T.R.
VAN YUZUNCU YIL UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF CHEMISTRY

DETERMINATION OF OXIDATIVE STRESS LEVELS AND SOME ANTIOXIDANT ACTIVITIES IN ACUTE AND CHRONIC RENAL FAILURE PATIENTS

M. Sc. THESIS

PREPARED BY: Seerwan Hamadameen SULAIMAN
SUPERVISOR: Prof. Dr. Halit DEMİR

VAN-2020

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ACCEPTANCE AND APPROVAL PAGE

This thesis entitled "**DETERMINATION OF OXIDATIVE STRESS LEVELS AND SOME ANTIOXIDANT ACTIVITIES IN ACUTE AND CHRONIC RENAL FAILURE PATIENTS**" and prepared by Seerwan Hamad Ameen Sulaiman under consultation of Prof. Dr. Halit DEMIR in Department of Chemistry, on date of 27/12/2019 it has been successful with a unanimous vote by the following jury and it has been recognized as a Master's Thesis, in accordance with Postgraduate Education and training regulation with the relevant provisions.

Supervisor: Prof. Dr. Halit DEMİR Signature:

Member: Prof. Dr. Suat EKİN Signature:

Member: Assist. Prof. Dr. Fikret TÜRKAN Signature:

The Board of Directors of the Institute of Science: 03/01/2020 date and is approved by decision No. 2020/1-I

Signature

Director of the Institute

THESIS STATEMENT

All information presented in the thesis obtained in the frame of ethical behavior and academic rules. In addition all kinds of information that does not belong to me have been cited appropriately in the thesis prepared by the thesis writing rules.

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Seerwan Hamadameen SULAIMAN

ABSTRACT

DETERMINATION OF OXIDATIVE STRESS LEVELS AND SOME ANTIOXIDANT ACTIVITIES IN ACUTE AND CHRONIC RENAL FAILURE PATIENTS

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M. Sc. Thesis, Department of Chemistry
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January, 2020, 91Pages

In this study, antioxidant activities and oxidative stress levels were determined from serum samples taken from patients diagnosed with acute and chronic renal failure at Department of Nephrology Training and Research Hospital Van Yüzüncü Yıl University Medical Faculty. During this study, activity of three antioxidant examined, they were superoxide dismutase (SOD), reducted glutathione (GSH), and catalase (CAT). And malondialdehyde (MDA) level it is the end product of lipid peroxidation, which is a free radical source. The blood samples were taken from 92 individuals, in which 31 patients were conducted with acute renal failure, 30 patients diagnosed to have chronic renal failure, and 31 individuals were used as control group, that they did not diagnosed with renal problems. The SOD activity of patient groups is significantly lower than that of healthy control group in acute and chronic renal failure ($p<0.05$). Also there are significant differences in the mean of serum GSH and CAT levels in patients group compared to that of control group. In the case of patients serum MDA level is found to be a significant difference when it is compared to those of control group, while the difference of SOD and MDA average for patient and control groups were found to be relevant ($p<0.05$). This study shows that the oxidative stress have strong influence on the cellular damage, and tissue in acute and chronic renal failure patients.

Keywords: Acute renal failure, Chronic renal failure, CAT, GSH, MDA, SOD.

ÖZET

AKUT VE KRONİK BÖBREK YETMEZLİĞİ HASTALARINDA OKSİDATİF STRES DÜZEYİ VE BAZİ ANTİOKSİDAN AKTİVİTELERİN BELİRLENMESİ

SULAIMAN, Seerwan Hamadameen
Yüksek Lisans Tezi, Kimya Anabilim Dalı
Tez Danışmanı: Prof. Dr. Halit DEMİR
Ocak, 2020, 91 Sayfa

Bu çalışmada, Van Yüzüncü Yıl Üniversitesi Eğitim ve Araştırma Hastanesi Eğitim Fakültesi Nefroloji Anabilim Dalında akut ve kronik böbrek yetmezliği teşhisi konulan hastalarından alınan serum örneklerinden antioksidan aktiviteler ve oksidatif stres düzeyleri belirlendi. Bu çalışma sırasında üç tane antioksidan aktivite incelendi, bunlar süperoksit dismutaz (SOD), redukte glutatyon (GSH) ve katalaz (CAT) idi. Malondialdehit (MDA) seviyesi, serbest oksijen radikal bir kaynağı olan lipid peroksidasyonunun son ürünüdür. Akut böbrek yetmezliği olan 31 hasta, kronik böbrek yetmezliği tanısı alan 30 hasta ve böbrek sorunları tanısı almayan 31 ağlıklı kontrol grubu olarak kullanılan toplam 92 kişiden kan örnekleri alındı. Hasta gruplarının SOD aktivitesi akut ve kronik böbrek yetmezliğinde sağlıklı kontrol grubundan anlamlı derecede düşük bulundu ($p < 0.05$). Ayrıca, hasta grubunda serum GSH ve CAT düzeylerinin kontrol grubuna göre anlamlı fark bulundu. Hastalarda, serum MDA düzeyinin kontrol grubuna göre anlamlı bir farklılık tesbit edildi, yine hasta ve kontrol gruplarında SOD ortalama farkının anlamlı olduğu bulundu ($p<0.05$). Bu çalışma, oksidatif stresin akut ve kronik böbrek yetmezliği hastalarında hücresel hasar ve doku üzerinde güçlü etkiye sahip olduğunu göstermektedir.

Anahtar kelimeler: Akut böbrek yetmezliği, Kronik böbrek yetmezliği, CAT, GSH, MDA,SOD.

ACKNOWLEDGMENT

At the end of this thesis, I would like to take some time to thank all the people without whom this project would never have been possible. Although it is just my name on the cover, many people have contributed to the research in their own particular way and for that I want to give them special thanks. I would like to acknowledge my indebtedness and render my warmest thanks to my supervisor, Professor. Dr. Halit DEMIR, who made this work possible. His friendly guidance and expert advice have been invaluable throughout all stages of the work. I greatly appreciate the freedom you have given me to find my own path and the guidance and support you offered when needed.Also with all my regards and respect to Prof.Dr. Suat EKIN during the studying course.Furthmore I want to take a moment to thank my other committee members, Assit.Prof. Dr Fikret Turkan. Thank you for investing time and providing interesting and valuable feedback. I feel proud and honoured that you have accepted to be on my committee.A special thanks to my family. Words can not express how grateful I am to my mother, and father for all of the sacrifices that you've made on my behalf. Your prayer for me was what sustained me thus far.Finally I thank my God, my good Father, for letting me through all the difficulties. I have experienced Your guidance day by day. You are the one who let me finish my degree. I will keep on trusting You for my future. Thank you, Lord.

January 2020

Seerwan Hamadameen SULAIMAN

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SYMBOLS AND ABBREVIATIONS

Some symbols and abbreviations used in this study are presented below, along with Descriptions.

Symbols	Description
Cu	Cupper
Fe	Iron
Zn	Zinc
O_2	Oxygen
O_3	Ozone
O_2^-	Superoxide
OH^-	Hydroxyl
ºC	Centigrade temperature
min	Minute
g	Gram
h	Hour
L	Liter
Ml	Milliliter
µl	Microliter
mg	Milligram
mM	Mill molar
M	Molar
N	Normal
rpm/min	Round/Minute
µg	Microgram
µm	Micrometer
β	Beta
α	Alfa
kDa	Kilo Dalton
U/L	Unit/liter

Abbreviations	Explanation
AKI	Acute kidney diseases
CKD	Chronic kidney diseases
RFI	Renal failure injury
ARF	Acute renal failure
CRF	Chronic renal failure
Apo	Apolipoprotein
ATP	Adenine triphosphate
CAT	Catalase
DNA	Deoxyribo nucleic acid
RNA	Ribonucleic acid
EDTA	Ethylenediamine tetra acetic acid
GPx	Glutathione peroxidase
GR	Glutathione reductase
HDL	High density lipoprotein
H2O2	Hydrogen peroxide
Kcal	Kilocalorie
LDL	Low density lipoprotein
MDA	Malondialdehyde
NADPH	Nicotinamide adenine dinucleotide phosphate
NO2	Nitrogen dioxide
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
RSS	Reactive sulphur species
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
UV	Ultraviolet
GSH	Reduced glutathione
Mn	Manganese
Ni	Nickel
GSSG	Oxidized glutathione

Abbreviations	Explanation
KOH	Potassium hydroxide
Na-azide	Sodium azide
Na2CO3	Sodium carbonate
BSA	Bovine Serum Albumin
NH4 (SO4)	Ammonium sulphate
CuCI2	Copper chloride
NaOH	Sodium hydroxide
DTNB	5,5, dithiobis-(2-nitrobenzoic acid)
BHT	Butylhydroxytoluene solution
TBA	Thiobarbituric acid solution
TCA	Trichloro acetic acid solution
DPPH	2,2-diphenyl-1-picrylhydrazyl
ABTS	2, 2'-azino-bis (3-ethylbenzothiazoline-6- Sulphonic acid)
TNF	Tumor necrosis factor
TH	T-helper
IL	Interleukin

1. INTRODUCTION

A most common definition of free radicals is "molecules or molecular fragments in atomic or molecular orbitals containing one or more unpaired electrons" (Halliwell et al., 2007). Free radicals are derived commonly from oxygen, nitrogen and sulfer molecules. These free radicals are parts of groups of molecules called reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive sulfer species (RSS) (Lu et al., 2010). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced in virtually all eukaryotic cells. These molecules regulate various physiological processes, including proliferation, migration, hypertrophy, differentiation, cytoskeletal dynamics, and metabolism, but when they are available in excess they interact with lipids, proteins, and nucleic acids, altering the structural and functional properties of target molecules and causing significant tissue dysfunction and injury (Griendling et al., 2016).

Reactive oxygen and nitrogen species ROS/RNS have significant roles in cell signaling and homeostasis. Free radical generated when our cells obtain energy from food such as microbial infections. However environmental stress (e.g., UV radiation, heat exposure, smoking, and ionizing radiation), their levels could increase during the times as dramatically. ROS at high concentration reacts readily with lipids, proteins, carbohydrates, and nucleic acids. This can cause major damage to cell structures and accumulate in a circumstance known as oxidative stress (Brieger et al., 2012; Conner et al., 1996). Oxidative stress is a condition in which the balance between oxidant production and antioxidant removal is disturbed resulting in increased production and oxidant accumulation in the body. This leads to cell aging, chronic inflammatory diseases, tumor growth, neurodegenerative diseases of diabetes, cancer, kidney disease, etc. (Salman et al., 2013).

All cells in the body have extensive antioxidant defense systems, as chemical substances can prevent or reduce the physiological system's oxidative stress. Antioxidants are our first line of defense against free radical damage and are important for optimum health and well-being (Percival, 1998). These protective systems are both

endogenous (body-produced) and exogenous (nutritionally obtained) (Demirci et al., 2013).

In the recent years, some types of research showed the correlation between oxidative stress, cellular senescence, and certain diseases. Also, our lifestyle is now giving rise to the overproduction of free radical and reactive oxygen species (ROS) in our body organs, increasing oxidative stress levels while reducing antioxidant activity (Jiménez-Zamora et al., 2016). Therefore, a more useful definition of oxidative stress could be a "condition where oxidation exceeds antioxidant systems due to the loss of balance among them."(Yoshikawa et al., 2002). Leading to increased oxidative stress in numerous human diseases such as diabetes mellitus, neurodegenerative diseases, rheumatoid arthritis, cataracts, cardiovascular diseases, cataracts, renal failure, Alzheimer's, respiratory diseases as well as in aging process (Marnett 2000).

Some conditions that may impact the function of the kidney include infections, kidney stones, acute kidney injury (AKI) and chronic kidney disease (CKD). When the kidneys are damaged or diseased, they can lose their ability to perform these vital functions suddenly or slowly, resulting in waste and fluid build-up and abnormal hormonal control of blood pressure and mineral homeostasis (Stevens et al., 2009). Kidney diseases are mainly classified into two types, either acute kidney injury (AKI) or chronic kidney disease (CKD). AKI is characterized by sudden and sometimes fatal loss of function in the kidneys resulting in the accumulation of nitrogen metabolism (urea) and creatinine end products and reduced urine production or both (Bellomo et al., 2012).

To present, the global incidence of kidney disease has been underestimated; many people are not aware of their impaired function in the kidney. Kidney diseases are generally "silent diseases," without any apparent early symptoms most often. Most patients with kidney disease are unaware of the high risk of kidney failure that may require dialysis or transplantation, as well as cardiovascular diseases, infections, and hospitalizations. We estimate that over 850 million people worldwide have some form of kidney disease, which is roughly double the number of people who live with diabetes 422 million and 20 times more than the prevalence of cancer worldwide 42 million or people living with AIDS/HIV 36.7 million. Thus, kidney diseases are one of the most common diseases worldwide (Richardson et al., 2017).

2. LITERATURE REVIEW

2.1. Free Radical And Reactive Oxygen Species Generation

Free radicals lead to the peroxidation of polyunsaturated fatty acids in the cell membrane and the resulting production of additional free radicals contributing to a chain of events. This attack leaks the cell membrane and the absorption and secretion functions are lost which eventually leads to cell death (Nagane et al., 2013). These radicals can be produced in cells by the losing or acceptance of a single electron, thus acting as oxidants or reductants (Chandrasekaran et al., 2017). Reactive species or free radicals include reactive oxygen and nitrogen species collectively and are called reactive oxygen nitrogen species (RONS). They are released from macrophages, neutrophils and dendritic cells in response to an inflammatory stimulus. RONS are highly reactive due to the presence of unpaired valence shell electrons or non-static bonds and are important for successful immune response and tissue damage limitation (Salman et al., 2013).

RONS are generated by all aerobic cells and play an important role in both aging and age-related diseases (Powers et al., 2011). The production of RONS is not limited to detecting damaging effects but is also involved in the extraction of energy from organic molecules, immune defense and signaling processes (Venkataraman et al., 2013). ROS describe free radicals and other nonradical reactive derivatives (Figure 2.1) .They include radicals such as superoxide anion ($O_2^{\bullet -}$), hydroxyl (OH•), peroxyl (RO_2•), nitric oxide (NO•), singlet oxygen ($^1O_2$), nitrogen dioxide (NO_2•) and lipidperoxyl (LOO•). These radicals are becoming increasingly implicated in human diseases. Non-radicals include hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3), peroxy nitrate ($ONOO^-$), nitrous acid (HNO_2), dinitrogen trioxide(N_2O_3) , lipid peroxide (LOOH) (Kumar V et al , 2009).

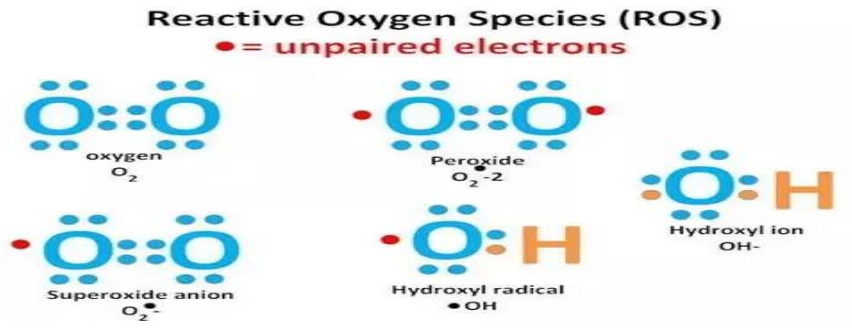

Figure 2.1. Reactive oxygen species (Dowling et al., 2009).

ROS and RNS show that they contain several carcinogens characteristics. Further, functional ROS concentrations may be required for normal cell functioning, and ROS is considered to be involved in plant and animal growth. Environmental or behavioral stressors (pollution, sunlight exposure, cigarette smoking, excessive alcohol consumption, chemicals/drugs, radiation etc.) or a tiny defect in antioxidant generation can cause a free radical excess known as "oxidative stress" and the dynamic redox balance between oxidants and antioxidants changes to oxidative potential (Figure 2.2) (cekic et al., 2013). Usually, cells could use intracellular enzymes to protect themselves against ROS damage, using them to maintain the ROS homeostasis low Whereas ROS levels increase in environmental stress and cell dysfunction periods and cell damage in the body is important (Lu et al., 2010) .

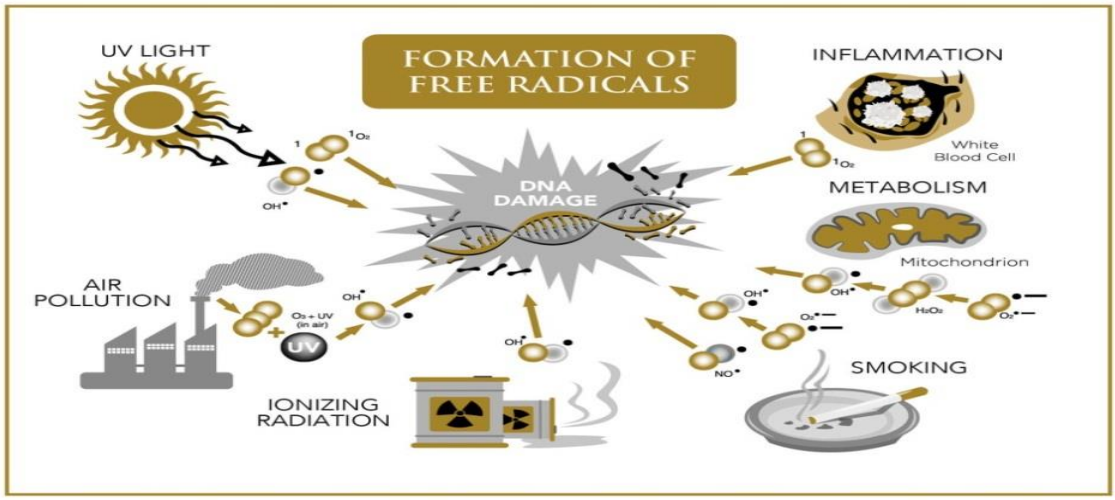

Figure 2.2. Formation of free Radicals (Lu et al ., 2010).

ROS generation can occur as a product of biochemical reactions, in the mitochondria, peroxisomes, cytochrome P450, and other cellular components, these major sources of endogenous in cells of mammals (Balaban et al., 2005). In normal aerobic metabolism and during exposure to radical-generating compounds, all organisms are exposed to ROS. Molecular oxygen in its ground state is relatively unreactive and harmless but can be partially reduced to form a number of ROS, including the superoxide anion and hydrogen peroxide (H_2O_2), which can further react to produce the highly reactive hydroxyl radical (Figure 2.3). ROS are toxic agents that can damage a wide variety of cellular components causing lipid peroxidation, protein oxidation, and genetic damage by modifying DNA.

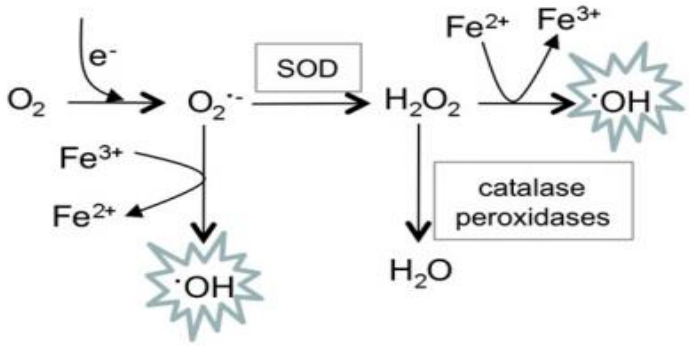

Figure 2.3. Generation of ROS.

The superoxide anion ($O_2^{\bullet}$) can be formed from electron transport chains by electron leakage to oxygen. The breakdown of superoxide catalyzed by superoxide dismutases (SODs) produces hydrogen peroxide (H_2O_2).Hydrogen peroxide can be reduced by iron (Fe^{2+}) in the Fenton reaction to produce the highly reactive hydroxyl radical. In the Haber-Weiss reaction, superoxide can donate an electron to iron (Fe^{3+}), Hydroxyl radical and Fe^{2+} production, which could further reduce hydrogen peroxide. Various antioxidant enzymes, including catalases and peroxidases, detoxify hydrogen peroxide to prevent the production of such ROS.

Oxidative stress can be defined as an excessive amount of ROS arising from an imbalance between ROS production and destruction. Oxidative stress is the result of increased free radical production and/or decreased antioxidant protection physiological activity against free radicals. However, if this defense mechanism does not control the

increased levels of ROS, oxidative stress occurs and is capable of injuring membrane lipids, proteins, and nucleic acids. Increased ROS reacts with polyunsaturated fatty acids to induce the release of toxic and reactive aldehyde metabolites, such as MDA, which is one of the lipid peroxidation process's end products (Aliahmat et al., 2012). ROS generation and antioxidant defense activity appear to be more or less in vivo balanced Also, as already mentioned, the balance can be tipped slightly in favor of the ROS so that there are continuous ROS formation and low level of oxidative damage in the human body (Figure 2.4).

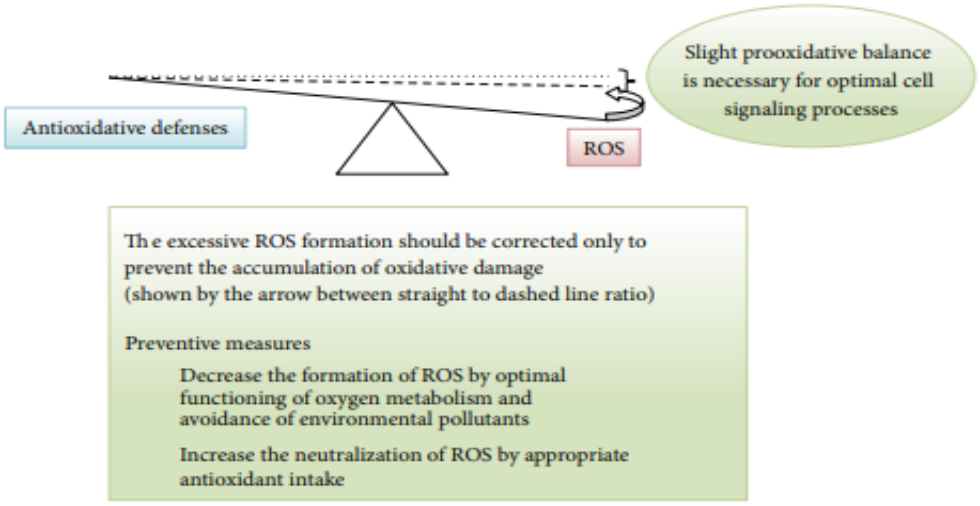

Figure 2.4. Model anti oxidative/oxidative balance of an adult person (Poljsak et al., 2013).

The balance is slightly moved towards the increased production of ROS (dashed line). The physiological balance is represented by the dashed line and not the dotted line (geometric balance) since the optimal immune system and cell signaling processes require a slight prooxidative balance. Several protective systems operate in mammalian cells to prevent formation of free radicals or to scavenge excessive amounts already formed. These are known as preventive and chain breaking antioxidants or radical scavengers like catalases, glutathione peroxidase, superoxide dismutase (SOD), α-tocopherol (Vit. E), ascorbic acid (Vit. C), β carotene (Vit.A), selenium. Although about 4000 antioxidants have been identified, the best known are Vit. E, Vit. C and the carotenoids. Many other non-nutrient food substances, generally phenolic or

polyphenolic compounds, display antioxidant properties and thus may be important for health (Hatwalne et al., 2012).

2.2. Free Radical Scavengers

Many protective systems operate in mammalian cells to prevent free radicals from forming or to scavenge excessive amounts that have formed. Free radical scavengers either prevent or eliminate reactive oxygen species before they can damage critical cell components. They are known as preventive and chain-breaking antioxidants. The first group includes catalases (CAT), glutathione peroxidases (GPx) and superoxide dismutase (SOD) i.e. the enzymatic mechanism of inactivation (oxygen-derived free radicals). The second group, chain-breaking antioxidants or 'radical scavengers' are compounds capable of transferring hydrogen to free radicals. This group includes physiological antioxidants such as ascorbic acid, α tocopherol, and β carotene. Preventive antioxidants remove the organisms involved in inducing free radical chain reaction, while chain-breaking antioxidants directly repair oxidizing radicals. Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) e.g. Vit.C or in lipids (hydrophobic) e.g. β carotene and Vit. E which are membrane-bound. In the cell cytosol and the blood plasma, water-soluble antioxidants interact with oxidants, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation, which can be synthesized in the body or obtained from the diet. Approximately 4000 antioxidants are found. During normal body metabolism, some of the antioxidants such as glutathione, ubiquinol, and uric acid are generated. For the formation or action of peroxidases, SOD, and catalase, several essential minerals, including selenium, copper, manganese, and zinc, are required. If the nutritional supply of these minerals is therefore inadequate, enzymatic defenses against free radicals may be impaired (Hatwalne et al., 2012).

Many methods that use DPPH (1,1-Diphenyl-2-picrylhydrazyl) to determine the radical scavenging activity. The most common work on the spectrophotometer to determine every antioxidant. The DPPH method is defined as a simple, fast and convenient method independent of sample polarity to screen multiple samples for radical scavenging. A wide variety of methods with the use of DPPH were established

to determine the radical scavenging behavior of different foods, beverages, and substrates (Marxen et al., 2007).

2.2.1. Superoxide scavenging and ROS

Superoxide ($O_2^{\bullet}$), Superoxide is the primary oxygen-free radicals produced in mitochondria during oxidative phosphorylation by slipping an electron from the ETC to molecular oxygen (Jin et al., 2017), a prevalent cellular free radical is involved in many deleterious changes often associated with an increase in oxidative processes and associated with low antioxidant concentrations. Although $O_2^{\bullet}$ it is not so reactive to biomolecules itself, this helps to create more $OH^{\bullet}$ and $ONOO^{\bullet}$. In phagocytes, the enzyme NADPH oxidase is produced in large quantities to kill pathogens. $O_2^{\bullet}$ is already a by-product of mitochondrial respiration as well as several other enzymes such as NADH oxidase, XO, mono oxygenases and cyclooxygenases (Steinbeck et al., 1991).

Direct scavenging of $O_2^{\bullet}$has been a model for determining the antioxidant activities .In the chemical systems, $O_2^{\bullet}$can be generated enzymatically or non-enzymatically from quinone derivatives, such as 6-anilino-5, 8-quinolinequinone (LY83583), 1,4-benzoquinone, 1,4-naphthaquinone, 2-methyl-1,4-naphthaquinone,riboflavin, etc. (Figure. 2.5). In the presence of enzymes such as NADPH-cytochrome P450 reductase and mitochondrial NADH-ubiquinone oxido reductase or thiol compounds such as glutathione and L-cysteine, LY83583 undergoes a one-electron reduction due to low redox potential (-0.3 V versus SCE), followed by formation of LY83583 semi quinone anion radical. Under an aerobic condition, this species interacts with molecular oxygen to form $O_2^{\bullet}$ and original quinones (Figure. 2.5).

$O2^{\bullet}$ is also generated in riboflavin/methionine /illuminate and assayed by the reduction of Nitro blue tetrazolium (NBT) to form blue formazan. Briefly, the reaction mixture is illuminated at 25C for40 min. and $O2^{\bullet}$ generated from the photo chemically reduced riboflavin can reduce NBT to form blue formazan which has absorbance at 560 nm. This system can be used to deter the radical scavenging activity of antioxidants. Antioxidants can be added to the reaction mixture to scavenge $O_2^{\bullet}$, thereby inhibiting NBT reduction. Reduced reaction mixture absorption indicates increased $O2^{\bullet}$ scavenging activity. The $O_2^{\bullet}$ scavenged percentage is calculated by the change in

absorption. NBT salt and other tetrazolium salts are chromogenic specimens that are useful in determining $O_2^{\bullet}$ (Lü et al., 2010).

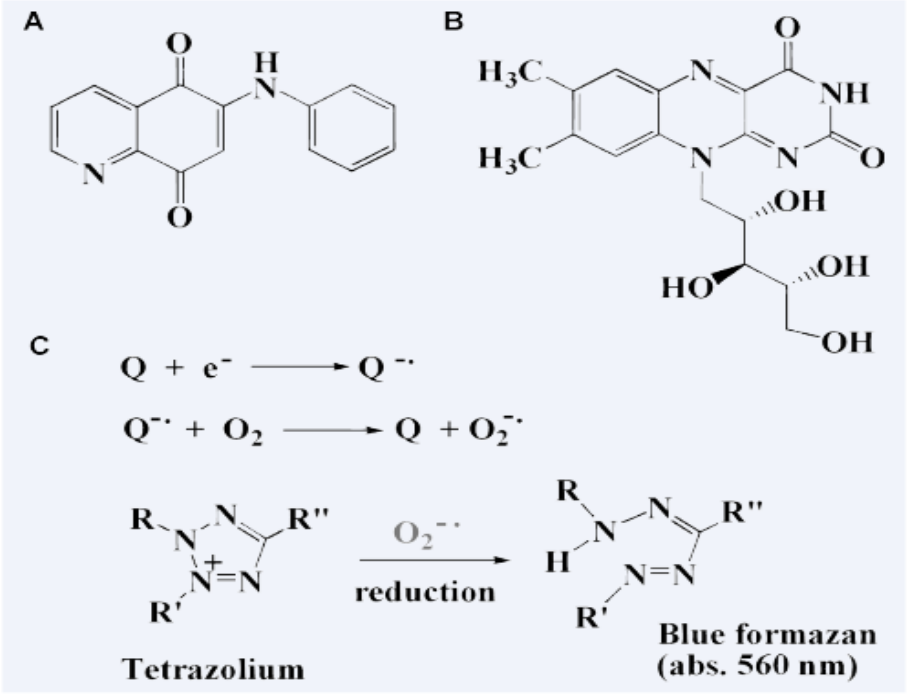

Figure 2.5. Structure of quinones.

(Q):LY83583 (A), riboflavin (B) and the formation of superoxide and its reaction with NBT (C), where R, R and Rrepresent p-nitrophenyl, o-methoxyphenyland phenyl groups, respectively (Lü et al., 2010).

2.2.2. Hydroxyl radicals scavenging and ROS

Hydroxyl radical ($OH^{\bullet}$) is highly reactive, more toxic than other radical species and can affect biological molecules such as DNA, proteins, and lipids. $OH^{\bullet}$ It is widely believed that by simply incubating $FeSO_4$and H_2O_2 in aqueous solution, the reaction system Fe^2 or C / H_2O_2Fenton is produced. Therefore, $OH^{\bullet}$ scavenging antioxidant activity can be achieved by direct scavenging or preventing the formation of $OH^{\bullet}$ by free metal ions chelation or converting H_2O_2 into other harmless compounds. The Gutteridge method which is monitored in the Fe^3 EDTA – H_2O_2–deoxyribose system, will determine the scavenging ability of antioxidants. The extent of deoxyribose

degradation induced by the $OH^{\bullet}$ can be measured in the aqueous phase directly by thiobarbituric acid reactive species (TBARS) assay at 532 nm (Figure. 2.6). This method is based on the fact that the degradation by $OH^{\bullet}$forms of deoxyribose is an active species of malondialdehyde, forming a thiobarbituric acid (TBA) adduct. The adduct, MDA–TBA, has a spectrophotometrically assayable absorption at 532 nm. Through this assay, the ability of several antioxidants to scavenge $OH^{\bullet}$has been studied and compared to that of $OH^{\bullet}$ DMTU, uric acid, trolox and mannitol (Lü et al .,2010).

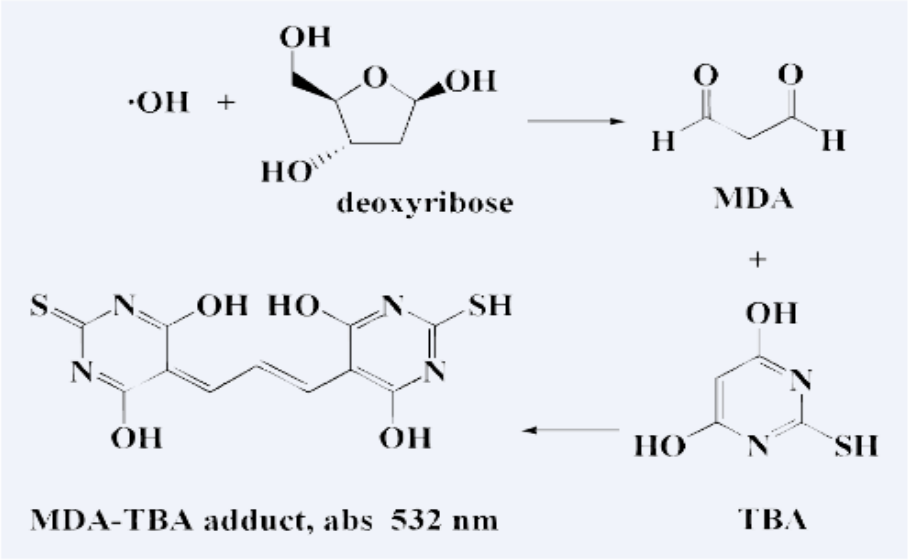

Figure2.6 Reaction pathway of TBARS assay of $OH^{\bullet}$ (Lü et al., 2010).

2.2.3. Biochemistry of ROS and RNS

The production of ROS can come in the mitochondria, peroxisomes, cytochrome P450, and other cellular components as well as a product of biochemical reactions. Most parts of the ROS produce as primarliy by the mitochondrial ETC. In the ETC nearly all of the cells and tissue that continually switch a small part of molecular oxygen to ROS. Maybe the ROS produced by other mechanisms such as the respiratory burst in activated phagocytes as ionizing have influence damage of the radiation on the cell membrane parts and many cellular enzyme by-products like as (NADPH oxidases, xanthine oxidase, nitric oxide synthase). The ROS generation has a natural byproduct of aerobic metabolism and integrated into the preserve of homeostasis of tissue oxygen.

If oxygen homeostasis is not controlled, the oxidative stress in the environment of cellular that is increased. Superoxide, hydrogen peroxide and hydroxyl radicals are normal metabolic by-products which are produced always by the mitochondria in growing cells. Microsomal cytochrome P450 enzymes, flavoprotein oxidases and peroxisomal enzymes are other important to intracellular sources of ROS (Ozcan et al., 2015).

The action of ROS with a large number of biomolecules (carbohydrates, lipids, proteins, and nucleic acids). After reacting with them, the ROS could irreversibly damage and change the function of these molecules. The ROS in the process of aging, after these ROS become as cell damaging agents in aging theory. ROS also work as a vital role in host protection because lacking in ROS production reduce the ability to kill leukocytes (Beckman et al., 1998).

2.2.3.1. Biosynthesis of ROS

2.2.3.1.1. Mitochondrial ROS production

The most amount of intracellular ROS is sourced from mitochondria. (Figure. 2.7). The superoxide radicals are generated from the two major sites, first in the electron transport chain, namely complex I (NADH dehydrogenase) as well as second is complex III (ubiquinone cytochrome c reductase). The transfer of electrons from complex I or II to coenzyme Q or ubiquinone (Q) the effect there decreased in shape forming of coenzyme Q (QH_2). The reduced form QH_2 regenerates coenzyme Q by an unstable intermediate semi quinone anion $^{\bullet}Q^{-}$ in the Q-cycle. The formed Q at once the convey of electrons to molecular oxygen resulting in the forming of radical superoxide. Superoxide production is non-enzymatic and so this higher the metabolic rate, the ROS produced is higher.

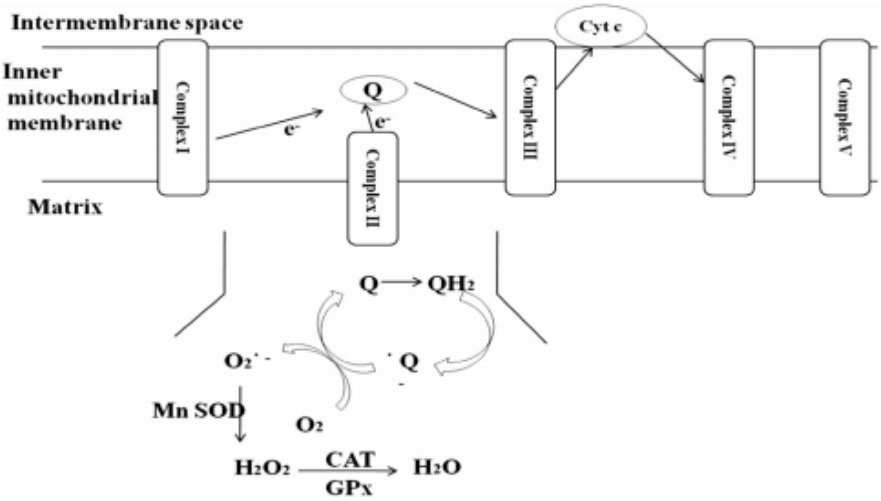

Figure 2.7 Mitochondrial ROS production (Phaniendra et al., 2015).

The change of superoxide anion to hydrogen peroxide via the work as mitochondrial superoxide dismutase (MnSOD). The H_2O_2 could be detoxified by the Catalase (CAT) and glutathione peroxidase (GPx). The other mitochondrial parts there which related to generation of ROS such as mono amino oxidase, aketoglutarae dehydrogenase, and glycerol phosphate dehydrogenase.

2.2.3.1.2. Other ROS production

In the cells of mammals, xanthine oxidase, lipoxygenase, cyclooxygenase, nitric oxide synthase, cytochrome P450 enzymes, mitochondrial NADH: ubiquinone oxidoreductase (complex I) and NADPH oxidase that should be similar as major sources of superoxide are specified. Through the reducing of oxygen, which it catalyzes superoxide generation, there NADPH use as donor electron (Bedard et al., 2007). The 5-lipoxygenase (5-LOX) The biosynthesis of very extremely bioactive in eicosanoids also has been catalyzed, such as leukotriene (LT) and hydroxy eicosatetra enoic acid (HETE). The biosynthesis of LT starting with the metabolism of arachidonic acid by 5-LOX. LTB4. In the last years ago, investigated that shown LTB4 treatment of fibroblasts and neutrophils in the production of ROS at result, as well as that LTB4-such as chemotaxis is mediated by a NADPH oxidase-dependent cascade (Ozcan et al., 2015).

2.2.4 Physiological role of ROS

Approximately all the body processes function have the correlated including the physiological role of ROS, such as related with the reproductive processes. During the physiological circumstances is assured the level of free radicals as well as reactive metabolites is needed, it is not sufficient for the whole repression of the free radical generation. There ROS is causing to the hypothalamic pituitary adrenal axis to be activated. The elevated high to the release of endogenous catecholamine did detect in of cold environmental conditions. The activity of succinate dehydrogenase will also become high showing the effect of ROS as obvious in the cold environmental conditions.

The useful of physiological cellular usage of the ROS is now existence confirmed in various fields, such as intracellular signaling and redox regulation.The fact which the low levels of ROS signal molecules, apoptosis, modulate cell proliferation, and gene expression by activating transcription factors has been well known,like NF kappa-B and hypoxia-inducible-factor-1α (HIF). The inducers of NF-kappa-B (NF-κB) contain also tumor necrosis factor alpha (TNFα) and interleukin 1-beta (IL-1β). ROS could work as signaling intermediates for cytokines, such as IL-1 and TNFα. These pro inflammatory cytokines, tumor necrosis factor (TNF)-α, interleukin-1β (IL-1β), and interferon-γ (IFN-γ), besides can elevated level of the oxidative stress in humans, its influence of the generation ROS (Renard et al., 1997). The $O_2^{\bullet-}$and H_2O_2 as produced by activated phagocytes is the typical instance for good intent in deliberate metabolic ROS production. H_2O_2 is generally recognized such as an intracellular messenger. Generation of $O_2^{\bullet-}$, HOCl, and H_2O_2 by phagocytes is the critical for protection against many bacterial and fungal strains. $O_2^{\bullet-}$ is produced via various cell types other than phagocytes, such as lymphocytes and fibroblasts. Besides ROS are vital role in signal transduction, there is seems to be no high seize of antioxidant defensive lines in mammals (Poljsak et al., 2013). The immune system uses the ROS For instance, the ROS has shown to cause T-cell reproduction by activating NF-kB. Macrophages and neutrophils produce ROS to destroy the bacteria by they swallow the phagocytosis (Fraunberger et al., 2016).

2.2.5 Determination of ROS

The free radicals have a very short of half-life, it that making as difficult to evaluate them in the laboratory. However, today have several methods can use for measuring oxidative stress, each of which methods has advantages and disadvantages. There are several of the possible approaches: detection of free radicals, such as directly by paramagnetic electron resonance (electron spin resonance, ESR),as well as indirectly methods, its some more stable intermediates have examined: the assessment of the effect of the free radical attack on biological molecules via high performance liquid chromatography, gas liquid chromatography, colorimetric tests. The amount measurement of antioxidant has status could be evaluated by colorimetric, immune, or enzymatic methods (Figure 2.8). The direct ROS detection methods to measure superoxide, H_2O_2, $OH^{\bullet}$. These were very reactive species as well as it is hard to measure them.

In vivo, ESR is comparatively insensitive and needs stable the concentrations free radical in the micromolar range, which has a reduce its usage to the determined quantity of ROS in the patient. ESR method is can only be used to in vivo specimens via the spin trapping technique. While the toxicity that does not appear to be a major problem to most traps, There are no efficient electron spin traps for human use. Indirect methods are used to solve these problems. Indirect methods act alike generally evaluate changes in endogenous antioxidant defense systems or measure cellular component damage caused by the ROS. It appears that reasonable to determine the damage induced by ROS rather than the direct ROS measurement,because of the damage caused by ROS that is critical rather than the total amount of generated ROS (Poljsak et al ., 2013).

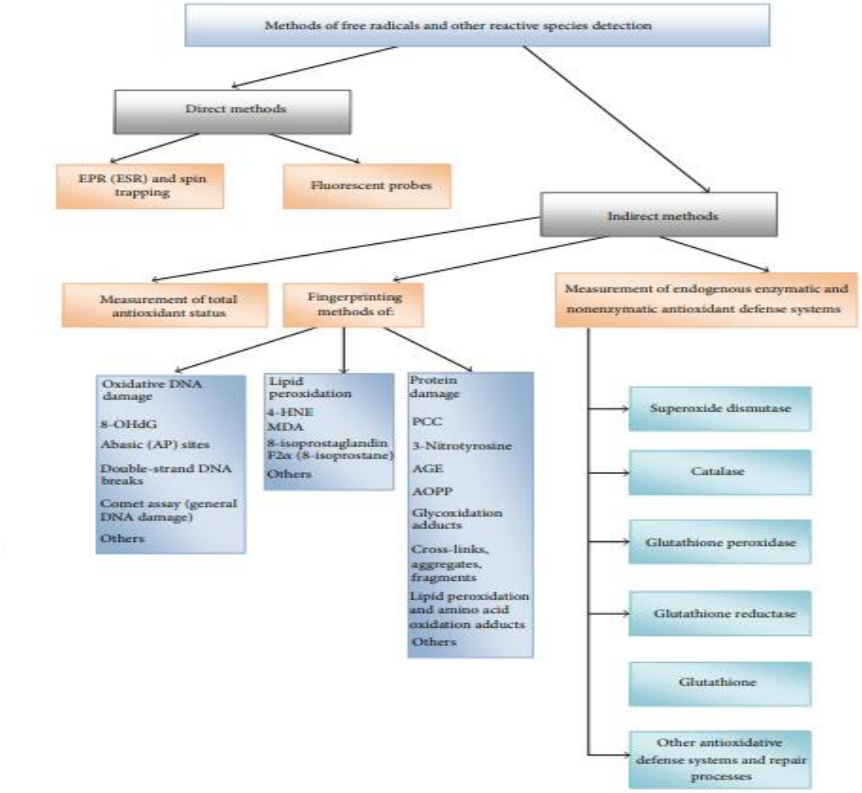

Figure 2.8. Scheme of Methods the oxidative stress determination. (8-OHG) = 8-hydroxyguanosine; 4-HNE = 4-Hydroxynonenal; MDA = malondialdehyde; PCC = protein carbonyl content; AGE = advanced glycation end products; AOPP = advanced oxidation protein products (Poljsak et al., 2013).

2.6.6. Inhibition of free radical generating enzymes

NADPH oxidases are a class of the enzymes connected with the plasma membrane that move one electron from the cytosolic donor NADPH to an extracellular oxygen molecule, generated $O_2^{\bullet}$ (Babior 1999). XO is the enzymes implicated in the synthesis of uric acid in the body, which catalysis the oxidation of hypoxanthine and xanthine to the submission of uric acid and the level of oxidative stress raised in the body (Figure. 2.9). These enzymes are the major sources of free radicals and have observed in the many conditions of physiology and pathology. $O_2^{\bullet}$ is also a generated by mitochondrial respiration, as well as numerous other enzymes such as NADH oxidase, mono oxygenases and cyclooxygenases. It is biologically very toxic and can use for killing invading microorganisms by the immune system. In phagocytes, the

enzyme NADPH oxidase produces $O_2^{\bullet}$ as large amount for use in the oxygen-dependent killing mechanisms to invade pathogens. The regulated generation of reactive oxygen derivatives when the respiratory burst is important to the protection of an organism toward attacking microorganisms without inducing a significant loss of the tissue function (Steinbeck et al., 1991). Although an extreme level of the ROS increases oxidative stress such as low-density lipoprotein (LDL) oxidation. Also is a direct correlation between the raised of phagocytic NADPH oxidase activity and increased endogenous oxidized LDL has observed in the patients who have metabolic syndrome. However, both NADPH oxidase control to avoid the excess of ROS and antioxidant supplementation was recommended as useful methods to prevent the harmful effects of oxidative stress in patients with hemodialysis (Lü et al ., 2010).

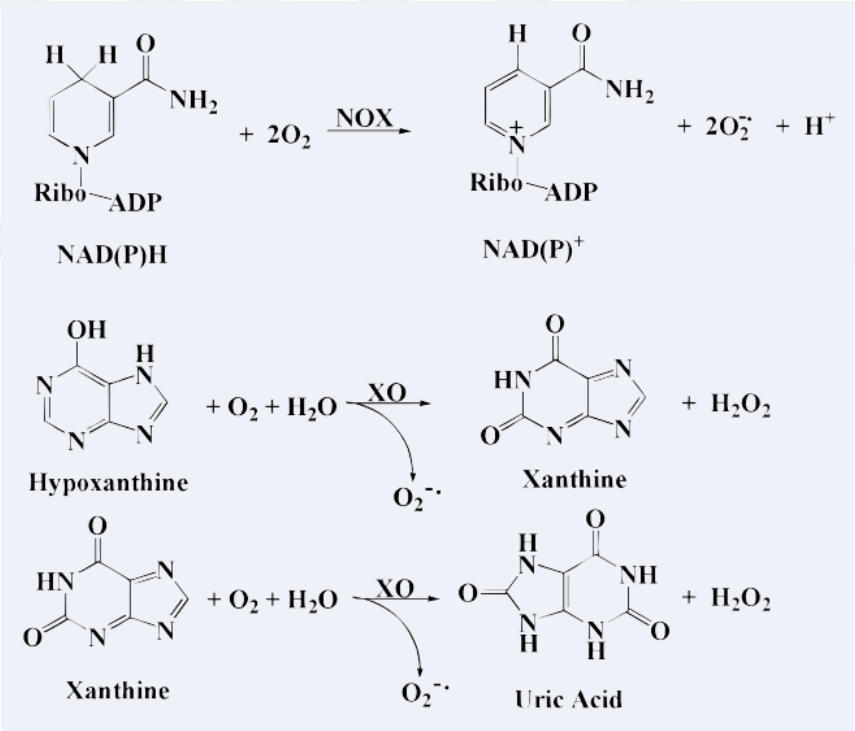

Figure. 2.9. Superoxide and hydroperoxide generation from NAD (P) H, oxidase (NOX) and XO (Lü et al., 2010).

2.3. Oxidative Stress

The imbalance between the free radicals and antioxidant enzymes of their stable agent in the body is indicating to oxidative stress. when free radical has a low level of

concentration performance as an important role in the cellular signaling and physiological regulation methods but the high level can affect the damage change in the cell. Also, oxidative stress has appeared as beneficial in some cases occurrences. For example, it acts alike reduce apoptosis to ready the birth canal for delivery. Besides, during suitable physical activity and ischemia, the biological defense processes are reinforced by oxidative stress. Thus, an even more good definition of oxidative stress may well be a "state where oxidation exceeds antioxidant systems due to the loss of balance between them has been lost." (Yoshikawa et al., 2002).

When the oxidative stress happens, cells are trying to reduce the oxidant influence as well as restore balance by activating or silencing genes that have to encode protective enzymes, transcription factors, and structural proteins (Scandalios 2004). One of the most significant determinants of oxidative stress in the body is the ratio between oxidized and reduced glutathione (2GSH/GSSG). The increased level of ROS produced in the body can alter the structure of DNA, result in changing the proteins and lipids, activate multiple stress-induced transcription factors, and generate inflammatory and anti-inflammatory cytokines (Birben, E., et al 2012).

Redox state is the overall reducing potential or ability reduction of every redox pairs including GSSG/2GSH, NAD^+/NADH, $Asc^{\bullet-}$/$AcsH^-$, etc. present in biological fluids, organelles, cells or tissues. Redox state does not just represent a redox couple's state, as well as redox the environment of a cell. The redox state from a biological system is preserved during normal circumstances against more negative possible of redox levels. When the high oxidative stress, the mitochondrial roles have impaired the function, there resulting in depletion of ATP and death of necrotic cells, although mild oxidation may cause apoptosis. The some of new reports have seen proof that the apoptosis or necrosis induction when oxidative stress is determined via the redox state of he cell. The influence damage of the free ROS and RNS radicals which causing of potential biological damage is called to oxidative stress as well as nitrosative stress,respectively. This is noticeable in the biological systems if there is each of an excessive ROS / RNS generation as well as a deficiency of an enzymatic and nonenzymatic of antioxidant. The redox state/oxidative stress are a difficult method.The effect on the organism depending on the type of oxidant, the place, and power of its

generation, the structure and function of the different antioxidants, also the capacity of repair systems (Schafer et al., 2001).

2.3.1 Redox homeostasis

Redox homeostasis is accomplished by wary regulation of both ROS production and the removal of the body system. Maintaining homeostasis and signaling of the redox event that needs significant control of synthesis and detoxification. The obstruction of the redox pathway that controlling the ROS as well as its redox signalling activities influences the cell physiology and it may eventually occur to abnormal signaling, unregulated of toxic by-products aggregation, oxidative deficiency, and cytotoxicity.The High levels of oxidative stress are usually associated with abnormalities that represent the tumor-specific alterations that reveal cancer cells to increase the ROS depending on the intensity of their defense of the antioxidant system (Panieri et al., 2016). Besides, there are many types of cells likely more obvious or the more responsive to oxidative stress correlated molecules such as hepatic stellate cells, Kupffer cells, and endothelial cells. A type of cytokines such as TNF-α can the generate in the Kupffer cells caused by oxidative stress, which might raise inflammation as well as apoptosis. In the event of hepatic stellate cells, which proliferation and synthesis of collagen is a hepatic stellate cell is induced via the lipid peroxidation triggered by oxidative stress (Sakaguchi et al., 2011).

In mammals, the system of antioxidant that has a work product for protecting the redox homeostasis (Figure 2.10). When the extreme of ROS, this homeostasis will also be disturbed, going to the result in oxidative stress,which plays an important role in liver diseases as well as other chronic and degenerative dysfunctions (Li et al., 2014). The oxidative stress is not only causing the hepatic damage by trying to induce irrecoverable changes in proteins, lipids,and DNA content but also, the more notable of modulating mechanisms that regulate the normal functions of biological.While these mechanisms control the transcription of genes,protein expression, the apoptosis of cells and activation of hepatic stellate cells (Feng et al., 2011). It has also been recommended

that there are difficult cross-talks with pathological circumstances, inflammation, free radicals as well as immune responses (Li et al .,2015).

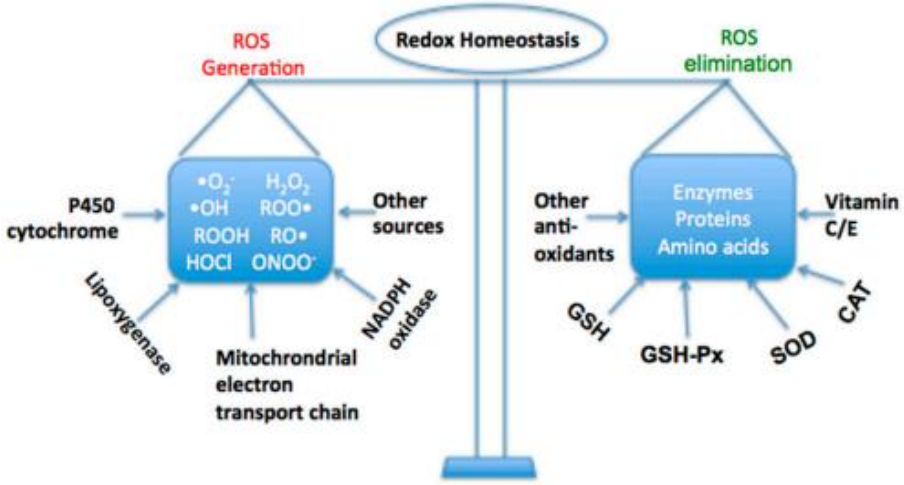

Figure 2.10. The redox homeostasis.

2.3.2. Oxidative stress and diseases

Oxidative stress can cause tissue injury or perhaps even the death of cells which can occur mostly by two pathways: necrosis and apoptosis (Gueteens et al., 2002). Oxidative stress leads to a large number of diseases as well as many of the diseases that influence lead to death such as (Figure 2.11) (Ozcan et al., 2015).

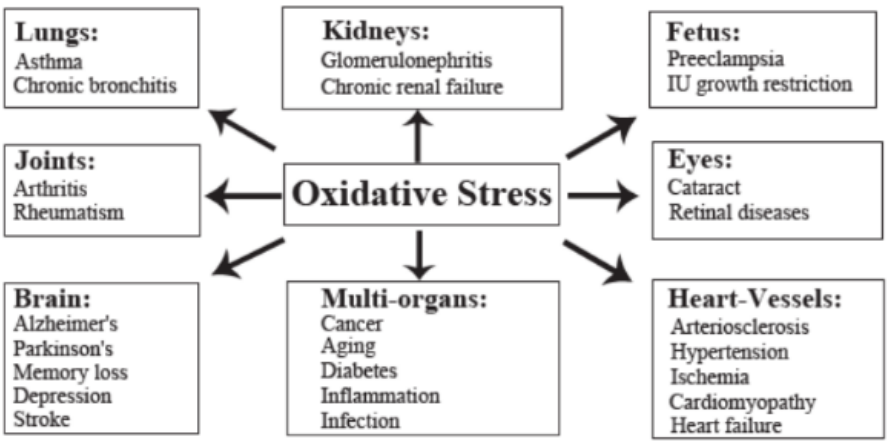

Figure 2.11 Oxidative stress-induced diseases in humans (Pham-Huy et al., 2008).

2.3.3. Biological actions of free radicals

Oxidative stress may a cause to destroy all of the molecular targets, including DNA, lipids, and proteins (Figure 2.12), the first line of the attack is not always noticeable because the injury of mechanisms overlaps greatly. The initial cellular target of OS can the alter; DNA is an influential the first target of damage (Gueteens et al., 2002). When the lipid peroxidation is started, there will be a generation of chain reactions until finished products are produced. Thus the end products of lipid peroxidation, like as malondialdehyde (MDA), F2-isoprostanes, and 4-hydroxy2-nonenol (4-HNE) are formed in the biological systems. The bases of DNA are highly sensitive to the ROS oxidation, as well as the main observable of the oxidation product of DNA bases in the vivo is 8-hydroxy-2- deoxyguanosine. Oxidation of the DNA bases can effect mutations and deletions in each of these nuclear and mitochondrial DNA (Rahal et al., 2014).

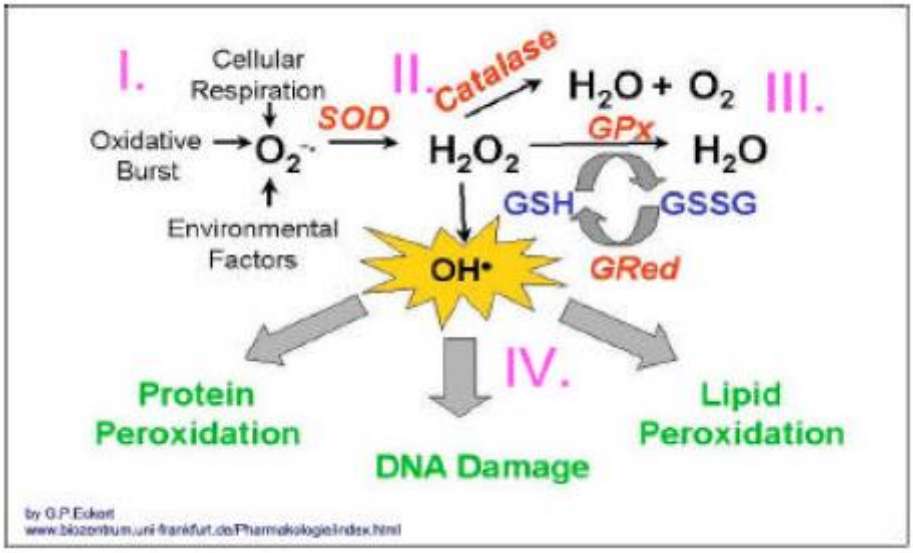

Figure 2.12. Biological actions of free radical (Gueteens et al., 2002).

2.3.3.1. Oxidative damage to DNA

Oxygen originated radicals can directly attack DNA, which either on the sugar, phosphate or bases of purine and pyrimidine. On the other side, the oxidative damage of DNA may also be indirect by the increase of the intracellular Ca^{++} ions. The structure of DNA changes by the influence of the free radical-mediated reactions (e.g., deletions, rearrangement, base-pair mutations, nicking, insertions and sequence amplification). The bases of degradation can produce various products, such as 8-OH-Gua, hydroxy methyl urea, thymine glycol; thymine and adenine ring opened and saturated products (Rowe et al., 2008). The nucleic acids damaged by the oxidatively from both ROS / RNS. The mitochondrial DNA is more susceptible to the ROS attack than the nuclear DNA since this is the positioned are close easy reach to the ROS produced site. ROS most significantly, the $OH^{\bullet}$ radical that directly interacts with all of the pieces the DNA including purine and pyrimidine bases, the base of deoxyribose sugar and the causes several interchanges such as single and double-stranded breaks in the DNA. The $OH^{\bullet}$ radical work like this the hydrogen atoms to generate a number of changed purine and pyrimidine base via products and the cross links of DNA protein. The pyrimidine base attack by $OH^{\bullet}$ produces various pyrimidine compond such as thymine glycol, 5-hydroxydroxydeoxy uridine, uracil glycol, hydantoinand, 5-hydroxydeoxy cytidine among others. The purine base compound generated by hydroxyl radical attack like as, 8-hydroxydeoxy guanosine, 2, 6-diamino-4-hydroxy-5-formamidopyrimidine, 8-hydroxy deoxy adenosine (Halliwell et al., 1999).

2.3.3.2. Oxidative damage to proteins

The free radicals injury outcomes in the inactivation and denaturation of necessary proteins, with the highest risk of proteins, are these amino acids including sulphur (as methionine and cysteine), like certain enzymes and membranes ion carriers. The radical has abstracts a proton, thereby oxidizing the moiety of the sulphydryl.When enzymes in the risk including alpha-1 antiprotease, calcium ATPase, calmodulin glucose-6-phosphate dehydrogenase as well as glyceraldehydes-3-phosphate

dehydrogenase (Cabiscol et al., 2000). ROS can trigger peptide chain of fragmentation, the modification of electrical charge protien, the cross-linking of proteins, and oxidation of particular amino acids, as well as consequently has to lead to higher sensitivity to the proteolysis through degradation by special proteases (Kelly et al., 2003). The types of protein residues are cysteine and methionine are especially very sensitive to oxidation (Dean et al., 1985). Oxidation of sulfhydryl groups or protein residues methionine produces to the alterations of conformational, protein unfolding, as well as degradation. Enzymes that also have metals on or near their active sites are particularly more sensitive to oxidation catalyzed by metals. Oxidative alteration of the enzyme has proved to prevent their activity (Birben et al., 2012).

2.3.3.3. Lipid peroxidation and malondialdehyde (MDA)

Lipid peroxidation is a free radical as a mediated chain reaction at the started that appears like the oxidative decay of polyunsaturated lipids. The most popular purposes are pieces of the biological membranes. When generated in the biological membranes, certain that reactions can be started either improved by several numbers of this toxic product, such as end peroxides as well as aldehydes (Ayala et al., 2014).

Malondialdehyde (MDA) is composite of the three-carbon formed by the peroxidized polyunsaturated fatty acids that mostly arachidonic acid. Also, this is one of the end products from membrane lipid peroxidation. Since the level of MDA is elevated in several diseases among the abundance of oxygen free radicals, the multiple associations with free radical damage were detected. Esterbauer and his partners widely researched in the 1980s from the many various aldehydes that can be produced as secondary products through lipid peroxidation, like as malondialdehyde (MDA), hexanal, propanol, as well as 4-hydroxynonenal (4-HNE). The MDA views like the most abundant mutagenic product of lipid peroxidation, while 4-HNE was the most toxic product (Ayala et al., 2014). The lipids of the membrane, particularly the polyunsaturated fatty acid are remainder of the phospholipids which is very sensitive to oxidation by free radicals (Siems et al., 1995). The lipid peroxidation is extremely necessary for vivo because of its relationship in the numerous pathological

circumstances. The results of lipid peroxidation in the membrane that the loss of functioning such as reduced fluidity, inactivation of membrane-bound enzymes as well as receptors (Bast et al., 1993).

MDA has also been frequently used as a beneficial biomarker for omega-3 as well as omega-6 fatty acid of lipid peroxidation for many years due to its simple reaction with thiobarbituric acid (TBA). The TBA technique is based on the reactivity of TBA toward the MDA to produce a strongly colored chromogenic fluorescent red adduct; its the test was the initial usage in the food chemists to determine the auto oxidative degradation of oils also fats. Furthermore, the thiobarbituric acid reacting substances test (TBARS) is particularly unspecific which has to start to an abundant controversy over its performance for the quantification of MDA in the vivo samples (Sinnhuberet al., 1958). Each one of them enzymatic or non-enzymatic pathways (Figure 2.13). The production of MDA through enzymatic systems is very well-noted but MDA biological functions and working dose-dependent double usage have not been observed while MDA was more chemically stable as well as the membrane is permeable than ROS and have much less than toxic compared to 4-HNE or methylglyoxal (MG). To determine the production of MDA in vivo and biological function critical as reveals by the many of research work on this compound (Ayala et al., 2014).

The production of MDA by enzymatic ways, it could be formed in vivo as a byproduct in enzymatic processes throughout the biosynthesis of thromboxane A_2 (Figure 2.13)(Ayala et al., 2014).TXA_2 is a biologically active metabolite of arachidonic acid generated by the performance of the thromboxane A_2 synthase in the prostaglandin end peroxide or prostaglandin H_2 (PGH_2). PGH_2 beforehand is produced through the action of the cyclooxygenases within arachidonic acid (Massey et al., 2011). On the other side production of MDA by nonenzymatic processes is a blend of lipid hydroperoxides that generated throughout the lipid peroxidation process. The peroxyl radical of the hydroperoxides with a homoallylic cis double bond to the peroxyl group allows for their simple cyclization by adding intra-molecular radical to the double bond as well as forming a new radical. After cyclization, the intermediate of the free radicals can cyclize repeat to produce bicycle end peroxides, which are structurally similar to prostaglandins, also undergoes cleavage to produce MDA.

After produced MDA, the fate of MDA is can either be enzymatically metabolized or it can bind to cellular as well as tissue proteins or DNA to create adducts that effect to damage of biomolecular. New studies have shown that a possible biochemical pathway for MDA metabolism includes oxidation by mitochondrial aldehyde dehydrogenase accompanied by decarboxylation to generate acetaldehyde,which this oxidized to acetate as well as more CO_2 and H_2O by aldehyde dehydrogenase(Figure 2.13)(Marnett et al.,1985). On the other side, phosphoglucose isomerase is probably to be effective for metabolizing cytoplasmic MDA to methylglyoxal (MG) and thus to D-lactate by enzymes of the glyoxalase way via utilizing GSH such as a cofactor (Agadjanyan et al., 2005).

MDA could be produced in vivo by decomposing of the arachidonic acid (AA) as well as larger PUFAs(polyunsaturated fatty acids) as a side product via enzymatic methods when the biosynthesis of thromboxane A_2(TXA_2) and 12-l-hydroxy-5,8,10-heptadecatrienoic acid (HHT) (blue pathway), or by nonenzymatic methods through bicyclic end peroxides produced throughout the lipid peroxidation (red pathway). Single generated MDA could be enzymatically metabolized (green pathway). The essential key enzymes involved in the formation and metabolism of MDA: cyclooxygenases (1), prostacyclin hydro peroxidase (2), thromboxane synthase (3), aldehyde dehydrogenase (4), decarboxylase (5), acetyl CoA synthase (6), and tricarboxylic acid cycle (7) (Ayala et al., 2014). MDA measurement is absolutely important in pathological states, but it also has played a major role in the toxicological impact of pollutants in humans as well as animals like metals, solvents, and xenobiotics. Also, the quantification of MDA was commonly utilized during studies including toxicity processes of many substances like those of parquet, carbon tetrachloride as well as exposure to metals such as cadmium, aluminum (Abd-Elghaffar et al., 2005).

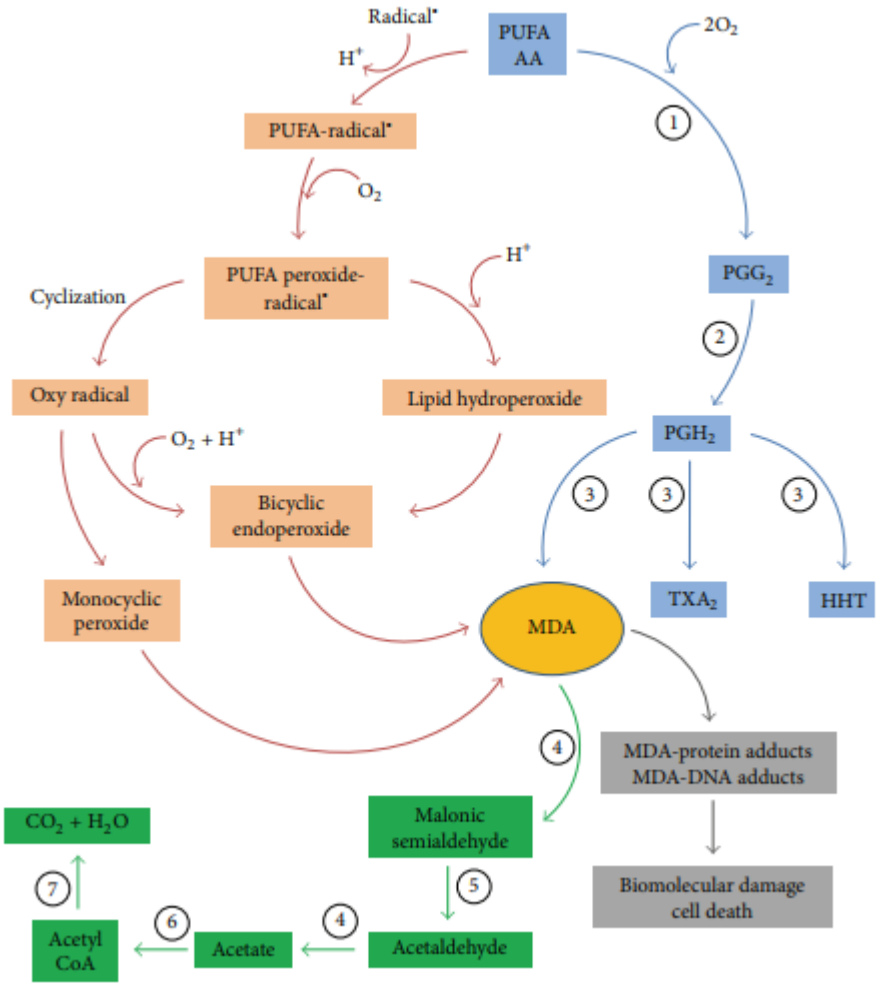

Figure 2.13. MDA formation and metabolism.

However, the MDA is detected by these techniques generally used are the determination of MDA-TBA complex by either indirect spectrophotometry or fluorimetry as well as via direct HPLC (high performance liquid chromatography) with various detectors. MDA molecule is small, polar and highly water-soluble, that making it hard to extract. It contains no eletrophore, chromophore or fluorophore that would allow to detection at the necessary sensitivities for investigation. Therefore, analytical derivatization is a general explanation to these problems, although chromatographic methodologies have been regarded as a higher special measure than the commonly used method for the determination of thiobarbituric acid reactive substances (Grotto et al., 2009).

2.4. Antioxidants

The antioxidant is the first line of defense in the body that works against free radical damage as well as is important for optimal health with well-being (Percival, M. 1998). Antioxidants are the molecules that have inhibitting cellular damage induced by the oxidation of other molecules. The oxidation process is a chemical reaction that happens when carrying electrons of one molecule to an oxidizing agent. Also oxidation reactions are known to generate free radicals. Antioxidant interacts with certain free radicals, therefore, it prevents this chain reaction by removing free radicals in the intermediate position as well as inhibits other oxidation reactions by oxidizing itself. When free radicals attacking the cells, the antioxidants have able to stabilize or deactivate these free radicals. Antioxidants are essential for optimum cellular and healthy physiological (Percival, M. 1998).

Antioxidants are a type of natural chemical element when these discovered in the food which able to prevent or reduce the oxidative stress of the physiological system.The body is permanently generating free radicals due to the regular usage of oxygen. Those free radicals are accountable for cell damage in the body as well as related to many types of health problems, like heart disease, renal failure, macular degeneration, diabetes, and cancer. Antioxidants being excellent free radical scavengers help in the preventing as well as correcting the cell damage generated via these radicals. While oxidation reactions are essential to life, they can be dangerous as well. The found of antioxidants in the plants and animals have a difficult method of many types of antioxidants, like as vitamin C and vitamin E, as well as enzymes, including as catalase (CAT), superoxide dismutase (SOD), and several peroxidases (Hamid et al., 2010). Additionally, there have some antioxidants in the type of micronutrients which cannot be made by the body itself, for example, vitamin E, β-carotene, and vitamin C, as well as consequently these need be supplemented in the normal food (Teresa et al., 2011).

Antioxidants can divide into two type general classifications: endogenous and exogenous. Exogenous antioxidants perform an important role in this delicate balance linking the oxidation and antioxidation in living systems. The type of endogenous antioxidant defense system which has included endogenous (enzymatic as well as non-enzymatic)(Table 2.1) antioxidants like as superoxide dismutase (SOD), catalase

(CAT), glutathione peroxidase (GPx) and glutathione (GSH), with different and exogenous antioxidants included as vitamin C, vitamin E, carotenoids and polyphenols, there are the main source to the diet. The antioxidants inside the body are consist of antioxidant enzyme defenses as well as adding antioxidant substance like as melatonin and glutathione there are internally synthesized. The body could be supplied the antioxidant in the outside by the nutrition and many types of natural as well as synthetic compounds are obtained from the complex mixtures (like chocolate or olive oil) or separated to be taken such as a supplement. The mechanism of work each type of the antioxidant will alter depending on location, chemical structure, and bioavailability inside the body and the rate of redox modulation experienced by the cell. When the level of ROS is high that becomes harmful and presenting the pathophysiological actions, but at a low level of ROS they may be advantageous for normal physiological actions (Fraunberger et al., 2016).

Table 2.1.Some of enzymatic and non-enzymatic

Enzymatic antioxidants	Non- enzymatic antioxidants
A. Superoxide dismutase (SOD)	A. Vitamins
B. Catalase (CAT)	- α – tocopherol
C. Glutathione peroxidase (GPx)	- ascorbic acid
D. Glutathione-S-transferase (GST)	- carotenoids
E. Glutathione reducatse (GSH)	B. Glutathione
	C. Trace elements selenium, copper , manganese, and zinc
	D. coenzyme Q, dietary polyphenols
	E. other, uric acid, flavonoid, melatonin

Enzymatic antioxidants are uniquely generated in the human body also can be divided into two types such as primary and secondary antioxidants.Primary antioxidants mainly such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) Secondary antioxidant such as glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PDH) (Misra et al., 2014).

2.4.1 Beneficial use of antioxidants

Cellular redox homeostasis is carefully controlled by a comprehensive endogenous antioxidant defense system, which contains endogenous antioxidant enzymes like as superoxide dismutase (SOD), catalase , glutathione (GSH), glutathione peroxidase (GPx), proteins, and low molecular-weight scavengers, like coenzyme Q, uric acid, as well as lipoic acid. The human antioxidant protection is difficult and must reduce the levels of ROS while enabling useful roles of ROS to perform cell signaling as well as redox regulation (Halliwell 2011). Many of the necessary keeping repair systems become insufficient in senescent cells, cell damage accumulates, for instance, lysosomal accumulation of lipofuscin (Terman et al., 2006). Age is one of the factors that related to change oxidative is most obvious in the nonproliferating cells, like as cardiac myocytes and neurons since there is no "dilution" of damaged the structures via cell division (Terman et al., 2001). When the increased amount of fruit and vegetable intake that effects are correlated with decreased parameters of cell damage in vitro, for instance, lower oxidative stress, DNA damage, malignant transformation rate, as well as so ahead; epidemiologically they resemble to happen in lowered rate of certain classes of cancer and degenerative diseases, like as cataract and ischemic heart disease. On the other side, increased or prolonged free radical action that able to destroy ROS protection mechanisms, according to the increase of diseases and aging. Since oxidative damage of these cells enhances among age, the increased amount of intake of exogenous antioxidants of fruit as well as vegetables which may maintain the endogenous antioxidative defense. The antioxidants, such as vitamin C and E, carotenoids, and polyphenols (e.g., flavonoids), are instantly thought to be the main of exogenous antioxidants. Clinical investigations refer that eating a diet that has a rich in fruits, vegetables, whole grains, legumes, and omega-3 fatty acids that can benefit as humans body protect from disease prevention (Poljsak et al ,. 2013).

2.4.2 Antioxidant response against oxygen radicals of endogenous or exogenous sources.

Superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) these are antioxidant enzymes that do not only perform as a basic but essential role in the antioxidant defensive ability of biological systems against the free radical attack. Some radicals like singlet oxygen radical ($^1O_2$) or superoxide radical ($^{-}O_2$) that produced in tissues by metabolism either reactions in cells were catalytically changed to oxygen molecular (O_2) as well as hydrogen peroxide (H_2O_2) via superoxide dismutase (SOD). H_2O_2 is toxic to the body tissues or cells when it is collected. Also, in another compound as the existence of Fe^{2+}, it is changed to harmful the hydroxyl radical ($OH^{\bullet}$) via Fenton reaction. To prevent this phenomenon occurring in the body, the excess catalase in the peroxisomes that starts to break down H_2O_2 to water as well as molecular oxygen, thus reducing free radical-induced damage (Figure. 2.14). However, catalase is out in the mitochondria, therefore Glutathione Peroxidase (GPx) is utilized to the reduction of H_2O_2 to water as well as lipid peroxides to their similar alcohols. This collective defensive action is the called the first line of antioxidant defense, also these antioxidants perform as are a first line defense to protection body (Ighodaro et al., 2018).

In the human body, Nrf2 (The nuclear factor erythroid 2–related factor 2) is worked as a master regulator for most of the antioxidant defenses that included in the brain.A stressor can perform to influence the activation of the Nrf2 signal transduction process by directly or indirectly. The tripartite synapse has the neuronal activity acts that regulate Nrf2 activity in the astrocytes.While the increase in neuronal activity signaled by neurotransmitters like glutamate, the astrocytic signaling cascade of Nrf2 is activated through stimulation of group metabotropic glutamate receptors as well as Ca^{2+}. Although of the stressor in issue, the translocation of Nrf2 inside the nucleus can be performed in the main two ways: chemical alteration of cysteine residues on Keap1 another way phosphorylation of Nrf2.In considering the fact when the endogenous antioxidant response system is capable of tightly controlling the amount of reactive species and minimizing associated cellular damage, the function of exogenous antioxidants appears in the needless surface.

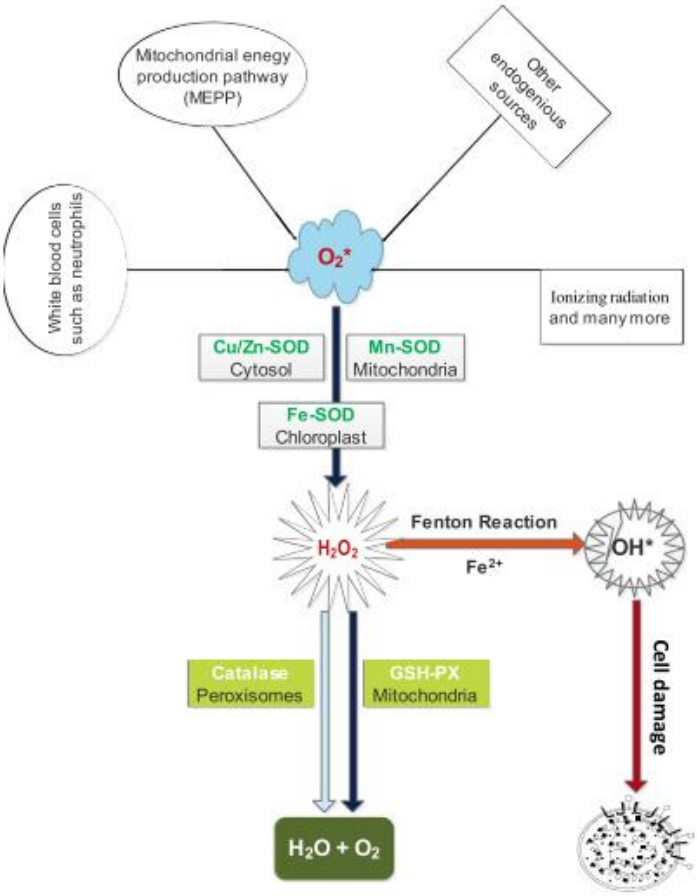

Figure 2.14. First line antioxidants defense against reactive oxygen species.

2.4.3. Enzymatic antioxidant defense system

2.4.3.1. Superoxide dismutase (SOD)

Superoxide dismutases (SOD) enzyme is located in both the dermis and the epidermis of the skin. SOD are enzymes that perform to catalytically change $O_2^{\bullet-}$ to oxygen (O_2) as well as hydrogen peroxide (H_2O_2) (Bertini et al., 1998). It acts removes the radical superoxide ($O_2^{\bullet-}$)as well as repair the body cells damaged through the free radical. SOD stimulate the conversion of superoxide anions to hydrogen peroxide. SOD is also known to compete among nitric oxide (NO) for superoxide anion, which inactivates NO to produce peroxynitrite. Since through scavenging superoxide anions, it elevates the activity of NO (Chakraborty et al., 2009).

$$2O_2^{\bullet} + 2H^{+} \xrightarrow{SOD} H_20_2 + O_2$$

Superoxide dismutase (SOD) is the first enzyme of detoxification as well as the usual effective antioxidant in the cell. It is an essential endogenous antioxidant enzyme that performs against reactive oxygen species (ROS) such as a part of the first-line defense system. It can catalyze the dismutation of two molecules of superoxide anion ($O_2^{\bullet -}$) to hydrogen peroxide molecular (H_2O_2) as well as molecular oxygen (O_2), thus giving the potentially harmful superoxide anion less hazardous. SOD is a metalloenzyme as well as, for its activity perform needs a metal cofactor. Based on the forms of metal ion needed as a cofactor by SOD, so have several forms of the enzyme (Fridovich et al., 1995). The metal ions which are ordinarily bound through SOD such as iron (Fe), zinc (Zn) copper (Cu) as well as manganese (Mn). In this context, SODs are categorized into three types like (i) Fe-SOD which is typically discovered in the chloroplasts of some plants as well as prokaryotes (ii) Mn-SOD which is present in prokaryotes as well as mitochondria of eukaryotes and the last type (iii) Cu/Zn-SOD is dominant in eukaryotes and more distributed, localized fundamentally in the cytosol but also these found in chloroplasts as well as peroxisomes (Karuppanapandian et al., 2011).

SOD enzyme deficiency is common. Consequently, the enzyme is necessary to cellular health, protecting body cells from extreme oxygen radicals, free radicals as well as other which harmful agents that promote aging either cell death. The levels of SODs decay with age, thus the formation of free radical to be increasing. It has been advised that proper daily SOD supplementation will to protect the immune system also is important to reduce the chances of diseases as well as eventually slow down the aging process. Krishnamurthy with encouraged the eating of fruits and vegetables such as cabbage, wheat grass, Brussels sprouts, broccoli and barley grass as natural sources of SOD (Ighodaro et al., 2018).

The use of the knockout models and systems where the SOD have been overexpressed by genetic manipulation have been essential in understanding the significance of the SOD antioxidant system below physiological as well as pathological conditions (Figure 2.15). SOD1 placed in the chromosome 21 (Tan et al., 1973), a finding that was critical in understanding the contribution of this enzyme down regulation of SOD1 in vitro as well as in vivo models has been correlated among

neuronal death (Troy et al., 1996). SOD2 placed in the chromosome 6 as well as the knockout of this gene is lethal (Lebovitz et al., 1996).The insufficiency of SOD2 happened in an increased generation of superoxide, which in change inhibits the respiratory chain by inactivating complex I and complex II (Lebovitz et al., 1996). SOD3 is the least characterized among the SOD family enzymes. It located in the chromosome 4 and part of chromosome 5, and has been shown to have high affinity for heparin. To date, the only acknowledged mutation has been demonstrated to be localized to the heparin-binding site (Folz et al., 1994).

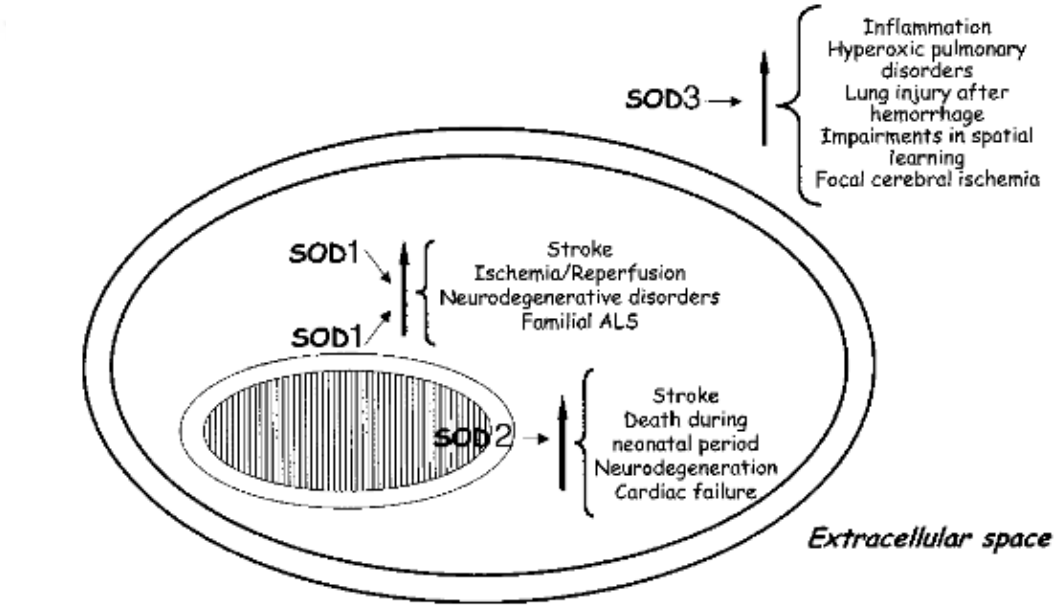

Figure2.15. Relative roles of SOD isoforms in disease (Muscoli et al., 2003).

SOD isoforms are well compartmentalized inside the cell (SOD1, cytoplasm; SOD2 in the mitochondria) as well as (SOD3) in the extracellular space. The role of the three isoforms in various pathological states has been unraveled by modulating the appearance of the three enzymes utilizing knockout and transgenic models.

Superoxide is generated from many sources, such as normal cellular respiration, activated polymorphonuclear leukocytes, endothelial cells as well as mitochondrial electron flux. Is that superoxide contributes to the pathogenesis of a wide array of diseases. Some important pro inflammatory works for superoxide include endothelial cell damage also increased micro vascular permeability. autocatalytic impairment of

neurotransmitters as well as hormones like as noradrenaline and adrenaline, each lipid peroxidation and oxidation, DNA damage (Dixet al., 1996), activation of the poly-ADP-ribose polymerase, so inactivation of nitric oxide (Gryglewskiet al., 1986) as well as formation of peroxynitrite (the reaction product of nitric oxide and superoxide (Beckmanet al., 1990), a potent cytotoxic and pro inflammatory molecule (Figure 2.16) Peroxynitrite nitrates endogenous SOD, the enzyme that provided superoxide under tight control. Once nitrated, MnSOD and/or CuZn SOD lose their enzymatic activity, a result promoting the accumulation of superoxide as well as superoxide driven damage.

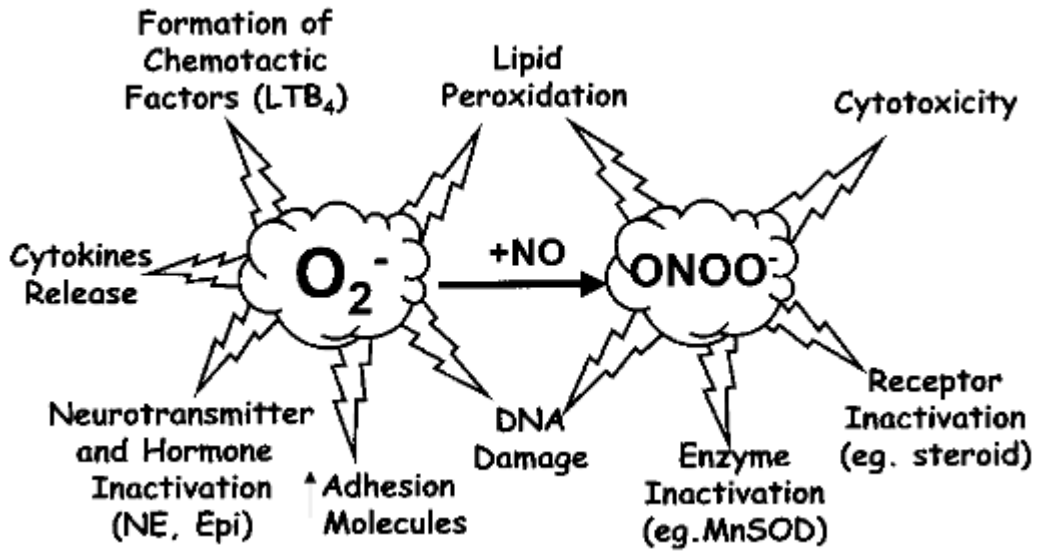

Figure 2.16. Impact of superoxide generation in inflammation (Muscoli et al., 2003).

Extreme the production of superoxide can lead to inflammation via several pathways, including the generation of destruction of useful nitric oxide (NO) and simultaneous generation of cytotoxic and pro inflammatory peroxy nitrite (ONOO).

2.4.3.2. Catalase (CAT)

Catalases are ubiquitous heme-containing enzymes that catalyze the dismutation of H_2O_2 into H_2O and O_2 (Figure 1.6).Catalase enzyme (CAT) is mainly found in the blood and most of the living cells and works as decomposes into water and oxygen.Catalase along with glucose peroxidase is also utilized commercially for the

maintenance of the fruit juices, cream composed of egg yolk, and salad by removing the oxygen (Chakraborty et al., 2009).

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

CAT is one of the common antioxidant enzymes present around in all living tissues that use oxygen. The enzyme uses each iron or manganese such as a cofactor as well as catalyzes the degradation or reduction of hydrogen peroxide (H2O2) to water and molecular oxygen, consequently completing the detoxification manner imitated through SOD. CAT is highly effective; it can breaks down millions of hydrogen peroxide molecules in one second. The enzyme is placed primarily in the peroxisomes but absent in mitochondria of mammalian cells. This indicates that the breakdown of hydrogen peroxide to water and oxygen is brought out by another enzyme known as glutathione peroxidase in mammalian cell mitochondria (Ighodaro et al., 2018).

CAT also reacts efficiently among hydrogen donors like as methanol, ethanol, formic acid, or phenols with peroxidase activity. The CAT activity gets in the two steps. A molecule of hydrogen peroxide oxidizes the heme to an oxyferryl species. A porphyrin cation radical is formed when one oxidation equivalent is removed of iron and one from the porphyrin ring. A second hydrogen peroxide molecule works as a reducing agent to reproduce the resting state enzyme, producing a molecule of oxygen and water. Hydrogen peroxide though at low concentration leads to control some physiological processes including signaling in cell proliferation, cell death, carbohydrate metabolism, mitochondrial function, and platelet activation and preservation of normal thiol redox-balance, however, at high concentrations it has been related to being very harmful to cells (Ercal et al., 2001). Consequently, the ability of CAT to effectively limit H_2O_2 concentration in cells underlines its importance in the physiological processes as well as being the first line antioxidant defense enzyme. The deficiency or mutation of the enzyme has been associated with many disease conditions and abnormalities (Góth et al., 2004).

2.4.3.3. Reductase glutathione (GSH)

Which catalyzes the reduction of oxidized glutathione (GSSG) to glutathione (GSH). Glutathione reductase is required for the glutathione redox cycle that maintains adequate levels of decreased cellular GSH. GSH works as an antioxidant, reacting with free radicals as well as organic peroxides, in amino acid transport. This enzyme one of the family members in the flavoprotein disulfide oxidoreductases. GR is that notice NADPH depended on oxidoreductase that changes GSSG to GSH through utilizing the pentose phosphate reaction pathways.

$$GSSG + NADPH + GR \rightarrow 2GSH + NADP+$$

GSH is widely abundant in all cell parts and is the major critical soluble antioxidant. GSH/GSSG ratio is the main determinant of oxidative stress. GSH exhibits its antioxidant effects in different ways. It detoxifies hydrogen peroxide and lipid peroxides by the action of GSH-Px. GSH donates its electron to H_2O_2 to reduce it into H_2O and O_2. GSSG is repeat reduced into GSH by GSH reductase that utilizes NAD(P)H like the electron donor. GSH-Pxs are also vital for the protection of cell membranes from lipid peroxidation. Reduced glutathione donates protons to membrane lipids also protects them from oxidant attacks. GSH is a cofactor for many detoxifying enzymes, such as GSH-Px and transferase. It has a role in changing vitamin C and E back to their active forms. GSH protects cells against apoptosis by interacting among proapoptotic and antiapoptotic signaling pathways. It also controls and activates various transcription factors, for example, AP-1 and NF-kB (Birben et al., 2007).

It is synthesized from three the amino acids include glycine, glutamate, and cysteine. Glutathione directly quenches ROS like as lipid peroxides, and also performs the main role in xenobiotic metabolism. Exposure of the organ to xenobiotic substances induces oxidative reactions within the upregulation of detoxification enzymes, i.e., cytochrome P-450 mixed-function oxidase. While an individual is revealed to high levels of xenobiotics, more glutathione is used for conjugation (a key step in the body's detoxification process) making it less ready to work as an antioxidant.Some investigation advises that glutathione and vitamin C act interactively to quench free radicals also that theirs have a sparing effect in each other (Percival, 1998; Jacob, 1995).

GSH is important, also when stress states in its function as a cofactor for stress defense enzymes, such as glutathione transferase (GSTs) and glutathione peroxidases (GPx). The gsh1 mutant has also been applied to model the effects of GSH depletion, which can be induced through many processes, like as conjugation to xenobiotics, excretion, and decreased synthesis as well as has been involved in degenerative diseases, cell aging, and apoptosis (Morano et al., 2012).

2.5. Kidney

The kidney is both structurally and functionally complex and acts as plays a central role in homeostasis. There are several possible varieties of renal malfunction which may induce a wide range of clinical situations. Manifestations of the renal disorder include fluid, electrolyte and pH imbalance, hemodynamic imbalance, the collection of drugs, toxins as well as waste metabolic products, loss of essential metabolites, and endocrine abnormalities like as anemia and bone disease. Important functions which the kidney acts, all of which are needed for metabolic homeostasis (Ahmad et al., 2013). When the kidney works normally in the body that does these functions, for example, 1-Removal of water, urea, creatinine, and other metabolic wastes and toxins from the blood. 2. Regulation of volume, composition, as well as pH of the body fluids. 3. Regulation of blood pressure. 4. Synthesis of erythropoietin, a hormone involved with red blood cell production. 5. Change of vitamin D to its active form, 1, 25 dihydroxy vitamin D, required for calcium absorption and bone health (Collister et al., 2009).

The kidney structurally consists mainly of three parts regions (Figure 2.17) the cortex, the medulla, and the pelvis. The cortex contains the glomeruli and the proximal and distal tubules, and the medulla includes the loop of Henle. Glomeruli in various areas have different-length loops of Henle to allow differential control over urine concentration. The loops of the juxtamedullary nephrons, closest to the medulla, extending nearly to the pelvis, the area in which urine flows from the collecting ducts. Throughout the kidneys, there are interstitial cells, apparently concerned among endocrine functions (Ahmad et al., 2013).

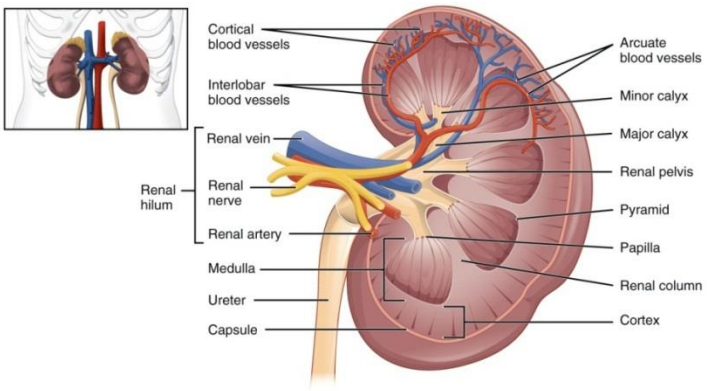

Figure 2.17. Kidneys.

The afferent arterioles aid about 1.3 million nephrons in each kidney. Nephrons are known as “functional units” of the kidney; they clean the blood and balance the components of the circulation. The glomerular filtration rate (GFR) indicates the amount of filtrate produced in all the renal corpuscles of both kidneys each minute. In adults, the average of the kidney is 125 mL/min for men and 105 mL/min for women. Homeostasis of body fluids dictates that an almost constant GFR is needed. If the GFR is too high, the needed substances may pass through the renal tubules and be lost in the urine. If the GFR is too low, all of the filtrates may be reabsorbed also too few waste products passed (Chopra et al., 2013). The kidneys, nervous system and hormones regulate GFR by stabilizing blood flow into and out of the glomerulus, modifying the glomerular capillary surface available for filtration.

2.6. Acute Kidney Injury (AKI)

Acute kidney injury (AKI, also known as acute renal failure (ARF) is the sign arising from a rapid fall in GFR (over hours to days). It is characterized by retention of both nitrogenous (such as urea and creatinine) and non-nitrogenous waste products of metabolism, as well as a confused electrolyte, acid-base, and fluid homeostasis (Makris et al., 2016). Also, It is defined as a sudden reduction in renal function or glomerular filtration rate (GFR), which may be causing to azotemia and/or deficient urine production induced through reduced renal blood flow, and kidney damage, inflammation, or obstruction. This can result appear from special diseases of the kidney

(such as interstitial nephritis, glomerulonephritis) either extra renal pathology (such as dehydration, heart failure, sepsis, obstruction) (Bellomo et al., 2004). In severe circumstances, this represents as metabolic acidosis, electrolyte abnormalities, fluid retention, hypertension, as well as in some cases, clinical signs of uremia like as confusion (Figure2.18) Acute kidney injury affects approximately 40% of critically illpatients and one third of them die within the first 90 days of admission (Mara li, 2015).

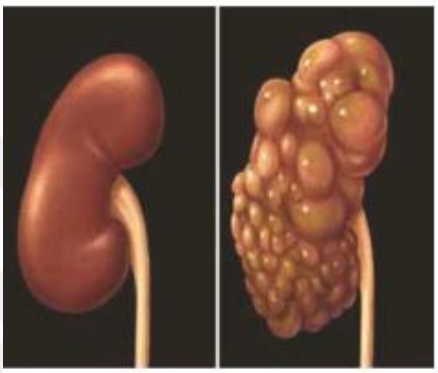

Figure 2.18. Acute renal failure

2.6.1. Causes and risk factors for AKI

Large differences are noted in the incidence and the effects of AKI among developing as well as developed countries. In urban districts of developing countries, main causes of AKI are hospital-acquired (such as renal ischemia, sepsis, and nephrotoxic drugs) while in rural districts it is more regularly an outcome of community-acquired disease (such as dehydration, diarrhea, infectious diseases, animal venoms, etc.) (Makris et al., 2016).

The main cause of acute renal failure is hypervolemia secondary to dehydration as a result of gastroenteritis (32%). Obstetric blood loss largely due to inadequate obstetric care is also an important cause (15%), acute glomerulonephritis and sepsis (12%). Other main causes are nephrolithiasis (10%). The very drug involved is a drug-induced acute renal failure because of over the counter sales of drugs (Anees et al., 2014). Acute kidney failure is an immediate and whole loss of kidney function. Many

things can cause acute kidney failures, for example, accidents, medicines, surgery, low blood pressure from shock, blockages of the bladder or kidney or dangerous infections. The kidneys may start working again by medical treatment. Patients with acute kidney failure may need dialysis therapy until the kidneys begin to work again. Also between 5% of -25% of all hospitalized patients develop ARF. A greater prevalence of ARF is found in critically ill patients. Despite the change in the medical care of individuals with ARF, mortality generally exceeds 50% (Ahmad et al., 2013).

Generally, the causes of AKI have been divided into three types: pre-renal, intrinsic, and post-renal. While there is significant overlap between these, particularly the first two types, it remains a useful clinical guide (Figure 2.19) (Fry et al., 2006). The first type of AKI induced is Pre-renal AKI is described through reduced blood delivery to the kidney.Causes of intravascular volume reduction such as hemorrhage, dehydration, or gastrointestinal fluid losses, reduced cardiac output, hypotension, NSAIDs, ACE, ARBs, renovascular obstruction, systemic vasoconstriction. Pre-renal AKI happens in approximately 10% to 25% of patients diagnosed with AKI. Intra-renal is the second type of AKI is caused by diseases that can influence the integrity of the tubules, glomerulus, interstitium, or blood vessels. Damage is inside the kidney; changes in kidney structure can be seen on microscopy. Acute tubular necrosis (ATN) represents a pathophysiologic state that occurs from toxic or ischemic insult to the kidney. The most frequent cause of intrinsic renal failure is ATN and it estimates about 50% of all cases of AKI. Finally, Post-renal AKI is due to the interference of urinary outflow. Causes include benign prostatic hypertrophy, pelvic tumors, and precipitation of renal calculi. Post-renal AKI considers for less than 10% of cases of AKI (Thadhani et al., 1996).

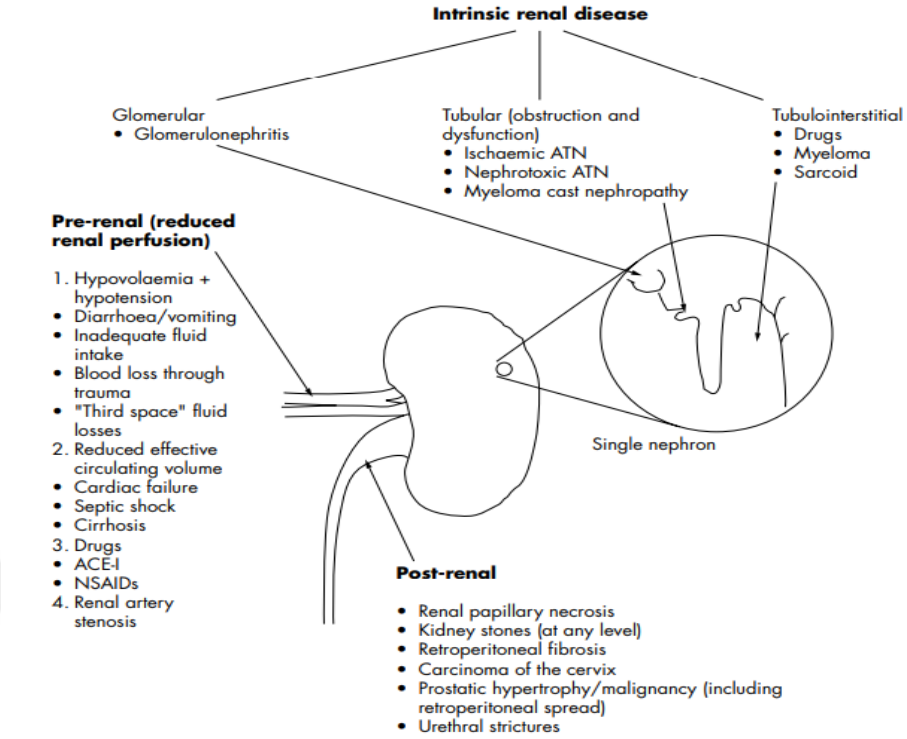

Figure 2.19. Etiology of acute renal failure.

2.6.2 Classification of AKI

In clinical, AKI is a fast decrease in kidney function, inducing failure to keep fluid, electrolyte and acid-base homeostasis. The Risk, Injury, Failure, Loss, and End stage Kidney (RIFLE) classification suggested through the Acute Dialysis Quality Initiative (ADQI) group divides AKI into five stages, as follows: (1) Risk; (2) Injury; (3) Failure; (4) Loss of function; and (5) End stage kidney disease (ESKD) (Van et al., 2006; Bellomo et al., 2007) (Table 2.2).

The RIFLE (an acronym meaning Risk of renal dysfunction; Injury to the kidney; Failure of kidney function, loss of kidney function as well as End-stage kidney disease) criteria also hold two clinical consequences: 'loss' and 'end-stage renal disease' (ESRD). These are separated to notice the important modifications that happen in ESRD that are not detected in persistent AKI. Persistent AKI (loss) is defined as the necessary for renal replacement therapy (RRT) for more than 4 weeks, thus ESRD is defined by the necessity for dialysis for longer than 3 months (Bellomo et al., 2004).

Table 2.2: The Risk, Injury, Failure, Loss, and End-stage Kidney (RIFLE) classification of acute kidney injury (Bellomo et al., 2007)

Stage	Glomerular filtration rate (GFR) criteria	Urine output(UO) criteria
Risk	Increased serum creatinine 1.5 times or GFR decreased > 25%	< 5 mL/kg/h for 6 h
Injury	Increased serum creatinine 2 times or GFR decrease > 50%	< 5 mL/kg/h for 12 h
Failure	Increased serum creatinine 3 times or GFR decrease > 75% or serum creatinine > 4 mg/dl	< 3 mL/kg/h for 24 h or anuria for 24h
Loss	Complete loss of kidney function > 4 weeks	
ESKD	End stage kidney disease (> 3 months)	

2.6.3. Diagnosis of AKI

The diagnosis of acute kidney injury is usually based on each a rise of serum creatinine or the detection of oliguria (Mehta et al., 2003). Serum creatinine is a marker of early to detected renal dysfunction because serum concentration is very affected by many non-renal factors (for example bodyweight, race, age, gender, total body volume, drugs, muscle metabolism, and protein intake). The use of serum creatinine is worse in AKI because the patients are not in a steady-state; hence, serum creatinine lags far behind renal injury. Thus, substantial rises in serum creatinine are often not observed until 48–72 h after the first insult to the kidney (Star, 1998). General Diagnostic Procedures such as Urinary catheterization (addition of a catheter into a patient's bladder; an increase in urine output may happen with post renal obstruction), Renal ultrasound (uses sound waves to evaluate size, position, as well as abnormalities of the kidney;dilation of the urinary tract can be seen with post renal ARF), Renal angiography (control of intravenous contrast dye to evaluate the vasculature of the kidney), Retrograde pyelography (injection of contrast dye into the ureters to evaluate the kidney and collection system), and Kidney biopsy (collection of a tissue sample of the kidney

for the purpose of microscopic evaluation; may aid in the diagnosis of glomerular and interstitial diseases) (DiPiro et al., 2014).

2.6.4. Treatment of AKI

Renal Replacement Therapy (RRT), patients with AKI as a segment of a systemic disease usually among failure of various organ systems need intensive care unit admission, given their probable needs for intensive monitoring, nursing and support of other organs systems. Patients with only one single organ renal failure can usually be arranged on a renal room, as well as nephrologists will often promote the role of the intensives in the intensive care unit. The severity of the AKI will determine the need for nephrology referral but this should be the same day if the intrinsic renal disease has a suspicion. It is common practice to start RRT (renal replacement therapy) at an ‘‘earlier’’ stage in patients with multi-organ failure because of their potential for extra degeneration and the benefits that RRT may bring. Before starting RRT, it must also be thought whether aggressive treatment is in the patient’s best benefits, particularly in those with significant pre-morbid functional deficiency as well as extra-renal comorbidity. The patient and their family must wherever tolerable be section of this process (Fry et al., 2006). RRT can be intermittent either continuous. Continuous dialysis can be blood-based or use the peritoneal way. However, peritoneal dialysis is now rarely used in the treatment of AKI in developed countries. It may still have a role where anticoagulation is not feasible, vascular access not achievable, or when very gentle fluid removal is needed, and where the resources for blood-based procedures do not exist. Consequences are more ordinary with peritoneal dialysis than among continuous hemofiltration in the treatment of infection correlated AKI. Intermittent treatment is delivered as intermittent hemodialysis (IHD), and continuous renal replacement therapy (CRRT) in several ways, arranged according to the method of obtaining the circulation and how solute removal is performed (Fry et al., 2006).

Hemodialysis (HD) this is the absolute means by which potassium can be removed from the body, and is shown in refractory severe hyperkalemia. Hemodialysis is more efficient than haemo filtration or peritoneal dialysis at potassium removal and

has an urgent effect once began. Maximum extraction happens in the first hour of dialysis. Serum potassium may reduce through 1.2–1.5 mEq/l/h if potassium free dialysate is utilized, which can accelerate hypokalemia as well as arrhythmias. Generally, a dialysate potassium concentration of 1.0 or 1.5 mmol/l is used. If the higher concentrations may be used in those at risk of arrhythmias (particularly if taking digoxin), and when a lower concentration there is a renewed hyperkalemia drive like rhabdomyolysis, tumor lysis, and hemolysis. Some rebound of potassium levels happens later dialysis, especially if primary levels were high or if treatments to enhance cellular uptake were applied pre-dialysis (Throssell et al., 2002).

2.7. Chronic Kidney Disease (CKD)

Chronic kidney diseases (defined as abnormalities of kidney structure or function, persistent for greater than 3 months) are the most popular form of kidney diseases, with an evaluated prevalence around the world of approximately 10.4% among men and 11.8% of women (GBD 2013). Between 5.3 and 10.5 million people need dialysis each transplantation, though there are many dies because they do not take these treatments due to loss of resources or economic barriers (Liyanage et al., 2015; Wang et al., 2015). Acute kidney injury (AKI) is undergone via 13.3 million patients each year; it may propose or lead to chronic kidney disease in the future. “Using all these sources of data, and surviving estimates of acute and chronic kidney diseases, there are nearly 850 million kidney patients in the world (GBD 2013). Inappropriate, the cardiovascular death exaction from CKD is huge: In 2013, there were 1.2 million cardiovascular deaths associated with CKD (Wang et al., 2015). “The death rate in CKD is strangely is high! AIDS, for example, estimates for “only” 1.9 deaths per 100,000 but with all the effective campaigning about HIV, it is acknowledged as a priority health problem. There is little active campaigning for kidney diseases, even though the number of people who die from kidney disease is eleven times higher.” (GBD 2013).

Chronic kidney disease is characterized by the failure of the kidneys to excrete waste products and surplus fluid from the body. Varying degrees of renal dysfunction from the earliest kidney damage to end-stage renal disease has been divided into five stages on the basis of markers of kidney damage as well as a level of kidney function

(glomerular filtration rate) (National Kidney Foundation 2002). The major causes of chronic kidney disease are diabetes mellitus, glomerulonephritis, renal vascular diseases and hypertension (ERA-EDTA Registry 2005). The risk of chronic kidney disease increases with ageing(National Kidney Foundation. 2002) but also lifestyle circumstances may perform a role in the increase of chronic kidney disease. It is understood that obesity influences to chronic kidney disease through diabetes mellitus and hypertension, but emerging evidence shows that obesity may also contribute directly to kidney damage through a cascade of additional hemodynamic, metabolic, and inflammatory mechanisms as well as by mechanical compression (Iseki K et al,. 2004). Besides,smoking is one a risk factor for CKD (Vupputuri et al., 2003). Furthermore, the prevalence of CKD is 1.5 times increased in men compared among women (ERA-EDTA Registry 2005).

Hypertension as well as diabetes mellitus are the two main causes of CKD in worldwide. However, chronic glomerulonephritis and interstitial nephritis are the main causes of CKD in developing countries of the world. This is a representation of the high prevalence of bacterial, parasitic, and viral infections (communicable diseases) that influence the kidneys in these countries. The prevalence of CKD is also increasing at a more accelerated rate in developing countries. The prevalence of some diseases included as obesity, hypertension, and diabetes mellitus has increased in the developing countries of the world, for example, Nigeria due to many factors (Chukwuonye et al., 2018).

While the information regarding the incidence and prevalence of ESRD is available, there is no enough data for the prevalence of CKD in Turkey. While there is a continuing study conducted by Turkish Nephrology Association (i.e., the CREDIT study), there are no available data about CKD prevalence in Turkey so far (Sahin et al., 2009). The reason for progressive rises in the prevalence of CKD is considered to be due to the epidemic of obesity during the developed world (Choi, 2011). Primary CKD the risk factors are; hypertension, diabetes and dyslipidemia (Nugent et al., 2011) all of which are closely associated with obesity.

CKD can also be the final outcome of untreated acute kidney injury (AKI) caused by infections, medicines, toxic substances heavy metals such as lead, cadmium,

mercury, and chromium. The choice of renal replacement therapy (RRT) modality depends on the physical and sociodemographic symptoms of the patient. Renal transplantation (RT) is the best RRT choice because it confirms the better condition of life as well as longer survival; nevertheless, due to the deficiency of transplants, peritoneal dialysis (PD) and mainly hemodialysis (HD) are utilized in most cases (Tzanakaki et al., 2014).

2.7.1. Classification of CKD

Classification for CKD the staging in National Kidney Foundation and Kidney Disease Outcomes Quality Initiative (NKF/KDOQI) has classified CKD into five stages (Levey et al., 2003). These are based on measured or evaluated Glomerular Filtration Rate (GFR). The acquired definition of CKD is kidney damage for ≥3 months, explained via structural or functional abnormalities of the kidney (pathological abnormalities or abnormalities of imaging or the composition of blood or urine), with either without decreased GFR. CKD is also described as GFR <60 ml/min/1.73m for ≥3 months, with each without kidney damage. There are currently five stages of CKD (Table 2.3).

Table 2.3. Definition and classification of CKD stages

Stage	Description	GFR ($mL/min/1.73m^2$) ↓
(1)	Kidney damage with normal or ↑ GFR	≥ 90
(2)	Kidney damage with mild ↓ GFR	60-89
(3)	Moderate ↓ GFR	30-59
(4)	Severe ↓ GFR	15-29
(5)	Kidney failure	< 15 (or dialysis)

2.7.2. Diagnosis of CKD

According to the KDIGO CKD guidelines (and the English National Institute for Health and Care Excellence (NICE) CKD guidelines), a patient is classified with CKD if abnormalities of kidney structure or function were present for a minimum of 3 months. The abnormalities are shown in Table 2.4.

Table 2.4 Diagnostic criteria for CKD (Fraser et al., 2016)

One of the following needs to be present for at least 3 months:
a) Decreased eGFR (<60 mL/min/1.73m^2) b) One or more marker of kidney damage: - Albuminuria (urinary albumin – to – creatinine ratio (ACR) ≥ 30 mg/g (3 mg/ mmol) - Structural abnormalities (from imaging) - Urine sediment abnormalities (hematuria, red or white blood cell casts, oval fat bodies, and renal tubular epithelial cells) - Electrolyte and other abnormalities due to tubular disorder - Histological abnormalities - Pervious history of kidney transplantation

2.7.3. Treatment of CKD

Patients who proceed to ESRD need a renal replacement therapy (RRT). The modalities that are applied for RRT are dialysis, such as HD and peritoneal dialysis (PD), and kidney transplantation. Dialysis is the artificial process of obtaining cleared of waste also unwanted water from the blood. This method is naturally done by our kidneys. Some people, though, may have failed or damaged kidneys which that kidney cannot carry out the function accurately; they may need dialysis. In other side, dialysis is the artificial replacement for lost kidney functions (renal replacement therapy). when hemodialysis first became a practical treatment for kidney failure, many studies have been taken out to make hemodialysis treatments more effective and decrease side effects. In hemodialysis, an artificial kidney (hemodialyzer) is utilized to remove waste and extra chemicals and fluid from the blood. To get the blood into the artificial kidney, the doctor requires to make passage into the blood vessels. This is made by minor surgery to the leg or arm (Daugirdas et al., 2012). Peritoneal Dialysis (PD) is type of dialysis, the blood is cleaned within the body. Minor surgery will have to be completed to place a plastic tube termed a catheter into the abdomen to make an way. During the treatment, the abdominal area (called the peritoneal cavity) is slowly filled with dialysate through the catheter. The blood stays in the arteries as well as veins that line the peritoneal cavity. Extra fluid and waste products are moved out of the blood and into the dialysate (Plantinga et al., 2010).

3. MATERIALS AND METHODS

3.1 Sample Collecting

During this investigation, blood samples has been taken from 31 healthy individuals, and 31 patients of acute kidney failure, 30 from chronic kidney failure patients in both male and female, from each of healthy, and acute, chronic patients, we took 4ml of blood from an antecubital venous vein and added 2ml to the biochemistry tube and the other 2ml to the biochemical serum tube.

3.1.1. Devices and materials

Vortex
Tubes
Ice bath
Centrifuge
Supernatant (filtrate)
Hot water bath
Spectrophotometer
Deep Freezing Tubes
Serum Storage Tubes
Pipettes
Oven
Stopwatch
Incubator
Spectrophotometer cuvette
Automated Pipette Tip
PH-meter
Beaker
Flask

Erlenmeyer flask

Volumetric flask

Spatula

Funnel

Filter paper

3.1.2. Reagents and chemicals

Xanthine

Ethylene demine tetra acetic acid solution

Nitro blue tetrazolium

Sodium carbonate

Hydrogen peroxide

Bovine serum albumin

Cupper chloride

Disodium phosphate

Xanthine oxidase

Butylhydroxy toluene solution

Thiobarbituric acid solution

Trichloroacetic acid solution

Phosphate Buffer

Hydrogen peroxide solution

Sodium hydroxide solution

Sodium citrate

Sodium chloride

Water

Elman’s reagent (5, 5’-dithiobis-(2-nitrobenzoic acid).

Methanol

Ammonium sulfate

3.2. Method

In this study the total population of the study that diagnosed and monitored was ranging from 18-65 years of age in male and female which included 31patenits acute kidney failure and 30 patients chronic kidney diseases who had been diagnosed and 31 healthy individuals without any additional. In the study biochemical parameters were determined by serum samples. Before the collection of blood samples for this research, local ethical committee approval was obtained from Van Yüzüncü Yıl University Medical Faculty of Educational Research and Training Hospital Department of Nephrology And Laboratory Research Center. During the study, 4 ml blood was taken from venous blood sample was collected as the study subject and centrifuged with 5000r/min for 10 minutes and then serums were separated from plasma. The separated serums were used to determine the superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT), and malondialdehyde (MDA) levels.

3.3. Analysis Method

3.3.1. Determination of superoxide dismutase (SOD) activity

SOD activity was determined by using the proposed method of (Popov et al., 2004).SOD accelerates the dismutation of hydrogen peroxide and molecular oxygen of superoxide radicals ($O_2^{\bullet-}$) formed during the oxidative energy production. This method is based on the reading of topic density resulted from using of xanthine and xanthine oxidase in which superoxide radicals that generated from the blue colored formazan dye of the nitro blue tetrazolium (NBT) in the optical density wavelength of 560 nm. The SOD that exists in the sample serum inhibits the formazan reaction by excluding superoxide radicals from the environment. Under the experimental conditions, 1unit of SOD is the %50 inhibition of NBT reduction rate.

%Inhibition = [(Blank OD – Sample OD) / Blank OD] × 100

3.3.2. Determination of reduced glutathione (GSH) activity

In the hemolysis of EDTA blood which prepared with distilled water, all the proteins that don't carry sulfhydryl (GSH) group are precipitated with precipitation solution. The reduced glutathione (GSH) was measured as the final product of the reaction was achieved, that was the formation of the yellow color, of obtained clear liquid of sulfhydryl groups and DTNB (5,5'-(dithiobis 2-nitrobenzoic acid). Measurement of the reduced glutathione level in the EDTA blood was done in 412 nm wavelength in the spectrophotometer (Beutler 1963).

Activity (mg/dl) = [(OD2-OD1)/13600 ×E1 1.25] ×1000

OD1: First absorbance before addition of DTNB at 412 nm.

OD2: Second absorbance after addition of DTNB at 412nm.

E1: 1 in the calculations

13600 is the molar extinction coefficient of the yellow color that formed during the interaction of GSH and DTNB.

3.3.3. Determination of catalase (CAT) activity

Catalase activity was determined according to the Aebi method in this study, which is used as a hydrogen peroxide substrate. The activity is carried out in the following manner, First, add1.4ml of 30mM H_2O_2 to the two tube bilnd and add 0.1 ml of phosphate buffer. Add 1.4ml of 30 mM H_2O_2 to the sample tube. Then 0.1 ml of enzyme was added to the vortex. Absorbance's were read at 240 nm twice at 30 second intervals, and activity was thus determined (Aebi 1984).

Activity account:

Activity= $(2.3/\Delta X) \times [(\log A1 / \log A2)]$

ΔX: 30 seconds

2.3: 1mmol optical density of H_2O_2 in 1cm light path.

3.3.4. Determination of malondialdehyde (MDA) level

The reaction of fatty acids with free radicals result in malondialdehyde, which is the final product of lipid peroxidation, is measured with thibarbituric acid that gives a colored form (Gutteridge 1995). 200ml from the blood is taken and put into 1 tube.800ml phosphate buffer, 25ml BHT solution, and 500ml of %30 TCA were added. The tubes were stirred with vortex and kept on ice for 2 hours. Then centrifuged at 2000 rpm for 15 minutes, 1ml from supernatant was taken and transferred to other tubes. Then 75ml of EDTA and 250ml of TBA were added. Tubes were mixed in the vortex and kept in a hot water bath for 15 minutes. Then, they were brought to room temperature and their absorbance was read at UV/V spectrophotometer at 532nm.

Calculation of malondialdehyde level:

C=F× 6.41× A

C: Concentration

F: Dilution factor

A: Absorbance

3.4. Statistical Data Analysis

Descriptive statistics for the study parameters were Mean, Standard deviation, Minimum and Maximum values are expressed. One way was used for normal distribution conditions and Kruskal Wallis test statistic was used for cases where normal distribution condition was not provided. The Statistical significance level was taken as 5% in the calculations and SPSS statistical package program was used for the calculations.

4. RESULTS

During the examination of SOD (superoxide dismutase) enzyme activity (Table 4.1) it could notice that the relationship between level of control group (7.35161±0.381952 U/L) and level of acute patient (1.31274±0.754314U/L), as well as the level of chronic patient (2.01267±0.661518 U/L) the statistically significant differenc was found ($p < 0.05$).

In addition to this, CAT (catalase) enzyme activity was examined (Table 4.1), it could notice that the relationship between level of control group (0.4154516 ± 0.12360066 U/L) and level of acute patients (0.0755855 ± 0.00058186 U/L), besides the level of chronic patient (0.0766340 ± 0.00585831 U/L), was also found to be a statistically significant difference ($p < 0.05$).

When GSH (reduced glutathione) enzyme activity (Table 4.1), it could notice that the relationship between level of control group (0.000286774 ± 0.0000658983 µmol/L) and level of acute patient (0.000021737 ± 0.0000164011 µmol/L) as well as the level of chronic patient (0.000006076 ± 0.0000053343 µmol/L) the statistically significant also was found differently ($p < 0.05$).

In the another hand, MDA (malondialdehyde) level was concluded, as shown in (Table 4.1), the correlation between level of control group(0,70839 ± 0,065069 µmol/L) and level of acute patient (2.15542 ± 0.753084 µmol/L), the level of chronic patient (1.92060 ± 0.497356 µmol/L) were statistically significant differences ($p < 0.05$).

Table 4.1. Comparison control and patients with acute and chronic kidney failure

Parameters	Controls Mean±SD(n=31)	Patients Acute Mean±SD(n=31)	Patients Chronic Mean±SD(n=30)
SOD(U/L)	7.351±0.381	1.312±0.754	2.012±0.661
CAT(U/L)	0.415±0.124	0.075±0.005	0.077±0.006
GSH(µmol/L)	0.00028±0.000065	0.000021±0.000016	0.0000061±0.0000053
MDA(µmol/L)	0.708±0.065	2.155±0.753	1.921±0.497

$p < 0.05$ The P-Value for all parameters.

Descriptive statistics and comparison results for SOD, CAT, GSH and MDA levels.

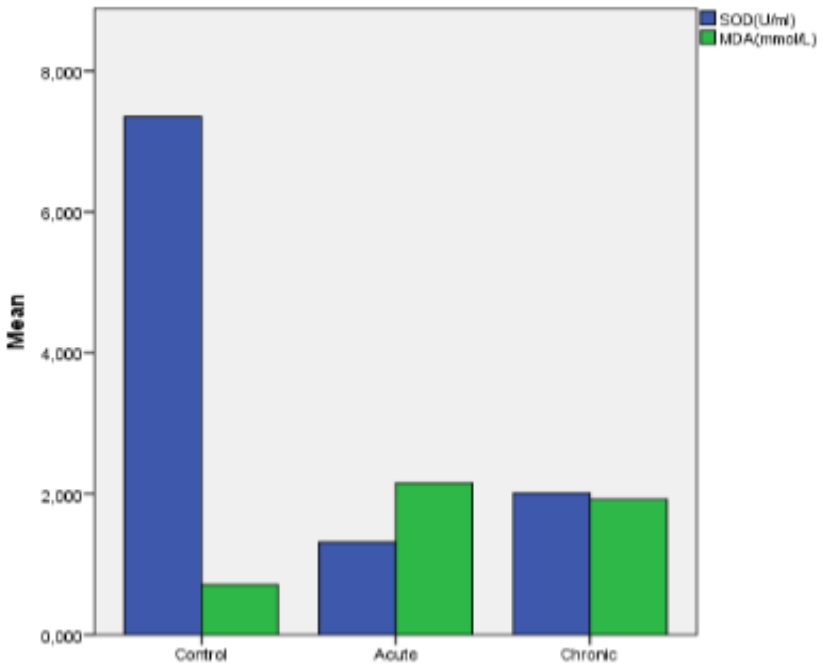

Figure 4.1. The level of SOD and MDA activity compared between control and acute with chronic renal failure.

According to the results obtained during the study SOD (superoxide dismutase) enzyme activity as in (Table 4.1). So the level of control group has (7.35161±0.381952 U/L) and level of acute patient was found (1.31274±0.754314U/L), the level of chronic patient (2.01267±0.661518 U/L) were compared to control that shown ($p < 0.05$) statistically significant difference was found. Moreover, the results of level MDA (malondialdehyde) enzyme activity as in (Table 4.1), a statistically significant differently ($p < 0.05$) among level of control cases (0,70839 ± 0,065069 µmol/L) and the patient have acute level (2.15542 ± 0.753084 µmol/L), as well as level of chronic patient (1.92060±0.497356 µmol/L), was taken.

In addition, CAT (catalase) enzyme activity level was reviewed in (Table4.1).that could notice the level of control patients (0.4154516 ± 0.12360066 U/L) to compare the level of acute patients (0.0755855 ± 0.00058186 U/L), and level of chronic patients (0.0766340 ± 0.00585831 U/L) was statistically significant ($p < 0.05$).

Finally, GSH (reduced glutathione) level of enzyme activity was analyzed as shown in (Table 4.1), which that level of control group (0.000286774 ± 0.0000658983 µmol/L) with the levels of acute patient has (0.000021737 ± 0.0000164011 µmol/L) and the levels of chronic patients (0.000006076 ± 0.0000053343 µmol/L) was also found to have a statistically significant difference ($p < 0.05$) to compared control groups.

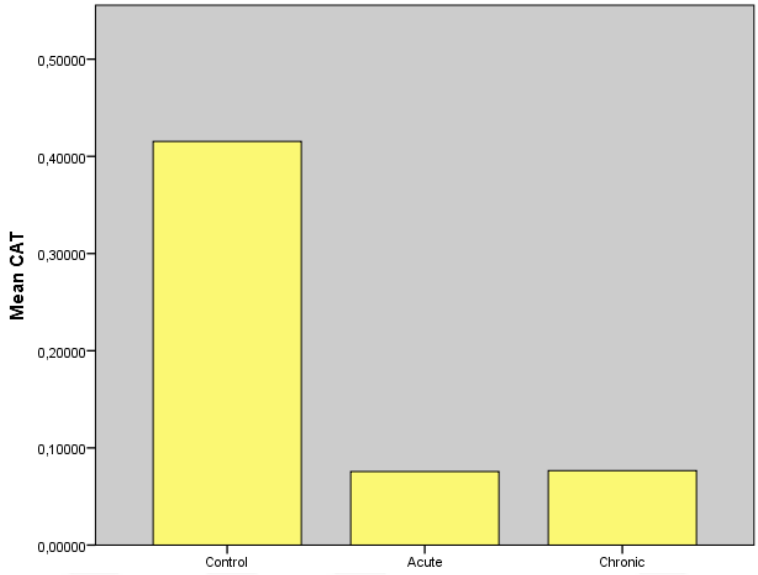

Figure 4.2. Level of CAT enzyme activity in cooperation control patients and acute with chronic renal failure has patients.

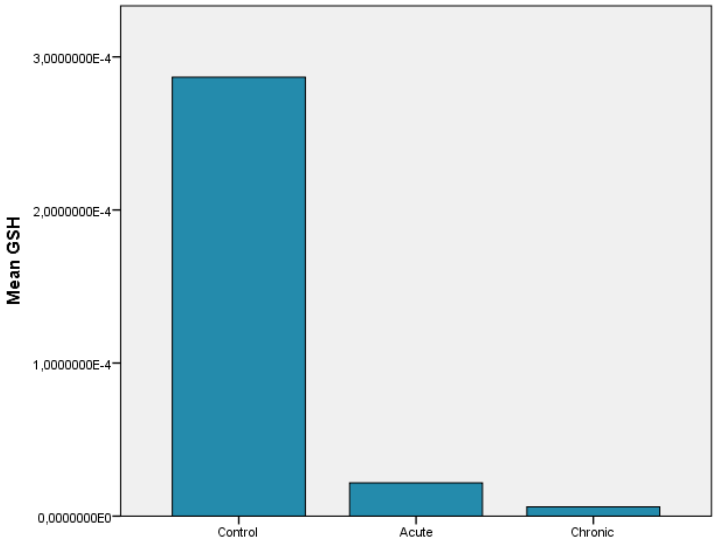

Figure 4.3. The level of GSH enzyme activity compared with control patients and acute with chronic renal failure patients.

5. DISCUSSION AND CONCLUSION

The present study aimed to determine plasma malondialdehyde (MDA) level for lipid peroxidation product which is shown as a marker of the oxidative stress in acute and chronic renal failure and erythrocyte superoxide dismutase (SOD), glutathione reduced (GSH) and catalase (CAT) activities as antioxidants.

Enzymology is one of the Advances fields of biochemistry, which have guided to the usage of different types of enzymes, and low molecular weight endogenous and exogenous antioxidants, that they have the ability to perform inhibition effect on oxidants. It is obvious that the results from different research will finally indicate the interaction of free radicals, as well as their role in the system of growth, genomics, proteomics, finally cancer. On the other hand, there are other environmental variables such as oxygen tension, redox status, and transition metal concentrations are also counted as an original variable (Rahal et al., 2014).

The Oxidative stress measurement could be done in both cases healthy and the one who has clinical conditions. In the case of renal failure, (ROS) will attack cell membranes, thus causes the change in the antioxidant enzymatic mechanism, and lipid peroxidation products such as MDA (Montazerifar et al., 2012). Directly and indirectly, oxidative stress will influence the kidney, for example, vascular reactivity, glomerular filtration, renal hemodynamics, and tubular reabsorption and secretion in all pieces of the nephron. Oxidative stress signaling changes all of these mechanisms during injury or disease and encourages mechanisms of cellular apoptosis, tissue injury, progression necrosis, fibrosis prevention, altered gene expression and unusual function of the kidney (Ratliff et al., 2016).

In the case of overproduction of ROS by a metabolic reaction which is using oxygen thus results in arising of oxidative stress, due to change in balanced oxidant/antioxidant state to oxidants. The ROS can hurt the DNA, proteins, and lipids, due to they readily react. There are some cellular metabolic actions, as well as, other environmental factors like air pollutants, and smoke may lead to high production of ROS. It is also has been reported that ROS also affects the expression of multiple genes

due to redox-sensitive transcription factors, as well as performing multiple gene expression by regulating redox-sensitive transcription factors and remodeling chromatin by changing histone acetylation/deacetylation. Regulation of the redox state is needed for the activation, proliferation, viability, and function of the cells (Birben et al., 2012).

It is suggested that ROS and RNS play an important role in the initiation and progression of kidney diseases and injuries, such as diabetic nephropathy, and hypertension. While they will produce in several tissues, also cell pressures in both renal and vascular cells. In the kidney and vascular system, there are many sources of ROS and RNS, that may present in ordinary and disease conditions, while mitochondrial and NADPH oxidase cumulating is the major factor of oxidative stress in the kidney (Brodsky et al., 2002). In the ordinary situation of redox signaling ROS and RNS are required for proliferation and development, kidney vasoreactivity, promoting cell survival (Ratliff et al., 2016).

Impairment of antioxidant defense leads to oxidative stress that can lead to complications such as those observed in chronic kidney disease (CKD) and acute kidney injury (AKI). Insights into kidney damage caused by oxidative stress can be acquired by observing the effects of sepsis pro-inflammatory processes on this organ. This disease causes serious tubule interstitial damage to fast and progressive renal disease. The existence of pro-inflammatory ROS, cytokines and apoptosis due to interstitial tubular dysfunction and loss of renal epithelial cells by damage renal tubular injury, That, in turn, is closely tied to the microvascular structure and renal blood flow that affect functional modification.

Oxidative stress due to kidney damage related AKI occurring in the oxidation of numerous macromolecules (e.g., protein, DNA and lipid). Malondialdehyde (MDA) and 4-hydroxynonenics are in large products in Lipid peroxidation production (Cristol et al., 1996). Also OS in the occurrence of CKD, a section of non-hemodynamic factors play a role, either by glomerular damage and ischemia of the kidney are directly or related indirectly with hypertension, inflammation, and endothelial dysfunction, characteristics that stimulate excessive oxidative stress in CKD are including inflammation, malnutrition, excessive activity of oxidase, increased phagocytic activity, and decreased mechanisms of oxidative defense.

One of the most sensitive organs that influenced by ROS regarded to be a kidney, because it is rich in blood supply, vascular tissues, and the organ that has high metabolic activity. OS could be dramatically increased in the CKD cases of hemodialysis before and after compared with the control group. It is reported that in the CKD cases, OS magnitudes are extremely high. For instance, patients who have CKD cases there OS level were three times higher. Furthermore, many researchers noted extremely high levels of MDA in pre- and post-dialysis cases compared to controls (Nagamma et al., 2014).

It is noted that high amount of ROS in AKI, will lead to damage and injury of renal oxidative. Ischemia and hypoxia will do obstruction of microcycles, cellular enzymes and a mitochondrial role, which leads to intracellular ROS generation, such as $O_2^{\bullet-}$ and H_2O_2, that finally drive to mitochondrial injuries, cell death activity pathways, and ATP depletion. ROS will help in reperfusion after ischemia (Dennis et al., 2017).

Meanwhile the investigation, one lipid peroxidation parameter has used. MDA is used for biological samples in inspection lipid peroxidation because it has low carbon aldehyde which could be produced by the free radical invasion of the polyunsaturated fatty acid biological membrane. MDA is the end product of lipid peroxidation; many researchers have found extremely high levels of MDA in CKD patients with HD (hemodialysis) cases. Although this is a perfect notice of oxidative stress, though, MDA has a small molecular weight, hydrosoluble molecule that means it can be removed by the kidneys. Recently it has been proposed that high MDA concentrations observed in these cases are partly due to the low glomerular filtration rate (Puchades et al., 2009). As some investigations, show that an interaction between both growth of atherosclerosis and OS in CKD and hemodialysis cases with CVD, the level of serum malondialdehyde increased, compare to cases without CVD (Modaresi et al., 2015).

MDA appeared significantly increased in acute and chronic renal disease patients when compared to the control group. It is reported that there are many factors which influencing oxidative stress in renal failure, such as increased production of oxidative metabolism agents, and reduced defense of antioxidants (Hacis¸evki et al., 2008). However, during the examination, which suggested a significant increase in statistically ($p<0.05$) between acute and chronic renal patients according the healthy

control group. In past years, a few reports have published of CKD and kidney replacement thereby with associated OS state and multiple diseases. Although, it has found that with the severity of atherosclerotic changes in the carotid arteries, the serum MDA concentration increases slightly of both patient groups. On the other hand, as shown to the status of atherosclerotic alterations in the carotid arteries, it also reveals important changes in the serum MDA concentration between the distinct subgroups of CKD and PD cases (Rašić et al., 2015).

MDA levels increase with the progress of kidney dysfunction, all variables could obtain changed due to increased production of free radicals, so peroxidation of lipid and additional increase in serum MDA after dialysis. These variables are occurring in macrophage activation and output of ROS as well as a loss of antioxidants during the session on hemodialysis. As well as increase in serum MDA was in patients revealed through hemodialysis compared to the similar cases before initiating the treatment among hemodialysis, which that traced of membrane dialysis and hydrophilic diffusion from the dialysate (Rusu et al., 2016).

The level of SOD activity was examined, the result showed that its activity significantly was decreased ($p<0.05$) among acute and chronic renal patients when compared to the control group. When serum MDA and SOD levels were compared in patients with acute and chronic it was also noted that there was a significant increase in serum MDA of renal failure and decreased SOD in renal failure patients. SOD is the first defense line from front against superoxide anions and turns it into hydrogen peroxide. When ROS level increased such as hydrogen peroxide, which known as suppressant of SOD activity. Several studies indicate that the concentration of MDA, a lipid peroxidation by-product, is significantly increased in patients with CKD before initiation of dialysis and renal replacement therapy compared to the control group (Antoniadi et al., 2008). In this research, there was a rise in MDA concentrations in patients with CKD. With the severity of kidney dysfunction, this indicates that these patients have raised lipid peroxidation.

OS causes endothelial dysfunction and decreasing in plasma SOD. Therefore, many studies have shown that SOD and other antioxidant enzymes are reduced in pre-clinical AKI cases, and genetic impairment of SOD increases sensitivity to AKI in

ischemia and hypoxia. SOD and CAT activity are reduced by oxidative damage. That defect promotes kidney disease after acute renal ischemia. AKI is protective against oxidation and/or renal damage via endogenous or nutritional antioxidants. For instance, vitamin E and selenium (which can increase the activity of antioxidant enzymes dependent on GSH) reduce nephrotoxicity (Dennis et al., 2017). The OS could be lead to higher levels with CKD patients, which that vital characteristics of renal patients such as diabetes, hypertension, and advanced age, all of them increased OS compared to healthy. Also, another source of OS with CKD cases is inflammation. It is known that, there is a wide connection between kidney disease and indicators as well as mediators such as interleukin (IL)-6, C-reactive protein, fibrinogen, and tumor necrosis factor-α. Those were shown as an increased level of MDA concentrations and were detected serum SOD level decreased (Krata et al., 2018).

There are many investigations that went to opposite outcomes in the chronic renal patients. It is suggested that in chronic renal failure case, in order to a high generation of free radicals, cells may protected by increasing SOD activity, which is protective pathway for the cell. One of the factors which lead to elevate of super oxide dismutase activity is over proliferation of O_2^- macrophages reducing antioxidant plasma activity in patients with CKD may be contributing to higher oxidative damage and generation of renal problems (Hacis evki et al., 2008). In addition, primary protection against generation of free radicals is SOD, the enzyme that needs to convert superoxide radical to hydrogen peroxide and oxygen. Otherwise catalase, with glutathione peroxidase, will be deactivated. While detoxification performed, decreasing of SOD activity may take place, due to decreasing of ROS (Jira et al., 2007). Some investigations reported that low production of SOD may occur due to enzyme suppression which related to H2O2. In this phenomenon H2O2 production will enhanced as a part of dismution reaction (Kalpakcioglu et al., 2008).

While antioxidants have performed the main role in having physiological and pathophysiological homeostasis of the cell and tissue correlated among the prevention of increase of ROS and RNS, pathological damage to the tissue consequently appears as oxidative stress passes their neutralizing ability. For instance, nonregulation of antioxidants, particularly catalase, and peroxidases leads to hydrogen peroxide growth.

These could interact with the metals transition to produce very active and damaging species with hydroxyl radical reactions.

Further, several studies revealed that superoxide overproduction, mainly by NADPH oxidase and mitochondria, and reduced superoxide metabolism by SOD and other antioxidants, its reason or potentiate the formation of hypertension, antioxidant systems in these patients keep failing due to low levels of SOD, glutathione peroxidase and catalase Renal damage generates at a result (Redon et al., 2003).

Additional investigations have determined as the level of MDA is significantly more elevated and antioxidant parameters levels have reduced than normal control range, such as CAT activity. In gastric, kidney, breast, lung cancer and colorectal adenomas, oxidative stress alike to increase in MDA and antioxidant parameter levels decreased. There are situations in which plasma MDA will increase such as elevates alcohol, saturated fat and consumption of meat present. However it is known that high fiber nutrition is reversely proportionate to MDA (Didžiapetrienė et al., 2014).The increase in serum MDA indicated that OS could happen in free radical convinced in the cell membrane of lipid component, therefore MDA is work as a good indicator for detecting oxidative stress in degenerative disease such as CKD. The level of SOD enhanced is trying to fight oxidative stress. The early marker of acute renal failure is SOD activity (Ratliff et al., 2016).

It is thought that chronic kidney disease to be a prooxidant and a low -level inflammatory condition. Oxidative stress level in both intracellular and extracellular related with the degree of intracellular and extracellular oxidative stress is correlated with the riskiness of the renal failure. It is noted that peroxidation results in tissue damage by lipid peroxidation, which is plays an important role in development of various diseases, such as atherosclerosis, in which correlated with elevation of cardiovascular morbidity and mortality in CKD. Increased levels of ROS and RNS during CKD outcome in arylesterase/paraoxonase deficiency, higher plasma concentrations of thiobarbituric acid-reactive species (TBARS) and activation of polymorphic nuclear cells. There are two other factors inside the kidney, that they stimulate ROS, which they are tumor growth factor b (TGF-b) and tumor necrosis factor a (TNF-a). The two mentioned factors aid in the passage of albumin across the

glomerular barrier, on the other hand, the second one stimulates of O2 generation which results in glomerular cell apoptosis. It is obvious that ROS is participated in glomerular damage pathophysiology. Due to glomerulus is the most damageable compartment of the kidney (Tamay-Cach et al., 2016).

Chronic pathological procedures could causes CKD's disease results in decreasing of glomerular filtration (GFR), which damages renal parenchyma. There are many Causes of CKD, primary glomerulonephritis (26.4 %) takes highest percentage, diabetic nephropathy (19.2 %), tubulointerstitial nephritis (16.5 %), hypertensive nephropathy (8.9 %), and polycystic kidney disease (8.9 %) (Mastalerz-Mıgas et al., 2006). In CKD after progressing into ESRD, renal replacement therapy with continuous hemodialysis does not increase the conditions of OS in CKD patients. On the other hand, leading to ROS excretion on the surface of dialysis membranes, each hemodialysis session induces OS (Peuchant et al., 1994), partly due to phagocyte activation (Himmelfarb et al., 2001). Hemodialysis also tends to increase the body's antioxidant capacity (Jackson et al., 1995). It is interesting to note that vitamin E-coated dialysis membranes have recently been shown to reduce oxidative genetic damage levels in patients with hemodialysis (Rodriguez-Ribera et al., 2017).

Meanwhile this research, the resulting level of catalase (CAT) activity was statistically and significantly decreased when it is compared to the control group in acute and chronic renal failure patients ($P<0.05$). It is known that inside of the kidney there are aerobic cells in which they contain high amount of catalase. CAT has an important role in reducing ROS as well as it leads to inhibition of lipid peroxidation. The failure of mitochondrial function comes from catalase defection, which leads to accumulation of ROS inside of mitochondria and results of mitochondrial disfunction (Ratliff et al., 2016). The two important groups of antioxidant enzymes which they are glutathione peroxidase and catalase contribute in oxidation of H_2O_2 and results in water molecule and oxygen. Glutathione peroxidase comes in first line importance, while catalase comes secondly in prevention against H_2O_2 formation process. Both enzymes works together in significant way, in order to metabolizing of H_2O_2 that may formed during normal metabolism process, and in kidney disease circumstances (Kobayashi et al., 2005).

Catalase plays a major role in decreasing ROS and avoiding lipid peroxidation. Catalase deficiency results in accumulation of mitochondrial ROS, an impact that causes to structural impairment of mitochondria. Low levels of hydrogen peroxide lead to the maintenance of basal tone in vascular bodies throughout the body, including the kidneys, its removal impairing ordinary hemodynamic function (Ratliff et al., 2016).

In this study, the level of GSH activity was statistically and significantly decreased when it is compared to the control groups in acute and chronic renal failure ($P<0.05$). GSH plays a key role in cellular resistance against oxidative damage. In this study, glutathione levels were significantly decreased in the patients of acute and chronic when compared with a group of healthy individuals. The results indicate that chronic renal and acute renal patients have lower GSH levels.

It should be mentioned that the individuals of control group were not matched by age and sex with the group of patients which is the limitation of this study. Some studies have shown, moreover, that age and sex have no impact on antioxidant molecules levels. For instance, in 25 hemodialysis patients (18 males and 7 females), Samadian and colleagues evaluated oxidative stress indices consisting of glutathione, vitamin E, and TAC. The concentrations of antioxidant markers did not show important differences between males and females in their research (Ahmadpoor et al., 2009).

The main intracellular scavenger is reduced glutathione (GSH), it has been oxidized by inactivated free radicals to glutathione disulfide (GSSG). The glutathione reductase will then regenerate GSSG into GSH. The GSSG-GSH ratio is therefore regarded as an excellent indicator of intracellular oxidant stress. At the end, an increase in renal transport work could lead to a reduction in GSH cellular store, depending on the capacity of the body to synthesize and maintain GSH in its decreased form (Paller et al., 1988). GSH for renal is an important free radical scavenger during post ischemic reperfusion changes in renal work have resulted in reciprocal modifications in GSH in the renal. A decrease in renal work in renal artery stenosis resulted in a rise in renal GSH and protection from free radical-mediated ischemic injury.

It appears that renal GSH content can be altered by modifying the stress of renal oxidant. When renal work (and therefore the consumption of renal oxygen) was reduced by either reducing the filtered sodium load as in the renal artery or by

pharmacologically inhibiting renal sodium reabsorption in the entire nephron, facing a constantly filtered sodium load, renal GSH concentrations rose. We assume that renal GSH rose because the reduction in renal O_2 consumption that would follow the reduction in renal sodium reabsorption resulted in less oxygen-free radicals being formed. Less GSH was absorbed to protect the cell against these free radicals and therefore GSH levels rose (WEINBERG et al., 1987).

In the advance cases of chronic renal failure glutathione reductase (GSH) activity will reduce. Their role is to catalyze GSH formation so that GR is also impacted when the pentose path is impacted. This impact has not been contrasted with the patients undergoing hemodialysis and peritoneal dialysis. The same can be said for glutathione transferase, which in chronic, non-dialyzed renal patients are discovered to be raised (Martin-Mateo et al., 1999).

In the last years ago, the most important recognized marker that uses such as an indicator for several diseases, when that has affected cases the level of MDA is high (Kesavulu et al., 2001). One of the diseases reported that shown is malignant brain tumors caused by DNA damage and mutation that is produced endogenously via lipid peroxidation showed that OS increased due to the level of MDA be increased when compared to control. Therefore one of the markers that could be used for diagnosis malign tumors is MDA (Grotto et al., 2009).

One of the studies examined the level of IL-18(Interleukin-18) and MDA before and after surgical treatment in patients with renal cell carcinoma. It was seen after surgery, if MDA was greater, as well as the level of IL-18 was greater. Additional sex analysis the level of IL-18 was higher in men and the level of MDA was lower after surgery. The research showed the level of IL-18 in women higher, but the level of MDA is unique and the level of CAT activity was greater. The level of SOD activity is higher in men and women after surgery (Didziapetriene et al., 2012).

The kidney damage one of the factored by reducing lipid peroxidation and the antioxidant enzymes could increase the level of the protection system's scavenging ability. Antioxidants have been work such as increasing the level of activity of SOD to protect the kidney and decreasing ROS generation as well as reducing MDA. For example one of the antioxidants that were found in medicinal plants such as Troxerutin

has richly amount in cereal grain, tea, coffee, and fruit, and vegetable varieties work to increase antioxidant activity such as the level of SOD and CAT to decrease the level of MDA (Rafieian-kopaei et al., 2013).

The cell protected membrane by vitamin E against lipid peroxidation when making a low radical sensitivity tocopherol, with O2 and hydroxyl radical such as vitamin C scavenges. Vit E composed in the main compound as well as alpha and gamma-tocopherol there has increased level of antioxidant activity. Some antioxidants such Vit E, Vit C and β-carotene uses as decreased the level of serum 8-oxo2'-deoxyguanosine when uses for 1 month like the indicator of DNA damage in kidney failure (Modaresi et al., 2015). Vit E and Vit C could be decreased the level of MDA in the research has shown (Korish et al., 2008). The cases have CKD usage omega-3 for a reduced level of MDA and increased antioxidant activity such as (SOD, GPx, and CAT) in hemodialysis cases, obvious by lowering thiobarbituric acid reacting substances (TBARS) (Tayyebi-Khosroshahi et al., 2010).

This investigation showed the damage of kidney disease (acute and chronic) that changes by oxidative stress and antioxidant enzymes activity SOD, CAT and GSH have been decreased. The results show have related oxidative stress and kidney failure, previous literature findings as well as oxidative stress are influential in the kidney by a decreased level of antioxidants in kidney failure and the level of MDA was increased which the end product of lipid peroxidation. Antioxidants can help kidney protect from oxidative stress by supplementation to reduce the level of MDA and increased antioxidant enzymes level. Finally, there suggest needed a process find to have the level of antioxidants is more influence to reduce oxidative stress in kidney disease.

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EXTENDED TURKISH SUMMARY
(GENİŞLETİLMİŞ TÜRKÇE ÖZET)

AKUT VE KRONİK BÖBREK YETMEZLİĞİ HASTALARINDA OKSİDATİF STRES DÜZEYİ VE BAZİ ANTİOKSİDAN AKTİVİTELERİN BELİRLENMESİ

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ÖZ

Bu çalışmada, Van Yüzüncü Yıl Üniversitesi Eğitim ve Araştırma Hastanesi Eğitim Fakültesi Nefroloji Anabilim Dalında akut ve kronik böbrek yetmezliği teşhisi konulan hastalarından alınan serum örneklerinden antioksidan aktiviteler ve oksidatif stres düzeyleri belirlendi. Bu çalışma sırasında üç tane antioksidan aktivite incelendi, bunlar süperoksit dismutaz (SOD), redukte glutatyon (GSH) ve katalaz (CAT) idi. Malondialdehit (MDA) seviyesi, serbest oksijen radikal bir kaynağı olan lipid peroksidasyonunun son ürünüdür. Akut böbrek yetmezliği olan 31 hasta, kronik böbrek yetmezliği tanısı alan 30 hasta ve böbrek sorunları tanısı almayan 31 sağlıklı bireyden oluşan toplam 92 kişiden kan örnekleri alındı. Hasta gruplarının SOD aktivitesi akut ve kronik böbrek yetmezliğinde sağlıklı kontrol grubundan anlamlı derecede düşük bulundu ($p < 0.05$). Ayrıca, hasta grubunda serum GSH ve CAT düzeylerinin kontrol grubuna göre anlamlı fark bulundu. Hastalarda, serum MDA düzeyinin kontrol grubuna göre anlamlı bir farklılık tesbit edildi, yine hasta ve kontrol gruplarında SOD ortalama farkının anlamlı olduğu bulundu ($p<0.05$). Bu çalışma, oksidatif stresin akut ve kronik böbrek yetmezliği hastalarında hücresel hasar ve doku üzerinde güçlü etkiye sahip olduğunu göstermektedir.

1.GİRİŞ

Serbest radikallerin en yaygın tanımı, "bir veya daha fazla eşleştirilmemiş elektron içeren atomik veya moleküler orbitallerdeki moleküller veya moleküler fragmanlar" dır (Halliwell et al., 2007). Reaktif oksijen ve azot türleri ROS / RNS, hücre sinyali ve homeostazda önemli rollere sahiptir. Serbest radikal, hücrelerimiz mikrobiyal enfeksiyonlar gibi gıdalardan enerji aldığında oluşur. Bununla birlikte, çevresel stres (örneğin, UV radyasyonu, ısıya maruz kalma, sigara içimi ve iyonlaştırıcı radyasyon), seviyeleri zaman içinde önemli ölçüde artabilir. Yüksek konsantrasyonda ROS, lipitler, proteinler, karbonhidratlar ve nükleyik asitlerle kolayca reaksiyona girer. This can cause major damage to cell structures and accumulate in a circumstance known as oxidative stress (Nagane et al., 2013). Böbreğin işlevini etkileyebilecek bazı durumlar enfeksiyonlar, böbrek taşları, akut böbrek hasarı (AKI) ve kronik böbrek hastalığıdır (CKD). Böbrekler hasar gördüğünde veya hastalıklı olduğunda, bu hayatı fonksiyonları aniden veya yavaşça yerine getirme yeteneklerini kaybedebilirler, atık ve sıvı birikmesine ve kan basıncının ve mineral home ostazının anormal hormonal kontrolüne nedenile olur (Stevens et al., 2009). AKI, böbreklerdeki ani ve bazen ölümcül işlev kaybı ile nitrojen metabolizması (üre) ve kreatinin son ürünlerinin birikmesine ve idrar üretiminin azalmasına veya her ikisine birden sahiptir (Bellomo et al., 2012).

Bu çalışmada, Van Yüzüncü Yıl Üniversitesi Eğitim ve Araştırma Hastanesi Eğitim Fakültesi Nefroloji Anabilim Dalında akut ve kronik böbrek yetmezliği teşhisi konulan hastalarından alınan serum örneklerinden antioksidan aktiviteler ve oksidatif stres düzeyleri belirlendi.

2. MATERYAL VE YÖNTEM

2.1. Materyal

Bu araştırmada sağlıklı 31 erkek ve kadın ve 31 akut böbrek yetmezliği ve 31 sağlıklı bireyden oluşan kan alındı. Sağlıklı ve hasta bireylerin her birinden, antekubital venöz venden 4 ml kan alındı ve biyokimya tüpüne 2 ml, serum tüpüne ise 2 ml ilave edildi.

2.1.1. Cihazlar ve Malzemeler

Vortex
Derin Dondurucu Tüpler
Serum Tüpleri
Spektrofotometrea
Ayarlanabilir Otomotiv Pipetleri
Thermo ile belirtilen Su Banyosu
Cam pipet
Soğutmalı santrifüj
Derin dondurucu
Fırın
Kronometre
Hassas terazi
Spektrofotometre küveti
Otomatik Pipet Ucu
pH ölçer
deney şişesi
kuvvartz
Test tüpü
Cam şişe
Spatula
Huni
Filtre kağıdı

2.1.2. Reaktifler ve Kimyasallar

Potasyum hidroksit
Hidrojen peroksit
Potasyum mono fosfat
Disodyum fosfat

Sodyum hidroksit

Sodyum sitrat

Elman’ın reaktifi (5,5'-ditiobis- (2-nitrobenzoik asit)

Ksantin

Etilen tetra asidik asit

Sodyum bikarbonat

Sığır serum albumin

Ksantin oksidaz

Amonyum sülfat

Bakır klorür

Etilen diaminetetra asetik asit disodyum

Butil hidroksil toluen

Tiyobarbitürik asit

Trikoasetik asit

Sodyum klorit

Su

Metanol

Nitroblue tetrazolyum

2.2.Yöntem

Bu çalışmada, böbrek yetmezliği 30 hasta kronik böbrek ve 31 hasta akut böbrek teşhisi konulmuş ve 31 sağlıklı bireyden oluşan ve yaşları 18-65 arası bireylerden seçildi. Biyokimyasal parametreler serum örnekleri ile belirlendi. Bu çalışma için kan örneklerinin alınmasından önce, Van Yüzüncü Yıl Üniversitesi Eğitim ve Öğretim Hastanesi Tıp Fakültesi Nefroloji ve Laboratuvar Araştırma Merkezi kan örnekleri toplandı. Usulüne uygun olarak venözden 4 ml kan alındı ve 2000 rpm/dk ile 5 dakika santrifüj edildi ve ardından serumlar plazmadan ayrıldı. Ayrılan serumlar, süperoksit dismutaz (SOD), redükte glutatyon (GSH), katalaz ve malondialdehit (MDA) seviyelerini belirlemek için kullanıldı.

Süperoksit dismutaz (SOD) aktivitesi tayini

A-) (Manuel Yöntem)

Reaktif Çözeltisinin Hazırlanışı:

1. 0.3 mM Ksantin: 4.56 mg ksantin (Sigma X7375) önce birkaç damla 1N NaOH de çözüldü ve 100 ml bidistile suda çözüldü.
2. 0.6 mM EDTA: 4.46 mg EDTA 20 ml bidistile suda çözüldü.
3. 150 mg/L NBT: 12.3 mg NBT (Sigma N6876) 100 ml bidistile suda çözüldü.
4. 400 mM Na_2CO_3: 2.544 g Na_2CO_3 60 ml bidistile suda çözüldü.
5. Sığır serum albümin (1g/L): 12 mg BSA (Sigma A2153) 12 ml bidistile suda çözüldü.

Reaktif çözeltinin hazırlanışı: 40 ml ksantin çözeltisi, 20 ml EDTA çözeltisi, 20 ml NBT çözeltisi, 12 ml Na_2CO_3 çözeltisi, 6 ml BSA'yı karıştırıldı.(Koyu renkli bir şişede saklayınız)

- Ksantin oksidaz (167 u/L)(Sigma X1875) enziminden 16 µl alınıp, 1ml 2M $(NH_4)_2SO_4$ da çözüldü.
- 2M $(NH_4)2SO_4$: 2.643 g $(NH_4)_2SO4$ 10 ml'ye saf su ile tamamlandı (+4 ˚C'de muhafaza edildi).
- 0,8 mM $CuCl_2.2H_2O$ 13.6 mg $CuCl2._2H_2O$ hazırlandı, 100 ml'ye saf su ile tamamlandı.

Aktivite Hesabı:

% inhibisyon: [(Kör OD – Numune OD) / Kör OD] x 100

1 Ünite SOD: NBT redüksiyonunu %50 inhibe eden enzim aktivitesidir.

Aktivite= (% inhibisyon) / (50 x 0.1)

Aktivite; U/ml cinsinden hesaplandı.

Katalaz (CAT) aktivitesi tayini

Aktivitesi tayini Hidrojen peroksidin substrat olarak kullanılan bu çalışmada Aeibi yöntemine göre katalaz aktivitesi belirlendi. Aktivite şu şekilde yapıldı önce iki tüp alındı kör tüpüne 1.4 ml 30 mM'lık H_2O_2 ilave edilir ve üzerine 0.1 ml fosfat tamponu eklenir. Numune tübüne ise 1.4 ml 30 mM'lık H_2O_2 ilave edilir. Üzerine 0.1

ml enzim eklenerek vortexle karıştırıldı. 30 saniye aralıklarla iki defa 240 nm'de absorbanslar okundu ve böylece aktivite tayin edildi (Aeibi, 1984).

Kullanılan çözeltiler:

1. 30 mM H_2O_2'nin hazırlanışı: 10 ml bidistile suyun içine, % 30'lik H_2O_2'den 34 µl alınarak konuldu (% 35'lik H_2O_2'den 25,8 µl alınarak konuldu).
2. 50 mM Fosfat Tamponunun hazırlanışı: 6.81 ″ g ″ KH_2PO_4 ve 7.1″ g ″ Na_2HPO_4 bidistile suda çözülerek, tamponun Ph'ı 1N NaOH ile 7.4'e ayarlandı ve hacim 1 litreye tamamlandı.

Aktivite Hesabı:

E.Ü.= (2,3 / Δx) x [(log A1 / log A2)] Aktivite; U/L cinsinden hesaplandı.

Δx= 30 saniye

2,3= 1 µmol H_2O_2'nin 1 cm'lik ışık yolunda verdiği optik dansisite

Malondialdehit (MDA) düzeyi tayini

Kullanılan çözeltiler:

1-) 0.1 M EDTA çözeltisi (Etilen diamin tetra asetik asit disodyum): 37.224 gr EDTA-Na2H2O 1 litre bidistile suda eritildi.

2-) % 88'lik BHT çözeltisi (Bütil hidroksi toluen): 0.220 ″ g ″ BHT, 25 ml saf alkolde çözüldü.

3-) 0.05 N NaOH çözeltisi (Sodyum hidroksit): 2 ″ g ″ gr NaOH, 1 lt bidistile suda eritildi.

4-) % 1'lik TBA çözeltisi (Tiobarbitürik asit) : 1 ″ g ″ TBA 100 ml'ye 0.05 N NaOH ile tamamlandı.

5-) % 30'luk TCA çözeltisi (trikloroasetik asit) : 30 ″ g ″ TCA, 100 ml distile suda eritildi.

6-) Fosfat Tamponu: 8.1 ″ g ″ NaCl, 2.302 ″ g ″ Na2HPO4, 0.194 gr NaH2PO4 bidistile suda eritilerek 1 lt'ye tamalandı. pH'sı 1N NaOH ile 7.4'e ayarlandı.

Deneyin yapılışı:

Bir tüpe serumdan 200 µl alındı. Üzerine 800 µl fosfat tamponu ve 25 µl BHT çözeltisi ve 500 µl % 30' luk TCA eklendi. Tüpler vortekste karıştırıldı, kapakları kapatıldıktan sonra 2 saat buz banyosunda tutuldu. Tüpler oda sıcaklığına getirildi. Daha sonra, tüplerin kapakları çıkartıldıktan sonra, 15 dk 2000 rpm'de santrifüj edildi. Santrifüjden elde edilen süpernatantın (süzüntünün) 1 ml'si alınarak başka tüplere aktarıldı. 1 ml'si alınan süzüntülerin üzerine 75 µl EDTA, 25 µl TBA eklendi. Tüpler vortekste karıştırıldı ve 15 dk (70˚C) sıcak su banyosunda tutuldu. Sonra oda ısısına getirilerek 532 nm'de UV/Vis spektrofotometrede absorbansları okundu.

Malondialdehit düzeyi hesaplaması:
C= konsantrasyon
F=Seyreltme faktörü
A=Absorbans
C= F x 6.41 x A
Düzey hesabı; µmol/L olarak hesaplandı.

Redükte glutatyon (GSH) tayini

Kullanılan çözeltiler:
1. Fosfat tamponu: 0.3 M disodyum fosfat bidistile su ile hazırlanır.
2. Ellman's ayıracı:; %1 sodyum sitrat, 100 ml'ye bidistile su içinde eritilir. İçerisine 40 mg DTNB (5',5'-(2-ditiobis nitrobenzoik asit) eklendi.

GSH tayin yöntemi:
1-) 200 µl serum üzerine 800 µl fosfat tamponu eklendi. 412 nm'de ilk absorbans (OD1) kaydedildi. Aynı tübe 100 µl Ellman's ayıracı ilave edildi, 2.absorbans (OD2) kaydedildi.

Hesaplama:
Glutatyon derişimi mmol/g protein biriminden hesaplandı.

C / 1000 = (OD2-OD1) / 13600 x E1 x 5/2 x ½

13600: GSH ile DTNB etkileşimi sırasında oluşan sarı rengin molar ekstinksiyon katsayısı.

E1: Eni 6 nm'den büyük olan bant kullanılırsa hem ışık yolu hem de bant genişliği farklarını düzelten bir türev ekstrinksiyon katsayısı kullanılır. Bizim kullandığımız bantın eni 2 nm'dir. Hesaplamalarda E1=1 olarak alındı.

1000: mmol'e dönüşüm katsayısı.

C: mmol / glutatyon (mg/dl)

OD1: DTNB ilave edilmeden önce 412 nm dalga boyunda ölçülecek optik dansite.

OD2: DTNB ilave edildikten sonra 412 nm dalga boyunda ölçülecek optik dansite.

İstatistiksel Analiz

Çalışılan parametreler için tanımlayıcı istatistikler standart sapmada ifade edildi. Eşleştirilmiş grup karşılaştırmalarında normal sapmanın sağlandığı yerde T testi, olmadığı yerlerde Mann-Whitney U istatistikleri kullanılmıştır. Anlamlılık düzeyi% 5 olarak kabul edildi ve tüm hesaplamalar SPSS istatistik paket yazılımı ile yapıldı.

3. SONUÇLAR

Böbrek yetmezliği akut kronik MDA, CAT, SOD ve GSH düzeylerinin sağlıklı kontrollerle karşılaştırıldığında istatistiksel olarak anlamlı bir farklılık gösterdi. Malondialdehit (MDA) düzeyi incelendiğinde (Tablo 3.1), kontrol grubu ile hasta grubu düzeyleri arasında (0.708±0.065 µmol/L) akut hastalar (2.155±0.753 µmol/L) ve kronik hastalar (1.921±0.497 µmol/L) istatistiksel olarak anlamlı bir ilişki olduğu saptandı ($p<0.05$).

Buna karşılık, kontrol grubu ile hasta grubu arasında redükte glutatyon (GSH) arasında (0.00028±0.000065 µmol/L) ve akut hastalar (0.000021±0.000016 µmol/L),kronic hastlar (0.0000061±0.0000053 µmol/L) (Tablo 3.1) istatistiksel olarak anlamlı bulundu ($p<0.05$).

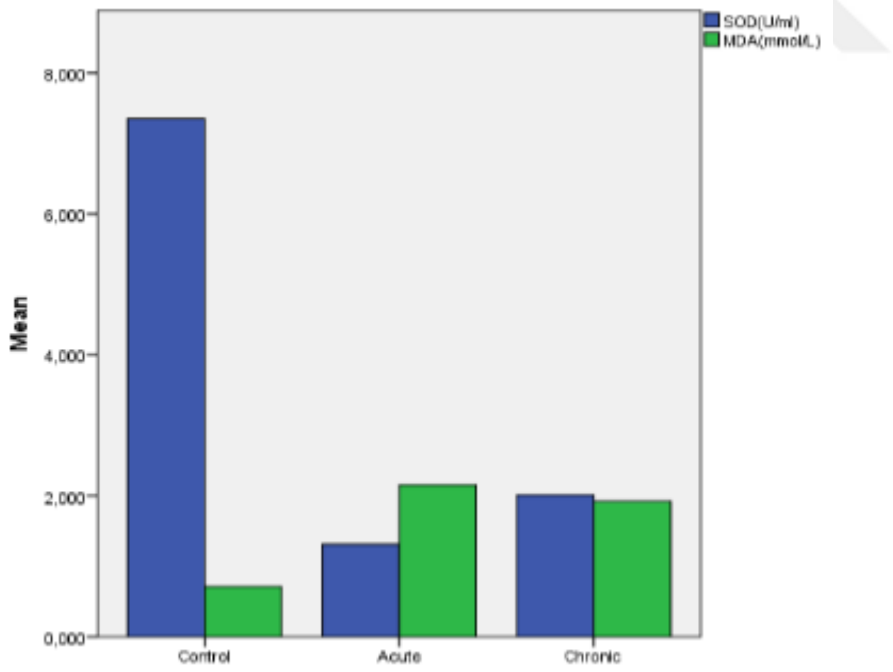
Ayrıca, süperoksit dismutaz (SOD) enzim aktivitesi (Tablo 3.1), kontrol grubu ile hasta grubu aktivitesi(7.351±0.381U/L)ve akut hasatlar (1.312±0.754 U/L),kronik hastalar (2.012±0.661 U/L farklılıklar da istatistiksel olarak anlamlı bulundu (p<0.05).

Öte yandan, katalaz (CAT) enzim aktivitesi (Tablo 3.1), kontrol ve hasta grubu seviyelerinin (0.415±0.124 U/L) ve akut hastlar (0.075±0.005 U/L), kronic (0.077±0.006 U/L) istatistiksel olarak anlamlı olduğunu göstermiştir (p< 0.05).

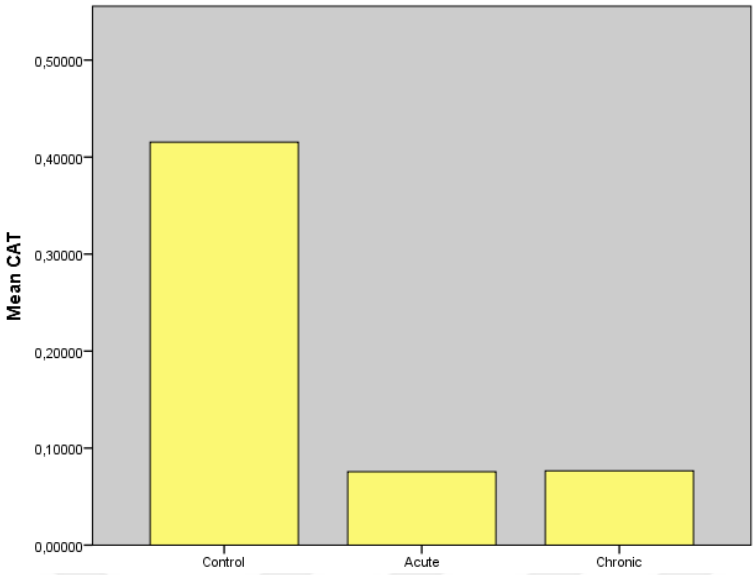
Tablo 3.1 Kontrol ve hasta gruplarında böbrek yetmezliğinin karşılaştırılması:

Parametreler	Kontrol grubu Mean±SD(n=31)	Akut hastalar Mean±SD(n=31)	Kronik hastalar Mean±SD(n=30)
SOD(U/L)	7.351±0.381	1.312±0.754	2.012±0.661
CAT(U/L)	0.415±0.124	0.075±0.005	0.077±0.006
GSH(µmol/L)	0.00028±0.000065	0.000021±0.000016	0.0000061±0.0000053
MDA(µmol/L)	0.708±0.065	2.155±0.753	1.921±0.497

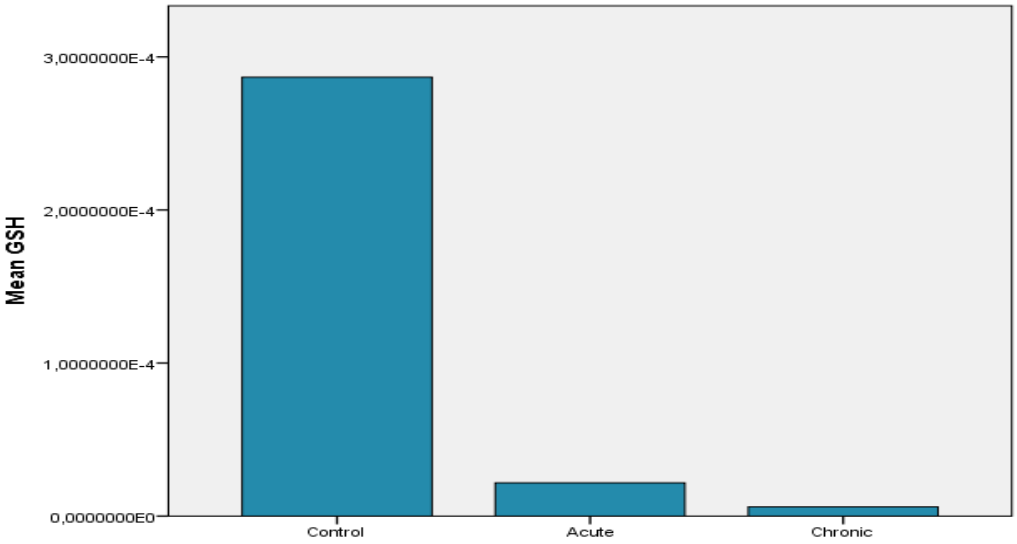
p< 0.05: anlamlı kabul edild



Şekil 3.1. Kontrol ile karşılaştırıldığında SOD ve MDA aktivitesinin seviyesi ve kronik böbrek yetmezliği ile akut.



Şekil 3.2. İşbirliği kontrol hastalarında ve kronik böbrek yetmezliği olan akutlarda CAT enzim aktivitesi seviyesi hastalara sahiptir.



Şekil 3.3. GSH enzim aktivitesinin seviyesi kontrol hastaları ile karşılaştırıldığında ve akut kronik böbrek yetmezliği hastaları ile karşılaştırıldığında.

4. TARTIŞMA VE SONUÇ

Bu çalışma, akut ve kronik böbrek yetmezliği ve serum süperoksit dismutaz (SOD), glutatyon azaltılmış (GSH) ve katalazda (CAT) oksidatif stresin bir göstergesi olarak gösterilen lipit peroksidasyon ürünü için plazma malondialdehit (MDA) seviyesini belirlemeyi amaçladı.

Çalışmanın bulguları, lipid-peroksidasyonunun bir götergesi olan MDA'nın, böbrek yetmezliğili hastalarda kontrol grubuna kıyasla MDA açısından istatistiksel olarak anlamlı şekilde arttığını göstermiştir ($p<0.05$). Böbrek yetmezliği hastalarında, oksidatif olan yüksek konsantrasyonlardaki oksidanlar, stresle aktive olan makrofajlar ve nötrofiller tarafından salınır. Bu, lipidlerin, proteinlerin, karbonhidratların ve DNA'nın zarar görmesine neden olabilir. Lipid peroksidasyonu ve MDA ile reaksiyona giren hücre zarlarının doymamış yağ asitleri serbest bırakılır. Oksidatif stres belirteci olarak işlev görür ve iltihaplanma etkilerini artıran immünojenik moleküllerin daha fazla üretimine yol açan lisin kalıntılarıyla reaksiyona girer (Ratliff et al., 2016). ROS oluşumu devam ettikçe kronik böbrek hastalığı büyük yayılım gösterebilir ,ayrıca dokularda gereksiz hasara neden olabilir. ROS saldırıları, membran lipidleri de çoklu doymamış yağ asitleri vasıtasıyla meydana gelir ve hücrenin işlevini bozabilir, sonuçta lipit peroksidasyonuna neden olabilir. Ayrıca, malondialdehit gibi lipid peroksidasyonun son ürünü, oksitlenmiş lipidlerin ayrıştırılmasıyla üretilecektir (Redon et al., 2003).

Ortalama SOD aktivitesi düzeyi, böbrek yetmezliği akut ve kronik kontrol grubuyla karşılaştırıldığında istatistiksel olarak anlamlı bir düşüş gösterdi ($P<0.05$). Azalan SOD aktivitesi, ROS etkisine bağlı olarak detoksifikasyon işleminin bir parçası olarak SOD bozulmasının bir sonucu olabilir.

Bu çalışmada CAT aktivitesi, böbrek yetmezliği akut ve kronik kontrol grubuyla karşılaştırıldığında istatistiksel olarak ve anlamlı bir şekilde azaldı ($P<0.05$). Böbrek yetmezliği grubundaki bu azalmış katalaz aktivitesi, katalazın H_20_2 ile etkisizleştirilmesinden dolayı meydana gelmiş olabilir. Her ikisi de azalmış katalaz aktivitesi gösterir (Ratliff et al., 2016). böbrek yetmezliği hastasının azalmış katalaz seviyesi, artan inflamasyonla açıklanabilir. Katalaz H_20_2'nin H_2O ve O_2'ye değişmesine

nedenle olur. Sonuç olarak, hücreleri biriken hidrojen peroksitin zararlı etkilerinden korur. Bu sonuç diğer yapılan litaratür bulgularla uyumludur (Kobayashi et al., 2005).
Bu çalışmada GSH aktivitesi böbrek yetmezliği akut ve kronik kontrol grubuyla karşılaştırıldığında istatistiksel olarak ve anlamlı derecede azaldı ($P < 0.05$). Glutatyonun azaltılması bir flavoenzimdir ve GSSH'nin glukoz-6-fosfat dehidrojenaz tarafından sağlanan GSH'ye indirgenmesinde rol oynayan NADPH'ye bağlıdır (Ahmadpoor et al., 2009).

Sonuç olarak, Bu çalışmada, SOD, CAT ve GSH gibi antioksidant azaldığı ve böbrek yetmezliği akut ve kronik hastalarda malondialdehit (MDA) gibi yüksek lipid peroksidasyon seviyesinin arttığı görülmüştür. Sonuçlar, oksidatif stresin böbrek yetmezliği ile ilgili olarak hastalığın olumsuz yönde etkilerini artırabileceğini göstermektedir.

Bu çalışmada, oksidatif stresin, böbrek yetmezliği hastalarında dokunun hücresel hasarını çok iyi etkilediğini göstermektedir.

CURRICULUM VITAE

Seerwan Hamadameen SULAIMAN was born in 1994, in Erbil province, Iraq. He completed primary, secondary and high school in Erbil district. He graduated from Clinical of Biochemistry Department in Health Sciences college in Hawler Medical University in 2017. He started his MSc. the department of Chemistry (Biochemistry) Institute of Science of Van Yuzuncu Yil University in Van -Turkey on February 2018.

T.C
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Tez Başlığı / Konusu: **"Akut ve Kronik Böbrek Yetmezliği Hastalarında Oksidatif Stres Düzeyi ve Bazı Antioksidant Aktivitelerin Belirlenmesi"**

Yukarıda başlığı/konusu belirlenen tez çalışmamın Kapak sayfası, Giriş, Ana bölümler ve Sonuç bölümlerinden oluşan toplam 90 sayfalık kısmına ilişkin, 12/12/2019 tarihinde şahsım/tez danışmanım tarafından yerleştirilen intihal tespit programından aşağıda belirtilen filtreleme uygulanarak alınmış olan orijinallik raporuna göre, tezimin benzerlik oranı % 10 (yüzde on) dır.

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- Kaynakça hariç,
- Alıntılar hariç,
- Tezden çıkan yayınlar hariç,
- 7 kelimeden daha az örtüşme içeren metin kısımları hariç (Limit inatch size to 7 words)

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Gereğini bilgilerinize arz ederim.27.12.2019.

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