. TELOMERE DISINTEGRATION

Besides the DNA damage with age-related deterioration, other regions, telomeres, are especially vulnerable (Blackburn et al., 2006). At the same time deactivation of telomerase expression results in loss of telomere protection, which is observed in some somatic mammalian cells (Bodnar et al., 1998). By the way, telomere shortening occurs during the normal aging process in both humans and mice (Blasco MA., 2007).

Telomeres can be considered as DNA fragments involved in the repair mechanism through a compound, known as shelterin (Palm W, de Lange T., 2008). This gives telomeres a distinctive feature. In the absence of telomerase, it gradually shortens. Moreover, in case of the existence of telomerase, the effect of (exogenous) DNA impairment is unnoticed due to shelterins. Therefore, DNA damage observed at telomeres causes lasting effects leading to detrimental cellular effects including aging and/or cell death. Telomere degradation follows the normal course of aging (Fumagalli et al., 2012). Beyond that telomere dysfunction triggers aging in mice and humans. However, in studies it has been suggested that telomerase can delay aging in mice. This information can serve as a criterion for the hallmark of aging (López-Otín et al., 2013).

### **3.EPIGENETIC MODIFICATIONS**

Epigenetic modifications include both changes in DNA methylation and post-translational alterations. Especially, enzyme systems are important regulators in both creating and providing continuity of epigenetic models in chromatin modifications (Fraga MF, Esteller M.,2007).

#### **3.1.Histone Remodelling**

Histone methylation appears to be a marker of aging in invertebrates. One of the life-extending features in nematodes and flies is the deletion of histone methylation complex components (Greer et al., 2010). Also, histone demethylases may modulate longevity by targeting pathways such as the insulin signaling pathway IGF-1 (Jin et al.,2011). The effect of histone modifications on aging has not yet been fully determined. In several studies of mammals, it has been reported that mammalian sirtuins exhibit some delaying effects on aging processes in mice. It has been reported that, transgenic overexpression of mammalian sirtuin (SIRT1) has a curative effect on health parameters during aging but has no effect on longevity (Guarente L.,2011). The potentially positive mechanisms of SIRT1 involve a wide variety of cellular actions. Therefore, these mechanisms are complex and interdependent. The role of sirtuin role in mammalian longevity has been derived from SIRT6. In a study of SIRT6-deficient mice, these mutant mice showed a rapid aging pattern. Despite that in mice overexpressing Sirt6, mice showed longer lifespan due to reduced serum IGF-1 values. On the other hand, mitochondrial SIRT3 mediates some positive mechanisms of dietary restriction (DR). Even if its effects are more dependent on deacetylation

rather than histone modifications, mitochondrial SIRT3 affects longevity (Someya et al.,2010).

### **3.2.DNA Methylation**

Linking aging and methylation of DNA is quite complicated. In previous studies they reported a global age-related hypomethylation. However, later analysis showed that in particular, loci of some tumor suppressor and Polycomb-targeted genes experience hypermethylation reactions with age (Maegawa et al.,2010).

### **3.3. Chromatin Remodeling**

t DNA and histone modifying enzymes act with chromosomal proteins. For example, hetero- chromatin protein 1 $\alpha$  (HP1 $\alpha$ ) and chromatin remodeling factors such as polycomb group proteins or (NuRD) complex (Pollina EA, Brunet A., 2011). The relationship between chromatin changes and aging is based on:

1. Loss-of-function mutations in HP1 $\alpha$ . (Flies have a shorter lifespan)
2. Overexpression of heterochromatin protein (Prolongs lifespan in flies) (Larson et al.,2012).

Telomeric repeats for chromatin modifications are increased in mammals. This shows:

chromosome ends are joined to heterochromatin domains (Gonzalo et al.,2006).

## **4. DEPRIVATION OF PROTEOSTASIS**

Aging process and related diseases are associated with damaged homeostasis of protein or associated with proteostasis (Powers et al.,2009). All cells try to maintain the constancy and viability of their proteomes. Proteostasis participates in the process necessary for the constancy of proteins (Hartl et al.,2011). One of the molecules of age-associated toxicity such as MOAG-4 uses a different pathway from molecular chaperones and proteases (van Ham et al., 2010). All these systems function to revitalize the formation especially of dysregulated polypeptides. By removing and degrading them, avoiding the damaged structures, and ensuring of renewal of intracellular proteins are possible (Koga et al.,2011).

Many studies have shown that proteostasis changes with aging. In diseases such as Alzheimer's disease, Parkinson's disease and cataracts misfolded or aggregated proteins contribute to the occurrence of these diseases (Powers et al.,2009).

### **4.1. Chaperone-mediated Protein Folding and Stability**

With aging, stress-related disruptions in the formation of cytosol and organelle-based chaperones are observed (Calderwood et al., 2009). Animal experiments demonstrated that chaperone can be effective in longevity. Especially, overexpression of chaperones in transgenic worms and flies has been reported to prolong their lifespan (Morrow et al., 2004).

## **4.2. Proteolytic Systems**

Autophagy-lysosomal and ubiquitin-proteasome systems are reported to decrease with aging, reinforces that, breaking of proteostasis accounts for a usual aspect of old age (Rubinsztein et al., 2011). Based on studies in transgenic mice of chaperone-mediated Autophagy receptor (LAMP2a), it will not cause a decrease in autophagic functions. Thus, it may have positive effects on liver functions (Zhang and Cuervo, 2008). Macroautophagy, another type of autophagy, started to arouse interest after the (mTOR) inhibitor rapamycin became effective in longevity in experimental animals (Blagosklonny, 2011). Especially, rapamycin delays many features of aging in mice (Wilkinson et al., 2012). The impact on longevity of rapamycin is due to the initiation of autophagy in yeast, nematodes and flies (Bjedov et al., 2010). However, similar effects have not yet been seen in mammals (Selman et al., 2009), spermidine, another macroautophagy promoter, also has a longevity effect in yeast, flies and worms. (Eisenberg et al., 2009). In addition, dietary supplement containing polyamines. Preparations containing spermidine or polyamine-producing intestinal flora have also been reported to be effective in longevity (Matsumoto et al., 2011). Beyond that, there is some information that can be considered as evidence of aging. proteostasis-related and experimental disruption of proteostasis as it can accelerate age-related pathologies (Zhang and Cuervo, 2008).

## **5. DEREGULATED NUTRIENT-SENSING**

Growth hormone (GH) and insulin-like growth factor (IGF-1) produced by mammals, especially hepatocytes, synthesized under the influence of growth hormone. Some intracellular signaling pathways may be the same. Therefore, inducing of IGF-1 and insulin, are called the pathway of insulin and IGF-1 signaling (IIS). The IIS pathway in evolution is the most preserved pathway of aging. (FOXO) and mTOR complexes account also for conservation of path in aging (Barzilai et al., 2012; Fontana et al., 2010; Kenyon, 2010). According to many studies diet restriction (DR) increases life expectancy or health duration (Fontana et al., 2010). Besides IIS, other systems are also involved in glucose-related pathways. Such as, mTOR, AMPK and sirtuins (Houtkooper et al., 2010). The mTOR kinase belongs to the multiple protein structures whereas mTORC1 and mTORC2 control features of biosynthesis metabolism (Laplante and Sabatini, 2012). Down regulation of mTORC1 activity in yeast, worms and flies increases lifespan (Harrison et al., 2009).

In addition, mTOR activity increases in the hypothalamus neurons, which is especially related to age-related obesity during the aging process in mice. This condition is reversible on Rapamycin therapy (Yang et al., 2012). Along with this information, the pathway of IIS expresses a unique function via the signaling pathway of IIS or mTORC1. Other nutrient sensors such as AMPK and sirtuins have different effects. They affect on nutrient scarcity and catabolic reactions rather than anabolic processes. Up -regulation of AMPK promotes



normal aging. Furthermore, the stimulation of AMPK function affects metabolism, inhibits mTORC1 and may affect lifespan expansion after administration of metformin to worms and mice (Anisimov et al., 2011; Mair et al., 2011; Onken and Driscoll, 2010).

## **6. MITOCHONDRIAL DYSFUNCTION**

During the time course of aging of cells and organisms in order to diminish the respiratory chain efficacy, an increase of electron leakage and decrease of ATP generation is observed (Green et al., 2011).

### **6.1. Reactive Oxygen Species (ROS)**

According to the mitochondrial free radical theory with aging the progressive. Mitochondrial dysfunction results in increased production of reactive oxygen species (ROS) and causes mitochondrial and cellular damage (Harman, 1965). In contrast, elevation of ROS may promote longevity in yeast and *C. elegans* (Doonan et al., 2008). According to the studies in mice, genetic effects that increase mitochondrial ROS and oxidative damage do not trigger aging and antioxidant defence does not influence the longevity (Perez et al., 2009).

### **6.2. Mitochondrial Integrity and Biogenesis**

Studies in DNA polymerase  $\gamma$  deficient mice indicate that mitochondria affects aging in an independently manner (Edgar et al., 2009). (see above Genomic Instability). There are number of mechanism that supports the abovementioned idea. For example, the relation between mitochondrial deficiencies and apoptotic signaling pathways, the impact on cellular signaling and interorganellar crosstalk, with aging, multipl converging mechanisms may affect the efficiency of

mitochondrial bioenergetics such as telomere attrition because of p53-mediated repression of PGC-1 $\alpha$  and PGC-1 $\beta$ . Also observed during physiological aging. However, this process could be changed by triggering telomerase activity (Bernardes de Jesus et al., 2012). SIRT1 affects mitochondrial pathways. Also, SIRT3, targets many enzymes involved in energy metabolism. SIRT3 may also affect the ROS mechanism. To sum up, these results suggest that sirtuins may regulate mitochondrial activity and protect against age-related disorders.

Other causes of defective bioenergetics are as follows:

- modifications in mtDNA,
- deterioration of mitochondrial proteins
- differences in the lipid organization of mitochondrial membrane,
- changes in mitochondrial dynamics
- defective quality control by mitophagy (Wang and Klionsky, 2011).

### **6.3. Mitohormesis**

Mitohormesis is a biological response of a diminished mitochondrial stress that promotes viability in a cell, tissue, or organism. Activated by a damaging stimulus the mitochondrial stress response requires a network. This interaction is based on signals that are associated with each other. Inducing of activity of mitohormetic response increases lifespan in different species. Furthermore, it increases healthspan as well. Although various factors and stress inducers are proposed to increase the activation of this mechanism, mitohormesis also has beneficial consequences (Bárcena et al.,2018).

## 7. CELLULAR SENESENCE

According to the discovery of cellular senescence, the normal cells are not immortal. Thus, providing a base for a scientific view of the theory of aging. According to Darwinian theory, genes that are useful in young age are preferred over genes useful late in life. George C. Williams introduced a concept, the antagonistic pleiotropy, built on this model. (Williams G.,1957).

The perspective cellular aging has been reshaped in terms of that the immune system which brought the concept of immunosenescence to the forefront. Immunosenescence is related to decreased immune activity during aging. Aging in T cells is related to the insufficiency of the proliferation potential, but immune cell type associated with immunosenescence is still a question mark. Furthermore, some markers to study senescence of cells may be irrelevant such as proliferation arrest markers, p16 or  $\beta$ -galactosidase (Akbar et al.,2016). Cellular senescence is a delay of the cell cycle, previously described by Hayflick (Hayflick L and Moorhead Ps., 1961). Although, the phenomena observed by Hayflick is caused by telomere shortening, there are other aging-related inducers that trigger senescence individually. Noteworthy, non-telomeric DNA deterioration and effects of the INK4/ARF side have also the ability of stimulating senescence (Collado et al., 2007). Therefore, information about constituents of immuno-aging and specific definitions are needed. Specific cells involved in immune surveillance of senescent cells are natural killer (NK) cells, macrophages and T cells. It was stated that monocytes/macrophages are also involved in the clearance process

(Sagiv et al.,2016). High activity of senescent cells results in a specific messaging secretome, the (SASP). The result of this function is different from apoptosis in terms of programmed cell senescence during mammalian embryonic development (Muñoz-Espín et al.,2013).

Stress-related senescence and reactive oxygen species;

Increased level of reactive oxygen species (ROS) is observed after various types of stresses. The relation between oxidative stress and senescence is demonstrated by treatment with antioxidants. Mechanistically, increased level of intracellular ROS induced by the RAS–RAF–MEK–ERK cascade activate the p38 MAPK, which causes to increase transcriptional activity of p53 and upregulation of p21 (Muñoz-Espín et al.,2014).

High oxidative stress is an important factor of aging progression. Different types of cellular senescence are identified such as replicative, physiological, or stress-related premature senescence (Lozano-Torres et.al., 2019).

Aggregation of senescent cells, one of the indicator of aging, promotes the progress of aging and associated diseases (López-Otín., 2013). Neuronal cell death is a slow natural aging process of the central nervous system. During the aging process, simultaneously with the loss of water content of the body, rise in plasma osmolality have serious effects on blood, body systems and organs. Progressive high plasma osmolality affects viscosity of the blood circulation and hemodynamics of the organ systems as well (Jung Y. Hahr.,2019).

According to Reinhart et al. Differences in plasma osmolality cause either swelling or shrinking of the red blood cells (RBCs). They observed the impact of osmolality on features of RBCs. Additionally, their potential to perfuse an artificial microvascular network (AMVN). These authors also observed the highest perfusion rate to be at an osmolality of 290 mosm/kg H<sub>2</sub>O and that the perfusion rate decreased as osmolality increased (Reinhart et al.,2015).

However, some protective mechanisms of neuronal cell function occur during the early stages of hypoxia. These effects can be considered as protective mechanisms for cellular damages. Protective effects on neuronal cell activity that occur in early stages of hypoxia can be accelerated by Hypoxia- related signals. Suppression of synaptic function is reversible when there is adequate Glucose supplementation. If enough ATP cannot be synthesized from anaerobic glycolysis, continuing cell functions and survival processes are limited. In both oxygen and glucose deficiency, it cannot avoid the deadly effects of Ca<sup>2+</sup> flow and cellular protective mechanisms cannot be active. There are many factors that affect high plasma osmolality. One of them is the long-term dietary consumption of foods with high animal protein content. In addition, high alcohol consumption, low of physical activity, insufficient fluid and sodium intake also aggravate the natural aging process by increasing plasma osmolality. To a certain extent, cancer and aging share common elements, but cancer results from an abnormal achievement of cellular fitness, while aging results from loss of fitness (Krnjevic K. 1999).

## **8. STEM CELL DEPLETION**

Especially, decreased regeneration ability of tissues is an important feature of aging. For example, the decline of hematopoiesis causes reduced production of adaptive immune cells, known as immunosenescence (Shaw et al., 2010). During the aging process, telomere shortening also has an important impact on declining of stem cells in several tissues. An enormous proliferation of these cells can be harmful by triggering the depletion of stem cell properties (Rera et al., 2011).

## **9. ALTERED INTERCELLULAR NETWORK**

Aging also involves changes at the level of intercellular communication (Laplante and Sabatini., 2012). Thus, during aging neurohormonal signaling processes are changed as inflammation increases. Immunosurveillance against pathogens diminishes, and the structure of the environment changes, by which the properties of all tissues are affected (Zhang et al., 2013).

### **9.1. Inflammation**

A well-known aging-related modification is called ‘inflammaging’ (Salminen et al., 2012). It may result from various causes. The deficiency of the immune system, the propensity of senescent cells, or the occurrence of a failure of autophagy function (Salminen et al., 2012). Inflammation also affects the pathogenesis of obesity and type 2 diabetes, two conditions related with aging. Furthermore, insufficient inflammatory responses have an important effect on atherosclerosis. Also, there is a decline in the function of the adaptive immune system

parallel with inflammaging (Barzilai et al., 2012). This immunosenescence may provoke the aging process at the systemic level, as a result of the deficiency of the immune system. A connection between inflammation and aging arises from the responses of both the inflammation and stress as well. Studies show that the connection between inflammation and aging arises from the mRNA decay factor AUF1. AUF1 affects the maintaining process of telomere length by inducing the expression of the telomerase catalytic subunit TERT, suggesting that one single factor may influence various aging indicators (Pont et al., 2012).

### **9.2. Different Types of Intercellular Connection**

Besides inflammation, aggregation evidence showed that aging-associated changes in one tissue can cause aging-related damage of other tissues, revealing coordination between organs (Nelson et al., 2012). There are other factors in which senescent cells promote senescence in neighboring cells. The microenvironment induces age-related activation deterioration of CD4 T cells, as studied in mice (Lefebvre et al., 2012).

### **9.3. Revitalizing Damaged Intercellular Network**

There are some probabilities for restoring networks between cells. Furthermore, the long-term use of anti-inflammatory agents such as aspirin may rise longevity in different species (Rothwell et al., 2011). Also, the gut microbiome has an impact on the function of the host immune system. It exerts systemic metabolic effects, may affect

longevity by influencing the structure and activity of the bacterial ecosystem (Claesson et al., 2012).



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