T. R.
VAN YUZUNCU YIL UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF MOLECULAR BIOLOGY AND GENETICS

DETERMINATION OF HEPATOPROTECTIVE AND ANTIOXIDANT ROLE OF THYME (*Origanum onites* L.) INFUSION AGAINST ETHYL ALCOHOL INDUCED OXIDATIVE STRESS IN RATS

M.Sc. THESIS

PREPARED BY: Shreen Abdulkareem MUSA
SUPERVISOR: Prof. Dr. İsmail ÇELİK

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ACCEPTANCE and APPROVAL PAGE

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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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ABSTRACT

DETERMINATION OF HEPATOPROTECTIVE AND ANTIOXIDANT ROLE OF THYME (*Origanum onites L.*) INFUSION AGAINST ETHYL ALCOHOL INDUCED OXIDATIVE STRESS IN RATS

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In this study, we aimed to determine the hepatoprotective and antioxidants role of thyme (*Origanum onites L.*) against ethyl alcohol (EtOH) induced oxidative stress in rats. Rats were divided into 4 groups with 7 rats in each and conducted as contro, 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH groups. The hepatoprotective and antioxidant role of *O. onites* infusion supplementation was evaluated as liver damage biomarkers, antioxidant constituents and malondialdehyde. The obtained results, while there was a significant increase in the AST and ALT levels of 20% EtOH group in comparison with the control group and a significant decrease in the TP and TC content of 20% EtOH group according to control group. The 3% *O. onites* + 20% EtOH mixture infusion group caused a significant decrease in the AST levels in comparison with the 20% EtOH group whereas, a significant increase in the TP and TC content of 3% *O. onites* infusion + 20% EtOH group in comparison with the 20% EtOH group. Also, while EtOH significantly increased MDA contents in the liver, kidney and erythrocyte tissues in comparison to those of control. The *O. onites* Infusion supplemented groups significantly decreased MDA contents in all tissues comparison to those of 20% EtOH group. Furthermore, while EtOH caused fluctuations in the antioxidant defence system levels as a result of oxidative stress condition in rats. The definitive curative effects of the *O. onites* infusion on these fluctuations have not been determined. As a result, it was concluded that *O. onites* contents may have a hepatoprotective and antioxidant role against EtOH induced oxidative stress in rats.

Keywords: Antioxidant, Hepatoprotective, *Origanum onites* L., Rat

ÖZET

DENEYSEL OLARAK ETİL ALKOL İLE OKSİDATİF STRES OLUŞTURULAN SIÇANLARDA KEKİK (*Origanum onites* L.) İNFİZYONUNUN KARACİĞER KORUYUCU VE ANTİOKSİDAN ROLÜNÜN BELİRLENMESİ

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Bu çalışmada, etil alkol (EtOH) ile deneysel oksidatif stres oluşturulan sıçanlarda kekik (*Origanum onites* L.) karaciğer koruyucu ve antioksidan rolünün belirlenmesi amaçlanmıştır. Sıçanlar her birinde 7 sıçan olarak 4 gruba ayrıldı ve gruplar kontrol, %20 EtOH, %3 *O. onites* infüzyonu ve %3 *O. onites* + %20 EtOH grubu olarak belirlenmiştir. *O. onites* infüzyonun karaciğer koruyucu ve antioksidan rolünün değerlendirilmesi için karaciğer hasarı biyobelirteçleri, antioksidan bileşenleri ve malondialdehit içerikleri tespit edilerek değerlendirilmiştir. Sonuçlara göre; etil alkol grubunun serum AST ve ALT enzim seviyeleri kontrol grubuna göre önemli düzeyde artarken, %20 EtOH grubunun TP ve TC içeriğinde ise kontrol grubuna göre anlamlı bir düşüş görülmüştür. %3*O. onites* + %20 EtOH grubu, %20 EtOH grubuyla karşılaştırıldığında AST seviyelerinde önemli bir düşüşe neden oldu ve %20 EtOH grubuna kıyasla %3*O. onites* + %20 EtOH grubu TP ve TC içeriğinde önemli bir artışa sebep oldu. Ayrıca kontrol grubu ile kıyaslandığında etil alkol grubu karaciğer, böbrek ve eritrosit dokularında MDA miktarı anlamlı artarken, %3*O. onites* + %20 EtOH grubunda ise alkol grubuna göre tüm dokularda istatistiki açıdan önemli düzeyde azalma olduğu görüldü. Ayrıca, alkol sıçanların farklı dokulardaki oksidatif stres sonucu antioksidan savunma sistem unsurlarının seviyelerinde dalgalanmalara neden olurken, *O. onites* infüzyonunun bu dalgalanmalara karşı kesin iyileştirici etkileri belirlenememiştir. Sonuçlar kekik içeriğinin alkolün oluşturduğu oksidatif hasarına karşı karaciğer koruyucu ve antioksidan rolünün olabileceği sonucuna varıldı.

Anahtar kelimeler: Antioksidan, Karaciğer koruyucu, *Origanum onites L,*Sıçan

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Shreen Abdulkareem MUSA

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SYMBOLS AND ABBREVIATIONS

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

Symbols	Description
µ	Micron
α	Alpha
β	Beta
^{o}C	Celsius degree
Min	Minutes
g	Gram
L	Liter
Mg	Milligrams
mL	Milliliters
µl	Microliter
M	Molar
mM	Millimolar
mmol	Millimole
nmol	nanomoles
nM	nanomolar
µmol	Micromole
rpm	Rotation / min
sec.	Seconds
U	Unit

Abbreviations	Description
ΔA	Absorbance change
ADP	Adenosine di phosphate
ALT	Alanine amino transferase
AST	Aspartate amino transferase
ATP	Adenosine tri phosphate
BHA	Butylated hydroxy anisole
BHT	Butylated hydroxy toluene
CAT	Catalase
DNA	Deoxyribonucleic acid
DTNB	5,5 rob-dithiobis- (2-nitrobenzoic acid)
EDTA	Ethylenediamine tetra acetic acid
ER	Endoplasmic reticulum
GR	Glutathione reductase
GSH	Reduced glutathione
GSH-Px	Glutathione peroxidase
GSSG	Oxidized glutathione
GSSG-R	Glutathione reductase
GST	Glutathione S-transferase
Hb	Hemoglobin
H_2O_2	Hydrogen peroxide
HO_2	Perhydroxyl radical
INT	2-(- iodophenyl) -3- (4-nitrophenyl) -5-phenyl tetrazolium chloride
LDH	Lactate dehydrogenase
LOOH	Lipid Hydroperoxide
LPO	Lipid peroxidation
MDA	Malondialdehyde
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
$NO^{\bullet}$	Nitric oxide radical

$ONOO^{\bullet}$	Peroxynitrite
$O_2^{\bullet -}$	Superoxide radical
$^1O_2$	Singlet oxygen
$OH^{\bullet}$	Hydroxyl radical
R	Radicals
RNA	Ribonucleic acid
$ROO^{\bullet}$	Peroxyl radical
$RO^{\bullet}$	Alkoxyl radical
ROOH	Lipid Hydroperoxide
SD	Standard deviation
-SH	Sulfhydryl
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
UV	Ultraviolet Rays
Na_2HPO_4	Disodium hydrogen phosphate
NaH_2PO_4	Sodium dihydrogen phosphate
OD	Optical Density
TC	Total cholesterol
TP	Total protein
XO	Xanthine oxidase

1. INTRODUCTION

Over the past 40 years, there has been a tremendous amount of research on the dual role of free radicals as both toxic and beneficial species. Free radicals are produced as by-products of normal cellular metabolism or generated by chemicals in our external environment (e.g., cigarette smoke, air and water pollution, exposure to sunlight, gamma-irradiation, and certain chemotherapeutic drugs). At low to intermediate concentrations, free radicals exert their effects through regulation of cell signaling cascades. At high concentrations, they damage all macromolecules, inducing DNA damage, lipid peroxidation, protein modification, and eventually cell death. Free radicals have been implicated in the pathogenesis of a number of conditions, such as aging, atherosclerosis, ischemic heart disease, cancer, and Alzheimer's disease (Santo et al., 2016). Nature has bestowed the human body with a complex web of antioxidant defense system including enzymatic antioxidants as GPx, GR, CAT and SOD, and non-enzymatic antioxidants such as thiol antioxidants, melatonin, coenzyme Q, and metal chelating proteins, which are efficient enough to fight against excessive free radicals too. Also, nutrient antioxidants such as vitamin C, vitamin E, carotenoids, polyphenols, and trace elements are known to have high antioxidant potency to assist in minimizing harmful effects of reactive species. The immune system is also, extremely vulnerable to oxidant and antioxidant balance as uncontrolled free radical production can impair its function and defense mechanism (Aslani and Ghobadi, 2016). The ability to estimate chemical biomarkers of free radical damage in body fluids and tissues is an important step in understanding the mechanisms contributing to disease processes (Santo et al., 2016).

1.1. Oxidative Stress

In healthy aerobes, production of reactive species (RS) is approximately balanced with antioxidant defence systems (Halliwell and Gutteridge, 2007). The balance is not perfect, however, so that some RS-mediated damage occurs continuously. In other words, antioxidant defences control levels of RS rather than eliminate them, e.g. the OxyR system in *Escherichia coli* keeps H_2O_2 levels at approx. 0.2 μM (Pomposiello and Demple, 2002).

Why is this cause maintaining excess antioxidant defenses has an energy cost: it could be energetically 'cheaper' to repair or replace damaged biomolecules (Halliwell and Gutteridge, 2007). In addition, antioxidants may simply be unable to intercept some RS. For example, hydroxyl radical ($OH^{\bullet}$) generated by homolytic fission of water due to our background exposure to ionizing radiation (Von Sonntag, 2006) is so highly reactive that it will react with whatever it meets first and so is virtually impossible to scavenge. Yet another factor is that RS play essential roles in *vivo*, not least in the redox regulation of gene expression and other cellular events (Pomposiello and Demple, 2002; Nathan, 2003; Cho et al., 2004; Rhee et al., 2005; Temple et al., 2005).

The term 'oxidative stress' refers to a serious imbalance between RS production and antioxidant defences. Sies (1991) defined it as "a disturbance in the pro-oxidant–antioxidant balance in favour of the former, leading to potential damage". Such damage is often called 'oxidative damage', another vague term. Whiteman and Halliwell (2004) have defined oxidative damage as "the biomolecular damage caused by attack of RS upon the constituents of living organisms" . Increased levels of oxidative damage can result not only from oxidative stress, but also, from failure of repair or replacement systems (Halliwell and Whiteman, 2004; Halliwell and Gutteridge, 2007)

In principle, oxidative stress can result from (Halliwell and Gutteridge, 2015) :

1. Diminished antioxidants, e.g. mutations decreasing the levels of antioxidant defenses, such as GSH or MnSOD. Depletions of dietary antioxidants and other essential dietary constituents (e.g. copper, iron, zinc, thiamine, nicotinamide, vitamin D, magnesium) can also, lead to oxidative stress. For example, children

with the protein deficiency disease kwashiorkor suffer oxidative stress, involving low GSH levels (insufficient sulphur-containing amino acids in the diet) and the presence of 'catalytic iron ions' in body fluids (inability to make enough transferrin to bind iron safely). Whether giving them antioxidants would be of benefit is uncertain (Badaloo and Marshall, 2014).

2. Increased production of RS, e.g. by exposure to elevated O_2, the presence of toxins that produce RS, or excessive activation of 'natural' systems producing RS, e.g. inappropriate activation of phagocytic cells in chronic inflammatory diseases.

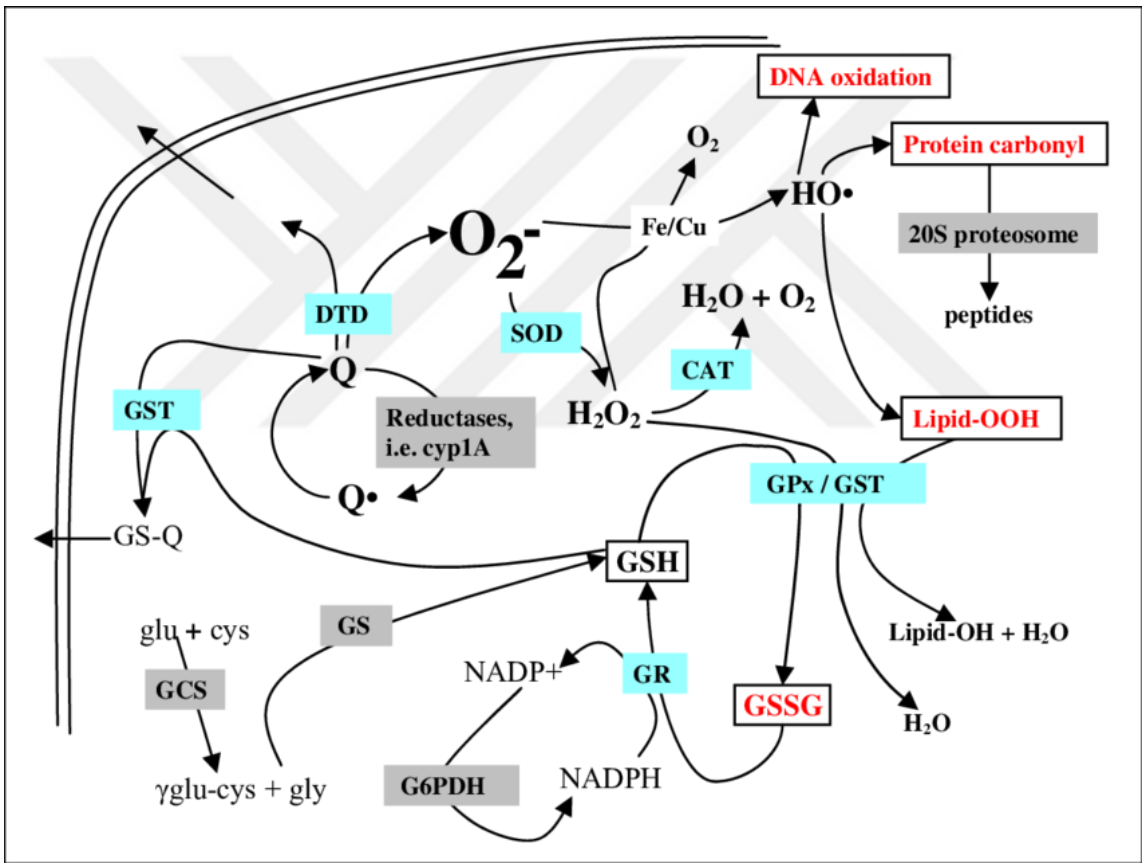


Figure 1. 1. Schematic diagram of oxidative stress. Antioxidant enzymes are shown in blue boxes, other enzymes in gray, oxidized molecules are shown in red text. Q represents a redox cycling compound (Almroth, 2008).

1.1.1. Consequences of Oxidative Stress

Consequences of oxidative stress can include any, or any combination of, the following, to an extent that depends on the cell/tissue being examined and the severity and time-course of the oxidative stress.

1. *Increased proliferation.* Many cells respond to mild oxidative stress by proliferating.
2. *Adaptation* of the cell or organism by upregulation of defense systems, which may: (a) completely protect against damage; (b) protect against damage to some extent but not completely; or (c) 'over-protect'-the cell is then resistant to higher levels of oxidative stress imposed subsequently.
3. *Cell injury.* This involves damage to some or all molecular targets: lipids, DNA, protein, carbohydrate, etc. Such damage can sometimes be the trigger leading to adaptation. Not all damage caused by oxidative stress is oxidative damage: secondary damage to biomolecules can result from oxidative stress-related changes in, for example, ion levels (e.g. rises in Ca^{2+} leading to activation of proteinases).
4. *Senescence.* The cell survives but can no longer divide.
5. *Cell death.* After injury the cell may: (a) recover from the oxidative damage by repairing it or replacing the damaged molecules; or (b) it may survive with persistent oxidative damage; or (c) oxidative damage, especially to DNA, may trigger death by apoptosis, necrosis, or cell death mechanisms intermediate between these extremes (Halliwell and Gutteridge, 2015).

1.2. Oxidation

Oxidation in a given biological system is basically involved in the production of energy at molecular level to be used for molecular, biochemical and physiological functions performed in a biosystem. Under normal conditions oxidation can involve either addition of oxygen, removal of hydrogen or removal of electron of these three, removal of electron is the most commonly takes place while removal of hydrogen is

moderately involved and addition of oxygen is not so common. During the mode of removal of electron an acceptor of the released/removed electron is required because electrons cannot remain stable in free-state; thus, under the influence of oxidoreductase enzymes, removal of electron (oxidation) and acceptance of electron (reduction) take place simultaneously. Hence, these as one unit may be regarded as redox/oxidation-reduction reaction. The endogenous and exogenous molecules may be grouped in to two sets, one which includes molecules with low redox potential and the other in which molecules having higher redox potential are included; these molecules are responsible for the oxidation-reduction potential/reduction potential in a system under investigation. Oxidation reduction potential is maintained at an equilibrated state by combined and coordinated interactions between prooxidants, antioxidants, and inactivated free radicals (Lahir, 2015). Suslow (2004) has suggested that redox potential or oxidation potential (ORP, ε, Eh) can be expressed as the quantitative ability of a chemical to accept electron and as a result of this the concerned chemical gets reduced.

Chemicals/substances having low redox potential release electron and a chemicals/substance having higher redox potential accept the released electrons. During this transfer of electron energy is liberated or yielded. The amount of energy released is proportional to the redox potential difference between the two chemicals acting as donor and acceptor; (Petrucci et al., 2002). In a biological system there is always competition between the action of prooxidants and the antioxidants; the equilibrium between these two rates is of great significance and must be coordinated meticulously to maintain normal vital functioning of cell and the molecules. In a given biological system this specific equilibrated state may be termed as redox potential and it is specific for each cell, cell organelle and biological site. If this coordinated balance gets disturbed there is every chance of derogated consequences of the cell and the given biosystem; (Repetto et al., 2012).

1.3. Free Radicals

Free radicals are the products of normal cellular metabolism. A free radical can be defined as an atom or molecule containing one or more unpaired electrons in valency shell or outer orbit and is capable of independent existence. The odd number of electron(s) of a free radical makes it unstable, short lived and highly reactive. Because of their high reactivity, they can abstract electrons from other compounds to attain stability. Thus, the attacked molecule loses its electron and becomes a free radical itself, beginning a chain reaction cascade which finally damages the living cell (Mukherji and Singh, 1986). Both reactive oxygen species ROS and reactive nitrogen species RNS collectively constitute the free radicals and other nonradical reactive species (Pham-Huy et al., 2008). The ROS/RNS play a twofold job as both beneficial and toxic compounds to the living system. At moderate or low levels ROS/RNS have beneficial effects and involve in various physiological functions such as in immune function (i.e. defense against pathogenic microorganisms), in a number of cellular signaling pathways, in mitogenic response and in redox regulation (Nordberg and Arner, 2001; Valko et al., 2007). But at higher concentration, both ROS as well as RNS generate oxidative stress and nitrosative stress, respectively, causing potential damage to the biomolecules. The oxidative stress and nitrosative stress are developed when there is an excess production of ROS/RNS on one side and a deficiency of enzymatic and non-enzymatic antioxidants on the other side. Furthermore, the excess ROS can damage the integrity of various biomolecules including lipids, proteins (Standman and Levine, 2000) and DNA (Marnett, 2000) leading to increased oxidative stress in various human diseases as diabetes mellitus, neurodegenerative diseases, rheumatoid arthritis, cataracts, cardiovascular diseases, respiratory diseases as well as in aging process.

1.4. ROS and RNS

In general, pro-oxidants/oxidants are termed as ROS/RNS. The most important free radicals produced during metabolic reactions are radicals derived from oxygen, ROS. Both the ROS and RNS can be classified into two groups of compounds namely;

radicals and non-radicals. Radicals are the species which contain at least one unpaired electron in the shells around the atomic nucleus and are capable of independent existence. The oxygen molecule itself is a radical, and because of the presence of two unpaired electrons it is referred as biradical. The examples for the radicals include superoxide ($O_2^{\cdot -}$), oxygen radical ($O_2^{\bullet\bullet}$), hydroxyl ($OH^{\bullet}$), alkoxy radical ($RO^{\bullet}$), peroxyl radical ($ROO^{\bullet}$), nitric oxide (nitrogen monoxide) ($NO^{\bullet}$) and nitrogen dioxide ($NO2^{\bullet}$) (Halliwell and Gutteridge, 1999). The high reactivity of these radicals is due to the presence of one unpaired electron which tends to donate it or to obtain another electron to attain stability. The nonradical species include hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), hypobromous acid (HOBr), ozone (O_3), singlet oxygen ($^1O_2$), nitrous acid (HNO_2), nitrosyl cation (NO^+), nitroxyl anion (NO^-), dinitrogen trioxide (N_2O_3), dinitrogen tetraoxide (N_2O_4), nitronium (nitryl) cation (NO_2^+), organic peroxides (ROOH), aldehydes (HCOR) and peroxynitrite (ONOOH) (Kohen and Nyska, 2002; Halliwell and Gutteridge, 1999). These nonradical species are not free radicals but can easily lead to free radical reactions in living organisms (Genestra, 2007).

1.4.1. Superoxide ion radical ($O_2^{\cdot -}$)

Superoxide anion radical is the most important widespread ROS formed by the enzymatic process, autooxidation reaction and by a nonenzymatic electron transfer reactions in which an electron is transferred to molecular oxygen (Michelson et al., 1977). It is mostly produced within the mitochondria and its reactivity with the biomolecules is low. The enzymes that can produce superoxide include xanthine oxidase (Kuppusamy and Zweier; 1989), lipooxygenase, cyclooxygenase (Kontos et al., 1985; McIntyre et al., 1999) and NADPH dependent oxidase. It can exist in two forms such as $O_2^{\cdot -}$or hydroperoxyl radical (HO_2) at low pH (Bielski and Cabelli,1996). The hydroperoxyl radical is the most important form and can easily enter the phospholipid bilayer than the charged form ($O_2^{\cdot -}$). Under physiological pH the most occurring form is superoxide. It can act as reducing agent and it reduces iron complexes such as cytochrome-c and ferric-ethylene diaminetetraacetic acid (Fe^+-EDTA), in which Fe^{+3} is

reduced to Fe^{+2}. It can Also,act as oxidizing agent and oxidize ascorbic acid and tocopherol.

$$O_2 + e^- \longrightarrow O_2^{\cdot -} \quad (1.1)$$

$$O_2 + Fe^{+2} \longrightarrow Fe^{+3} + O_2^{\cdot -} \quad (\text{auto} - \text{oxidation}) \quad (1.2)$$

Superoxide radical react with another superoxide radical in a dismutation reaction (Eq. 1.3), in which one radical is oxidized to oxygen and other is reduced to hydrogen peroxide (Bielski et al., 1985).

$$O_2 + O_2^{\cdot -} + 2H_2O \xrightarrow{Cu,Zn,Mn-SOD} H_2O_2 + O_2 \quad (1.3)$$

1.4.2. Hydroxyl radical ($OH^{\bullet}$)

Hydroxyl radical is the neutral form of hydroxide ion and is a highly reactive free radical (Bedwell, 1989). It can strongly react with both organic and inorganic molecules including DNA, proteins, lipids, and carbohydrates and cause severe damage to the cells than any other ROS can do (Halliwell, 1987). It is formed in a Fenton reaction (Eq. 1.4), in which H_2O_2 react with metal ions (Fe^{+2} or Cu^{+}), often bound in complex with different proteins such as ferritin (an intracellular protein that stores iron) and ceruloplasmin (plasma copper carrying protein) or other molecules (Fenton, 1894). Under stress conditions, an excess of $O_2^{\cdot -}$releases free iron from ferritin and the released free iron participates in Fenton reaction to form $OH^{\bullet}$. It is also, formed by the reaction between superoxide radical and H_2O_2 in a reaction called Haber–Weiss reaction (Eq. 1.5) (Haber and Weiss, 1934).

$$Fe^{+2} + H_2O_2 \longrightarrow Fe^{+3} + {}^{\cdot}OH + OH^- \quad (\text{Fenton reaction}) \quad (1.4)$$

$$O_2^{\cdot -} + H_2O_2 \longrightarrow O_2 + {}^{\cdot}OH + OH^- \quad (\text{Haber} - \text{Weiss reaction}) \quad (1.5)$$

1.4.3. Peroxyl radical ($ROO^{\bullet}$)

It is derived from oxygen in living systems. The simplest form of peroxyl radical is perhydroxyl radical ($HOO^{\bullet}$) which is formed by the protonation of superoxide (De Grey, 2002). About 0.3 % of the total $O_2^{\cdot -}$ in the cytosol of a typical cell is in the protonated form. It initiates fatty acid peroxidation and also, can promote tumor development (Cerruti, 1985).

1.4.4. Hydrogen peroxide (H_2O_2)

Hydrogen peroxide is formed in vivo in a dismutation reaction catalyzed by the enzyme superoxide dismutase (SOD) (Eq. 1.3). It is not a free radical but it can cause damage to the cell at relatively low concentration (10 μM), but at higher levels, the cellular energy producing enzymes such as glyceraldehhyde-3-phosphate dehydrogenase are inactivated. It can easily penetrate the biological membranes. H_2O_2 has no direct effect on DNA but can damage DNA by producing hydroxyl radical ($OH^{\cdot}$) in the presence of transition metal ions (Halliwell, 2000). The major antioxidant enzymes that can eliminate the H_2O_2 include CAT, GPx and peroxiredoxins (Chae, 1999; Mates, 1999).

1.4.5. Singlet oxygen ($^1O_2$)

It is an electronically high excited, meta-stable state of molecular oxygen and is a highly reactive toxic reactive oxygen species. Upon activation, the molecular oxygen is excited to first state $^1\Delta_g$ and then to next higher excited singlet state, $^1\Sigma_g$. The first excited state, $^1\Delta_g$, has two electrons with opposite spins in the same π^* orbital whereas, the second excited state, $^1\Sigma_g$, has one electron in each degenerated π^* orbital with opposite spins. The $^1\Delta_g$ state is extremely reactive and compared to the other electronically excited states. It is produced in vivo by the activation of neutrophils (Eq. 1.6) and eosinophils. It is also, formed by some of the enzymatic reactions catalyzed by

enzymes such as lipoxygenases, dioxygenases, and lactoperoxidase. It is a highly potent oxidizing agent that can cause DNA and tissue damage (Phaniendra et al., 2015).

$$HOCL + H_2O_2 \longrightarrow {}^1O_2 + H_2O + Cl\_ \quad (1.6)$$

1.4.6. Ozone (O_3)

Ozone is a powerful oxidant may be produced *in vivo* by antibody catalyzed water oxidation pathway which plays an important role in inflammation. It can form free radicals and other reactive intermediates by oxidizing the biological molecules. It can cause lipid peroxidation and oxidize different functional groups, such as, amine, alcohol, aldehyde and sulphydryl, present in proteins and nucleic acids. It can also, cause chromosomal aberrations which may be due to direct attack by O_3 or by the free radicals generated by it (Phaniendra et al., 2015).

1.4.7. Hypochlorous acid (HOCl)

It is a major oxidant produced by the activated neutrophils at the site of inflammation from H_2O_2 and chloride in a reaction catalyzed by the enzyme myeloperoxidase (Winterbourn and Kettle, 2000).

$$H_2O_2 + Cl\_ \longrightarrow HOCL + OH^{\cdot} \quad (1.7)$$

HOCl is a strong reactive species involved in oxidation and chlorination reactions. It can oxidize thiols and other biological molecules including, ascorbate, urate, pyridine nucleotides, and tryptophan (Albrich et al., 1981; Winterbourn, 1985). HOCl chlorinates several compounds such as amines to give chloramines; tyrosyl residues to give ring chlorinated products, cholesterol and unsaturated lipids to give chlorohydrins, and it can also, chlorinate DNA (Prutz, 1996).

1.4.8. Nitric oxide or nitrogen monoxide ($NO^{\bullet}$)

It is a small molecule generated in tissues by different nitric oxide synthases (NOS)s which convert L-arginine to L-citrulline (Andrew and Mayer, 1999). In this reaction one of the terminal guanido nitrogen atoms undergo oxidation and produces $NO^{\bullet}$. Three types of isoforms of NOS such as neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS) are involved in the formation of the NO radical.

$$\mathrm{L-Arginine} + \mathrm{O_2} + \mathrm{NADPH} \xrightarrow{NOS} \mathrm{L-Citrulline} + \mathrm{NO^{\cdot}} + \mathrm{NADP^{+}} \qquad (1.8)$$

It is both aqueous and lipid soluble and therefore it readily diffuses through cytoplasm and plasma membrane (Chiueh, 1999). The $NO^{\bullet}$ is an important intracellular second messenger stimulates guanylate cyclase and protein kinases and helps in smooth muscle relaxation in blood vessels. It is identical to endothelium derived relaxing factor (EDRF) produced by vascular endothelial cells which is an important mediator of vascular responses (Ignarro et al., 1987). It can also, act as an important cellular redox regulator (Wink and Mitchell, 1998) and regulate enzymatic activity by nitrosylating the proteins (Stamler, 1994). Since it is involved in many biological activities like blood pressure regulation, smooth muscle relaxation, neurotransmission, defensive mechanisms and immune regulation, this molecule was regarded as molecule of the year 1992 (Koshland, 1992).

1.4.9. Peroxynitrite (OONO-) and other reactive nitrogen species

Peroxynitrite (OONO-) is formed by the reaction between $O_2^{\cdot -}$ and $NO^{\bullet}$. It is highly toxic (Beckman and Koppenol, 1996) and can directly react with CO_2 to form other highly reactive nitroso peroxo carboxylate (ONOOCO2 -) or peroxynitrous acid (ONOOH). The ONOOH further undergo homolysis to form both OH• and NO_2 or rearrange to form NO_3. $OONO^-$ can oxidize lipids, oxidize methionine and tyrosine residues in proteins and oxidizes DNA to form nitroguanine (Douki and Cadet, 1996). The nitrotyrosine residues are considered as marker of peroxynitrite induced cellular damage (Ischiropoulos and Al-Mehdi, 1995).

NO reacts with O_2 and water to form nitrate and nitrite ions. One electron oxidation of $NO^{\bullet}$ results in nitrosonium cation ($NO_2^{\bullet+}$) while on electron reduction results in nitroxyl anion (NO^-). These two ions can react with NO and form N_2O and $OH^{\bullet}$. $NO^{\bullet}$ can react with a variety of radicals such as H_2O_2 and HOCl to form N_2O_3, NO^{2-} and NO^{3-} (Czapski and Goldstein, 1995).

1.5. Sources of Reactive Oxygen Species (ROS) Generation

The main sources of intracellular ROS are mitochondria, the endoplasmic reticulum (ER), peroxisomes, microsomes, and NOX complexes (seven distinct isoforms) in cell membranes. Specifically, mitochondria represent the main intrinsic source of ROS generation via the mitochondrial electron-transport system. Increased accumulation of calcium (Ca^{2+}) in the cytoplasm results in activation of the mitochondrial electron-transport chain and ROS generation. During mitochondrial production of adenosine triphosphate (ATP) and water, small concentrations of oxygen are produced, resulting in the early stages of ROS production. The $O_2^{\bullet-}$ anion, the first ROS element generated by mitochondria, is produced by complex I (NADH ubiquinone oxidoreductase) and complex III (co-enzyme Q, bc1 complex, and uniquinone/cytochrome c reductase) activity in the mitochondrial matrix and intermembrane space, respectively. In the intermembrane space, metals, such as Cu, Mn, and Zn-SOD, catalyze the conversion of superoxide anions into H_2O_2 (stable form). Monoamine oxidase and α-ketoglutarate dehydrogenase are also, potential sources of mitochondrial ROS generation. NOX represents a non-mitochondrial source of ROS generation and plays pivotal role in superoxide formation via oxygen reduction mediated by the electron donor NADPH. Mammalian NOX is composed of seven isoforms (NOX1-5, Dual oxidase 1 (DUOX1), and DUOX2), the majority of which generate superoxide, whereas NOX4, DUOX1, and DUOX2 generate H_2O_2.

The ER is a cellular organelle that also, plays a key role in ROS production. The ER lumen represents a suitable oxidizing environment (with a high ratio of oxidized-to-reduced forms of GSH) for protein folding and formation of disulfide bonds. Additionally, there are various cellular enzymes, including xanthine oxidoreductase,

nitric oxide (NO) synthase, cytochrome P450 monoxygenase, lipoxygenase, and cyclooxygenase, implicated in the process of ROS generation. $ONOO^-$, which is considered a potent oxidizing and nitrating agent, results from interaction between NO and O_2.

Extracellular sources of ROS generation include ROS-inducing agents, such as radiation, pollutants, and exposure to nanomaterials. Oxidative stress initiates defense strategies associated with macrophages and neutrophils against microbial invasion, cancer, and the exposure to pollutants. Given the role of iron in the Fenton reaction, which is implicated in the formation of hydroxyl radicals, free iron (Fe^{2+}) is a critical factor related to toxicity induced by ROS generation (Abdal Dayem et al., 2017).

1.5.1. Internal sources

These can be enzymatic reactions, which serve as a source of free radicals. These include those reactions involved in the respiratory chain, in phagocytosis, in prostaglandin synthesis and in the cytochrome P450 system. Some internal sources of generation of free radicals are mitochondria, xanthine oxidase, phagocytes, reactions involving iron and other transition metals, peroxisomes, arachidonate pathways, exercise, ischaemia / reperfusion, inflammation (Kumar, 2011).

1.5.2. External sources

These include non-enzymatic reactions of the oxygen with organic compounds. Free radicals also, arise in reactions, which are initiated by ionizing radiations. Some external sources of free radicals are cigarette smoke, environmental pollutant, radiations, ultraviolet light, ozone, certain drugs, pesticides, anesthetics and industrial solvents (Kumar, 2011).

1.5.3. Physiological factors

Mental status like stress, emotion etc. and disease conditions are also, responsible for the formation of free radicals (Kumar, 2011).

1.6. Free Radical Damages to Biomolecules

When there is an imbalance between the free radical production (ROS/RNS) and antioxidant defenses, the former will be produced in higher concentrations leading to oxidative stress and nitrosative stress. Since these free radicals are highly reactive, they can damage all the three important classes of biological molecules including nucleic acids, proteins, and lipids (Droge, 2002).

1.6.1. Carbonhydrates

Cells contain numerous sugars that are susceptible to attack by free radicals. These include the sugar moieties on DNA, RNA and proteins, as well as mono- and polysaccharides. Damage to sugars in DNA nucleotides appears to be repaired by fairly typical nucleases (Demple and Levin, 1991) and such damage is not generally considered a critical factor in toxicity. However, free glucose can undergo several oxidation reactions resulting in the formation of advanced glycation end products (AGEs) (Ott et al., 2014). Glucose can either slowly modify proteins to form schiff bases that may undergo further rearrangements, or be reduced to sorbitol, followed by further oxidation. Some of the resultant carbohydrate oxidation products can enolize in the presence of transition metals to become ketoaldehydes that interact with proteins to generate AGEs. These products may further stimulate the generation of ROS in cells by activating an NADPH-oxidase-coupled receptor of AGEs (RAGE) (Ott et al., 2014). AGEs may be involved in diabetic complications (Jomova and Valko, 2011; Rains and Jain, 2011; Ott et al., 2014).

1.6.2. Lipids

The membrane lipids, especially the polyunsaturated fatty acid residues of phospholipids are more susceptible to oxidation by free radicals. Peroxidation of membrane lipids is known to substantially alter the physical properties of lipid bilayers. In particular, the peroxidation alters lipid-lipid interactions, membrane permeability, ion gradients, membrane fluidity, and membrane permeability. The lipid peroxidation is initiated, when any free radical attacks and abstracts hydrogen from a methylene groups (CH_2) in a fatty acid (LH) which results in the formation of a carbon centered lipid radical ($L^{\bullet}$). The lipid radical can react with molecular oxygen to forma lipid peroxyl radical ($LOO^{\bullet}$). The resultant lipid peroxyl radical ($LOO^{\bullet}$) undergo rearrangement via a cyclisation reaction to form endoperoxides, which finally form (MDA) and 4-hydroxyl nonenal (4-HNA), the toxic end products of lipid peroxidation that cause damage to the DNA and proteins. These lipid peroxyl radicals can further propagate the peroxidation process by abstracting hydrogen atoms from the other lipid molecules. Isoprostanes (prostaglandin like substances produced by in the body by the esterfication of arachidonic acid) constitute the important product of lipid peroxidation of arachidonic acid and are considered as the makers of the oxidative lipid damage (Phaniendra et al., 2015).

1.6.3. Proteins

The protein oxidation can be induced by radical species such as $O_2^{\bullet -}$, $OH^{\bullet}$, peroxyl, alkoxyl, hydroperoxyl as well as by the non-radical species such as H_2O_2, O_3, HOCl, singlet oxygen, OONO-. ROS oxidize different amino acids present in the proteins, causing formation of protein–protein cross linkages, results in the denaturing and loss of functioning of proteins, loss of enzyme activity, loss of function of receptors and transport proteins. The sulphur containing amino acids such as methionine and cysteine are more susceptible to oxidation by ROS and are converted to disulphides and methionine sulphoxide respectively. However, in biological systems, only these two

oxidized forms of proteins can be converted back to their native form by two different enzymes namely disulfide reductases and methionine sulfoxide reductases respectively.

The ROS induced oxidative damage of amino acid residues such as lysine, proline, threonine and arginine yields carbonyl derivatives. The presence of carbonyl groups in proteins has been considered as the marker of ROS mediated protein oxidation. The other specific markers of protein oxidation are O-tyrosine (a marker for hydroxyl radical) and 3-nitrotyrosine (a marker for RNS). An increase in the levels of protein carbonyls is observed in a number of pathological conditions such as, alzheimer's disease, parkinson's disease, muscular dystrophy, cataractogenesis, rheumatoid arthritis, diabetes, progeria, atherosclerosis, respiratory dystrous syndrome, werner's syndrome, and ageing (Phaniendra et al., 2015).

1.6.4. RNA

ROS can attack different RNAs produced in the body. The RNAis more prone to oxidative damage than DNA, due to its single stranded nature, lack of an active repair mechanism for oxidized RNA, less protection by proteins than DNA and more over these cytoplasmic RNAs are located in close proximity to the mitochondria where loads of ROS are produced. Indeed, RNA is subjected to more oxidative damage than DNA in humans. 7, 8-dihydro-8-oxoguanosine (8-oxoG) is the most extensively studied RNA damage product and its levels are raised in various pathological conditions like alzheimer's disease, parkinson's disease, atherosclerosis, hemochromastosis and myopathies (Phaniendra et al., 2015).

1.6.5. DNA

ROS can oxidatively damage the nucleic acids. The mitochondrial DNA is more vulnerable to the ROS attack than the nuclear DNA, because it is located in close proximity to the ROS generated place. ROS, most importantly, the $OH^{\bullet}$ radical directly reacts with all components of DNA such as purine and pyrimidine bases, deoxyribose sugar backbone (Halliwell and Gutteridge, 1999.) and causes a number of alternations

including single and double stranded breaks in DNA. The $OH^{\bullet}$ radical abstracts hydrogen atoms to produce a number of modified purine as well as pyrimidine base by-products and DNA^{-} protein cross links. The pyrimidine attack by $OH^{\bullet}$ produces different pyrimidine adducts like thymine glycol, uracil glycol, 5-hydroxydeoxy uridine, 5-hydroxy deoxycytidine, hydantoin and others. The purine adducts formed by hydroxyl radical attack include, 8-hydroxydeoxy guanosine, 8-hydroxy deoxy adenosine, 2,6-diamino-4-hydroxy-5-formamidopyrimidine. The other free radical induced adducts of DNA bases include, 5-formyl uracil, cytosine glycol, 5,6-dihydrothyronine, 5-hydroxy-6-hydro-cytosine, 5-hydroxy-6-hydro uracil, uracil glycol, and alloxan. The major free radical induced adducts of the sugar moiety in DNA include glycolic acid, 2-deoxytetrodialdose, erythrose, 2-deoxypentonic acid lactone, 2-deoxypentose-4-ulose (Dizdaroglu et al., 2002). 8-hydroxy deoxyguanosine is considered as the biomarker of oxidative DNA damage and is involved in mutagenesis, carcinogenesis and ageing. The levels of 8-OHdG are higher in mitochondrial DNA than in nuclear DNA (Barja, 2000).

1.7. Lipid Peroxidation

Lipid peroxidation refers to a process under which free radicals attack lipids containing carbon-carbon double bond(s), especially polyunsaturated fatty acids (PUFAs). The greater the number of double bonds in a fatty acid, the more readily it undergoes peroxidation. Saturated fatty acids such as palmitic and stearic acid, and monounsaturated fatty acids such as oleic acid do not undergo peroxidation. The highly unsaturated fatty acids are very susceptible to peroxidation (Santo et al., 2016).

The two most prevalent reactive species that can affect profoundly the lipids are hydroxyl radical and peroxynitrite. The overall process of lipid peroxidation consists of three steps: initiation, propagation, and termination. The first step in lipid peroxidation by oxygen free radicals is abstraction of a hydrogen atom from the acyl chain to form a lipid radical. The next step is the addition of molecular oxygen to the lipid radical yielding lipid peroxyl radical. When lipid peroxyl radical abstracts a hydrogen atom from one of the adjacent PUFAs, a hydroxyperoxy fatty acid (also, called lipid hydroperoxide) is formed. Transition metals reduce hydroxyperoxy fatty acids yielding

alkoxyl radicals and hydroxy fatty acids. Another possible direction of peroxidation reaction is the generation of complex cyclic compounds via intra-molecular reactions yielding endoperoxides (Santo et al., 2016).

The ultimate direction of peroxidation reactions is the fragmentation of fatty acid carbon chain. Frag-mentation products include some of the most extensively studied products of lipid peroxidation, such as MDA, acrolein, and 4–hydroxy–2–nonenal (HNE). MDA is a naturally occurring end–product of lipid peroxidation that is mutagenic and carcinogenic. It is able to induce large insertions and deletions in the DNA, but base pair substitutions have also, been detected. MDA causes up to a 15–fold increase in mutation frequency compared to background levels (Niedernhofer et al., 2003)

1.8. Antioxidants

Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus, have diverse physiological role in the body. Antioxidant constituents of the plant material act as radical scavengers and helps in converting the radicals to less reactive species. A variety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables and tea, etc. (Mandal et al., 2009)

According to literature, these are "substance that when present in low concentration compared to those of the oxidisable substrates significantly delay or inhibit the oxidation of that substance" (Murthy, 2001).

The antioxidant can also, be defined as "A compound capable of inhibiting oxygen mediated oxidation of diverse substances from simple molecule to polymer and complex bio-systems" (Chi-Tang, 1994).

According to US Food and Drug Administration (FDA), antioxidants are defined as substances used to preserve food by retarding deterioration, rancidity or discoloration due to oxidation (Holliwell and Gutteridge, 1990).

1.8.1. Antioxidant defense systems

To fight excessive production of FR and RM, the organism has built protective systems and mechanisms against their toxic effects. Protection is organized at three levels: (a) systems preventing FR formation, such as inhibitors of enzymes catalyzing FR formation. Included in this category is xanthine oxidase producing superoxide, which can be inhibited by allopurinol, or chelating agents trapping ions of transition metals and eliminating them from their catalytical activity during production of FR. (b) When these primary protective systems are insufficient and FR and RM are already formed, scavengers and trappers of FR come into action and eliminate high reactivity of FR by turning them into nonradical and nontoxic metabolites. These compounds are called antioxidants and they prevent oxidation of biologically important molecules by FR or RM. (c) If protection of the organism fails at this level, then repair systems recognize impaired molecules and decompose them, as it is in case of proteinases at oxidatively modified proteins, lipases at oxidatively damaged lipids, or DNA repair systems at modified DNA bases (Ďuračková, 1998).

From the biological point of view, antioxidants are compounds which at low concentration prevent oxidative damage to molecules by oxidants (free radicals and reactive metabolites), while products of the reaction between oxidant and antioxidant should not be toxic and should not reinvigorate the radical reaction. For newly defined antioxidants, the source of oxidant and the method for detection of antioxidant ability should always be reported (Halliwell and Whiteman, 2004; Gutteridge and Halliwell, 2010).

Antioxidants can be categorized in multiple ways. Based on their activity, they can be categorized as enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants work by breaking down and removing free radicals. The antioxidant enzymes convert dangerous oxidative products to hydrogen peroxide (H_2O_2) and then to water, in a multi-step process in presence of cofactors such as copper, zinc, manganese, and iron. Non-enzymatic antioxidants work by interrupting free radical chain reactions. Few examples of the non-enzymatic antioxidants are vitamin C, vitamin E, plant polyphenol, carotenoids, and glutathione.(Shahidi and Zhong, 2010)

The other way of categorizing the antioxidants is based on their solubility in the water or lipids. The antioxidants can be categorized as water-soluble and lipid-soluble antioxidants. The water-soluble antioxidants (*e.g.* vitamin C) are present in the cellular fluids such as cytosol, or cytoplasmic matrix. The lipid-soluble antioxidants (*e.g.* vitamin E, carotenoids, and lipoic acid) are predominantly located in cell membranes.

The antioxidants can also, be categorized according to their size, the small-molecule antioxidants and large-molecule antioxidants. The small-molecule antioxidants neutralize the ROS in a process called radical scavenging and carry them away. The main antioxidants in this category are vitamin C, vitamin E, carotenoids, and glutathione (GSH). The large-molecule antioxidants are enzymes (SOD, CAT, and GSHPx) and sacrificial proteins (albumin) that absorb ROS and prevent them from attacking other essential proteins (Nimse and Pal, 2015).

The content of individual antioxidants is different in various organs and animal species. For example, the human eye lens contains little SOD and considerable amounts of ascorbic acid, while the rat eye is rich in SOD and has very little ascorbate. In rat peripheral nerve tissues, SOD has an activity of 90 U/mg nerve tissue, but in mice only 1 U/mg nerve tissue (Romero, 1996).

Plasma antioxidant capacity also, varies with age. For example, the concentration of urates increases by 25–30% with age, while ascorbic acid is more abundant in children. In adults, ascorbic acid represents about 15 % of the plasma total antioxidative capacity. Moreover, protein thiols (25 %), albumin (25 %), and vitamin E (5 %) also, contribute to antioxidative capacity. In addition to these main antioxidants, other low-molecular-weight as well as enzyme antioxidants also, participate in plasma antioxidative capacity to a lesser extent (Sies, 1991; Sies, 2001).

A compound is characterized as an effective antioxidant in vivo when it meets the following requirements:

1. it must react with biologically effective reactive metabolites;
2. the product of the reaction of prooxidant + antioxidant must not be more toxic for the organism than the removed prooxidant;

3. the potential antioxidant must be present in the organism at sufficient concentration;
4. the half-life of the antioxidant must be long enough to react with the oxidant (Halliwell and Whiteman, 2004).

Generally, an antioxidant in one system and under certain circumstances need not act as antioxidant under other circumstances and in other systems. An increasing body of evidence from experimental studies as well as clinical practice concerning their effect suggest that antioxidants need not always play a positive role. This fact has to be kept in mind especially during therapeutic administration of these compounds.

1.8.1.1. Exogenous antioxidants

1.8.1.1.1. Vitamins as exogenous antioxidants

1.8.1.1.1.1. Vitamin C (Ascorbic Acid, AA)

Vitamin C, Also, called ascorbic acid, is a powerful water-soluble antioxidant, naturally present in fresh fruits and vegetables. The synthesis of vitamin C occurs in most of the plants and animals from D-glucose or D-galactose. Human body is unable to synthesize vitamin C due to lack of the enzyme L-gulonolactone oxidase, thus it needs to be obtained through food or supplements. Its main antioxidant partners are vitamin E and the carotenoids, besides working along with the antioxidant enzymes.

The main biologically active form of vitamin C is its reduced form, L-ascorbic acid, which is a potent antioxidant due to its high electron-donating power and ready conversion back to its active reduced form. Vitamin C is capable of quenching free radicals cooperatively with vitamin E as well as regenerating the reduced state of vitamin E in membranes and lipoproteins. In addition, it has a role in raising the amount of intracellular GSH, hence protecting protein thiol groups against oxidation. Ascorbic acid can also, act as an important chain breaking antioxidant in the aqueous environment and scavenge $O_2^{\cdot -}$, H_2O_2, $^{\bullet}OH$, HOCl, aqueous peroxyl radicals, and singlet oxygen ($^1O_2$). During its antioxidant mechanism, ascorbate undergoes a two-electron reduction, initially to the relatively stable semi-dehydroascorbyl radical and then to a

relatively unstable dehydroascorbate. The latter can be easily hydrolyzed to diketogulonic acid, which is ultimately broken down to oxalic acid. Dehydroascorbate can be reduced back to ascorbate by either the selenoenzyme TrxR or the non-ezymatic reaction, which uses GSH. It seems that a very small amount of dehydroascorbate is present in plasma probably due to its quick absorption by red blood cells before recycling. Additionally, it has been reported that human subjects with low amounts of vitamin C, have higher contents of lipid peroxides in plasma compared to individuals with high levels of vitamin (Aslani and Ghobadi, 2016).

1.8.1.1.1.2. Vitamin E (α Tocopherol)

Vitamin E is a major lipid phase chain-breaking antioxidant that exists in nature in eight different stereoisomers including α, β, γ, δ tocopherol, and α, β, γ, δ tocotrienol. Tocopherols possess a chromanol nucleus and a phytyl tail, and their various isoforms are different in the number and position of the methyl groups present on the chromanol ring. Tocotrienols have similar structure to tocopherols, except they bear an unsaturated isoprenoid side chain. The α-, β-, γ- and δ-forms of both tocopherols and tocotrienols contain 3, 2, 2 and 1 methyl group, respectively. Alpha-tocopherol is the main bioactive form of vitamin E in humans with high antioxidant potency which is believed to be the main membrane bound antioxidant recruited by the cell. Due to its fat-soluble nature, α-tocopherol safeguards cell membranes from destructive effects of free radicals. Its vital property is the ability to protect against lipid peroxidation by scavenging peroxyl, oxygen, and superoxide anion radicals. Through an antioxidant mechanism, vitamin E can transfer its labile hydrogen to a lipid or lipid peroxyl radical and convert itself to the α-tocopherol radical which is then reduced back to its original form by ascorbic acid. Vitamin E can be found in natural sources including nuts and peanuts, eggs, fruits, cabbage, fish, and unrefined vegetable oils such as corn, cotton seed, wheat germ, and in whole grains (Aslani and Ghobadi, 2016).

1.8.1.1.1.3. Carotenoids (β-carotene)

Carotenoids are pigments of plant or microbial origin. At present, around 600 derivatives of carotenoids are known. Of these, 10%possess the activity of provitamin A and can be metabolized to retinol. Only a small number of carotenoids are present in plasma and tissue in sufficient amount.

Carotenoids can react with singlet oxygen and return the molecule of the excited oxygen into the basic energetic state. Due to this property, b-carotene is an important in vitro antioxidant that is part of a group referred to as quenchers. Carotenoids can Also, directly trap free radicals (Krinsky, 1993). Of the biologically important natural carotenoids, the most efficient quencher is lycopene (Devasagayam et al., 1992). Vitamin A exerts only a negligible antioxidative ability (Sundquist et al., 1994).

1.8.1.1.2. Synthetic antioxidants

Synthetic antioxidants have been developed to have a standard antioxidant activity measurement system and to compare with natural antioxidants that are incorporated into food. Synthetic antioxidants are added to food so that it can withstand various treatments and conditions to prolong shelf life and prevention of food oxidation, especially fatty acids. It has been reported that synthetic antioxidants are added to almost all processed foods, which are reported to be safe, although some studies oppose this fact.

The important synthetic antioxidants are BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole). The European Food Safety Authority (EFSA) between 2011 and 2012 classified a NOAEL (No Observable Adverse Effect Level) of 0.25 mg/kg BW/day for BHT and 1.0 mg/kg BW/day for BHA in terms of daily intake and admitted that the exposure of adults and children was unlikely to exceed these doses. TBHQ (*tert*-butylhydroquinone) stabilizes and preserves the freshness, nutritive value, flavor, and color of animal food products. Octyl gallate is considered as safe to use as a food additive because, after consumption, it is hydrolyzed into gallic acid and octanol, which are found in many plants and do not pose a threat to human health

(Joung et al., 2004). NDGA (nordihydroguaiaretic acid) despite being a food antioxidant is known to cause renal cystic disease in rodents (Evan and Gardner, 1979).

1.8.1.1.3. Exogenous antioxidants used as drugs

- Iron chelators and cytokines
- Endogenous antioxidant activity enhancers (ebselen and acetyl cysteine, which increase the activity of GSH-Px)
- Xanthine oxidase inhibitors (Allopurinol, Oxypurinol, Pterin aldehyde, Tungsten)
- NADPH oxidase inhibitors (Adenosine, Local anesthetics, Nonsteroidal anti-inflammatory drugs)
- Nonenzymatic free radical scavengers (Mannitol, Albumin)
- Recombinant superoxide dismutase
- Trolox-C (Vitamin E analogue) (Bayramoğlu, 2008)

1.8.1.2. Endogenous (natural) antioxidants

1.8.1.2.1. Enzymatic antioxidants

Enzymatic antioxidants are categorized into primary and secondary enzymatic defenses. Primary defense is composed of three important enzymes that prevent the formation or neutralize free radicals. Glutathione peroxidase, which donates two electrons to reduce peroxides by forming selenols and also, eliminates peroxides; catalase, which converts hydrogen peroxide into water and molecular oxygen and SOD, which converts superoxide anions into hydrogen peroxide as a substrate for catalase (Rahman, 2007).

Glutathione reductase and glucose-6-phosphate dehydrogenase are involved in the secondary enzymatic defense system. Glutathione reductase reduces glutathione (antioxidant) from its oxidized form to its reduced form, thereby recycling itself to continue neutralizing more free radicals. Glucose-6-phosphate regenerates NADPH (a

coenzyme used in anabolic reactions) creating a reducing environment (Gamble and Burke, 1984). These two enzymes do not neutralize free radicals directly but have supporting roles to other endogenous antioxidants.

Glutathione peroxidase, catalase, and SOD metabolize toxic oxidative intermediates and therefore form the primary antioxidant enzymes. These form the body's endogenous defense mechanism and help protect against free radical-induced cell damage. For optimum catalytic activity, these enzymes Also, require co-factors such as selenium, iron, copper, zinc, and manganese. It has been indicated that an inadequate dietary intake of these trace minerals may compromise the effectiveness of these antioxidant defense mechanisms. The consumption and absorption of important trace minerals may decrease with aging (Mehta and Gowder, 2015).

1.8.1.2.1.1. Superoxide dismutase (SOD)

SOD (EC 1.15.1.1) is the antioxidant enzyme that catalyzed the dismutation of the highly reactive superoxide anion to O_2 and to the less reactive species H_2O_2. Peroxide can be destroyed by CAT or GPX reactions.

$$O_2^- + O_2^- + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2 \qquad (1.9)$$

In humans, there are three forms of SOD: cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD (EC-SOD). SOD destroys $O_{2\cdot}$ by successive oxidation and reduction of the transition metal ion at the active site in a Ping Pong type mechanism with remarkably high reaction rates. All types of SOD bind single charged anions such as azide and fluoride, but distinct differences have been noted in the susceptibilities of Fe^-, Mn^- or Cu/Zn-SODs. Cu/Zn-SOD is competitively inhibited by N_3^- , CN^-, and by F^- .

Mn-SOD is a homotetramer (96 kDa) containing one manganese atom per subunit those cycles from Mn (III) to Mn (II) and back to Mn (III) during the two step dismutation of superoxide. The respiratory chain in mitochondria is a major source of oxygen radicals. Mn-SOD has been shown to be greatly induced and depressed by

cytokines but is only moderately influenced by oxidants. Inactivation of recombinant human mitochondrial Mn-SOD by peroxynitrite is caused by nitration of a specific tyrosine residue.

The biological importance of Mn-SOD is demonstrated among others by the following observations: (a) inactivation of Mn-SOD genes in *Escherichia coli* increases mutation frequency when grown under aerobic conditions ; (b) elimination of the gene in *Saccharomyces cerevisiae* increases its sensitivity to oxygen, (c) lack of expression in Mn-SOD knockout mice result in dilated cardiomyopathy and neonatal lethality; (d) tumor necrosis factor (TNF) selectively induces Mn-SOD, but not Cu/Zn- SOD, CAT or GPX mRNA in various mouse tissues and cultured cells; (e) transection of Mn- SOD cDNA into cultured cells rendered the cells resistant to parquet, TNF and Adriamycin-induced cytotoxicity, and radiation induced-neoplastic transformation; f) expression of human Mn-SOD genes in transgenic mice protects against oxygen induced pulmonary injury and Adriamycin-induced cardiac toxicity.

Cu/Zn-SOD (SOD-1) is another type of enzymes that has been conserved throughout evolution. These enzymes have two identical subunits of about 32 kDa, although a monomeric structure can be found in a high protein concentration from *E. coli*. Each subunit contains a metal cluster, the active site, constituted by a copper and a zinc atom bridged by a histamine residue.

Cu/Zn-SOD is believed to play a major role in the first line of antioxidant defense. Calves that were fed milk supplemented with 25 ppm Cu and 100 ppm Zn showed a stronger immune response and a higher SOD activity. Other recent reports involving SOD knockouts have revealed that Mn- SOD is essential for life whereas Cu/Zn-SOD is not. Cu/Zn-SOD knock-out mice appear normal and exhibit differences only after traumatic injury, whereas Mn-SOD knockouts do not survive past 3 weeks of age. Among various human tissues Mn-SOD contents were roughly one-half as large as the Cu/Zn-SOD contents. Extracellular superoxide dismutase (EC-SOD) is a secretory, tetrameric, copper and zinc containing glycoprotein; with a high affinity for certain glycosaminoglycans such as heparin and heparin sulphate. EC-SOD was found in the interstitial spaces of tissues and Also,in extracellular fluids, accounting for the majority of the SOD activity in plasma, lymph, and synovial fluid. EC-SOD is not induced by its

substrate or by other oxidants and its regulation in mammalian tissues primarily occurs in a manner coordinated by cytokines, rather than as a response of individual cells to oxidants (Krishnamurthy and Wadhwani, 2012).

1.8.1.2.1.2. Glutathione peroxidase (GPx)

The enzyme glutathione peroxidase (GPx) occurs in two forms: selenium dependent and selenium-independent GPx, differing in the number of subunits, in the selenium bond in the active center, and in the catalytical mechanism.

Selenium-independent GPx (glutathione S-transferase, GST) catalyzes detoxification of various xenobiotics. The selenium atom with oxidative number (II) which is present in the enzyme molecule does not participate in the catalytical mechanism (Holovska´, 1998). *Selenium-dependent glutathione peroxidase (GPx)* is composed of four subunits, while each subunit contains one selenium atom in the active center bound in the modified amino acid selenocysteine.

All GPx can reduce peroxides by two electrons producing selenols ⁻Se⁻OH. In the second stage of the catalytical cycle, selenols are reduced by two GSH back to –SeH. The significance of these selenoenzymes is based on elimination of peroxides – potential substrates for Fenton-type reaction. In addition, selenols react faster than thiols. During redox reactions, they transfer two electrons preventing the formation of the superoxide from the oxygen molecule. For the formation of superoxide, only one electron is required. A high GPx activity was detected in the liver; medium activity in the heart, lungs, and brain; and low activity in muscles (Ďuračková, 1998).

Glutathione peroxidase cooperates with the tripeptide glutathione (GSH) present in cells at relatively high (millimole) concentration. The substrate for GPx reaction is H_2O_2 or an organic peroxide. Glutathione peroxidase decomposes peroxides to water or alcohol and at the same time it oxidizes GSH (reaction 10 and 11). It is supposed that GSH reduces selenium in GPx and this reduced form of enzyme catalyzes decomposition of the hydrogen peroxide:

$$2\mathrm{GSH} + \mathrm{H_2O_2} \longrightarrow \mathrm{GSSG} + 2\mathrm{H_2O} \qquad (1.10)$$

$$2GSH + ROOH \longrightarrow GSSG + ROH + H_2O \quad (1.11)$$

The majority of glutathione present in cells is mostly in the reduced form (GSH) compared to the oxidized form (GSSG). A part of the total glutathione in cells is present in the form of "mixed" glutathione

$$\begin{array}{l} Y - glu - cys - gly \\ \quad\quad\quad\quad\quad\quad | \\ \quad\quad\quad\quad\quad\quad S - S - R \end{array}$$

where –R can be the cysteine residue, coenzyme A, or a protein containing –SH groups (Halliwell and Gutteridge, 2007). The ratio GSH: GSSG is 10–100:1 (Devlin, 2006). In cells, the enzyme glutathione reductase (GR) catalyzes GSSG reduction to GSH (Eq.12). Glutathione reductase can reduce not only GSSG but Also,"mixed" disulfides (GSSR). The cofactor of GR is NADPH, produced in the pentose cycle by glucose-6-phosphate dehydrogenase:

$$GSSG + NADPH + H^+ \longrightarrow 2GSH + NADP^+ \quad (1.12)$$

Glutathione peroxidase activity depends on the concentration of GSH. Therefore, its physiological activity requires sufficient concentration of glutathione.

1.8.1.2.1.3. Glutathione S-transferase (GST)

Glutathione Stransferases constitute a large family of multifunctional enzymes involved in GSH conjugation to xenobiotics and aldehydic products of lipid peroxidation (such as 4-hydroxyalkenals). Conjugates formed in hepatic cells are often excreted into bile using ATP-dependent pumps; they can also, be degraded and acetylated to form N-acetylcysteine conjugates (mercapturic acids), which are excreted in the urine.

GSTs are also, very important for the biogenesis of prostaglandins and leukotrienes. GSTs are widely distributed in all life-forms including bacteria, fungi, plants, and animals. All animals investigated to date have GST homo- or heterodimers that are active in the cytosol or nucleus. Mammalian liver is particularly rich in GST activity; 5 to 10% of its cytosolic protein is GST!

Structural and functional studies on vertebrate GSTs have divided the several isoenzymes in four major classes: alpha (α), mu (μ), pi (π), and theta (θ) (two minor classes are sigma (σ) and kappa). These classes are based upon N-terminal amino acid sequences, substrate specificities, sensitivity to inhibitors, isoelectric point, and immunological analysis. Eric Boyland and co-workers from England discovered the existence of multiple forms of GST in the 1960s. Most GSTs have molecular masses of 40 to 55 kDa. Measurement of "total GST activity" is based on the reaction of GSH with 1-chloro-2,4-dinitrobenzene (CDNB), forming S-(2,4-dinitrophenyl)-glutathione, which is quantified at 340 nm. This assay, however, does not measure the θ class of GSTs because of their lack of reactivity toward CDNB.

Glutathione S-transferases also, display selenium-independent GPx activity, designated as GST–Px, which is measured using the cumene hydroperoxide assay. Endogenous substrates of GST–Px include fatty acid hydroperoxides, Ch-OOH, and thymine hydroperoxide (formed upon oxidation of thymine), but not H_2O_2. The GST–Px activity is differentially distributed among the GST classes, being highest for GSTθ, moderate for GSTα, and low for the other isoforms.

Elegant studies in the late 1970s from Helmut Sies' laboratory in Germany showed that GST–Px activity is relevant in the detoxification of organic hydroperoxides in mammals. They studied GSSG efflux from rat liver under oxidative stress. When GSSG accumulates in the liver (due to cGPx-mediated oxidation of GSH to GSSG), it is pumped out into the circulation. The authors infused H_2O_2 into isolated livers from rats that had been fed a selenium-deficient diet (which severely depletes cGPx activity) and detected no GSSG efflux. However, significant GSSG efflux was observed from these livers when they infused tert-butylhydroperoxide (t-BOOH), which can be metabolized by GST–Px (reaction (13)).

$$t - BOOH + 2GSH \longrightarrow t - BOH + H_2O + GSSG \quad (1.13)$$

Glutathione S-transferases are very important in cancer resistance because many endogenously produced aldehydes and many P450-activated xenobiotic drugs can damage DNA. For example, a GSTα found in mice catalyzes the detoxification of exo-8,9-epoxide (formed by P450 activation of aflatoxin B1, a potent hepatocarcinogen found in badly stored peanuts), but rats do not express an enzyme with corresponding activity. This probably accounts for the much greater sensitivity to aflatoxin B1-induced hepatocarcinogenesis of rats compared with mice. Induction of GSTs has Also,been linked with resistance of cancer cells toward chemotherapy because many anticancer drugs (or their P450-activated forms) are GST substrates. Tremendous efforts have been made to develop rationally designed GST inhibitors for adjuvant treatment during chemotherapy. One candidate for clinical use is the diuretic drug, ethacrynic acid, a good inhibitor of most GST isoforms (Hermes-Lima, 2004).

1.8.1.2.1.4. Catalase (CAT)

Catalase (H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6) is a heme- containing enzyme that is present in virtually all aerobic organisms tested to date. In the cell, it is localized predominantly in the peroxisomes, where it is important in the removal of H_2O_2 generated by oxidases involved in β- oxidation of fatty acids, respiration, and purine catabolism. CATs from many species are known to be tetramers of 60- 65 kDa subunits with each subunit containing 1 Fe- protoheme IX moiety (4 heme groups per tetramer). Each tetrameric molecule of mammalian CATs contains four molecules of tightly bound NADPH, which does not seem to be essential for the enzymatic conversion of H_2O_2 to H_2O and O_2, but rather protects CAT against inactivation by H_2O_2 CAT has the highest turnover rate among all enzymes, one molecule of CAT can convert approximately 6 million molecules of H_2O_2 to H_2O and O_2 per minute and the pH optimum obtained from different sources is 6.8- 7.5. The enzyme can function in 2 ways: α and β phases.

The α- phase works catalytically (reactions 1.14, 1.15), breaking H_2O_2 down into H_2O and O_2 without the production of free radicals. The reaction takes place in two two- electron reactions. In the first, a H_2O_2 molecule oxidizes the heme to compound I (CI), removing one oxidation equivalent from the ferric iron, generating the oxoferryl species, and the other from the porphyrin ring, generating a porphyrin cation radical. The second H_2O_2 then reduces CI to regenerate the resting (ferric) enzyme while releasing H_2O and molecular O_2.

$$\mathrm{CAT(Por - Fe^{3+}) + H_2O_2 \longrightarrow CI(Por^{+\cdot} - Fe^{4+} = O) + H_2O} \quad (1.14)$$

$$\mathrm{CI(Por^{+\cdot} - Fe^{4+} = O) + H_2O_2 \longrightarrow CAT(Por - Fe^{3+}) + H_2O + O_2} \quad (1.15)$$

At limiting H_2O_2 concentrations, catalases may undergo a one- electron reduction (reactions 1.16, 1.17) to an inactive intermediate, compound II (CII), which can be subsequently converted to another inactive form, compound III (CIII) (Vašková et al., 2012).

$$\mathrm{CI(Por^{+\cdot} - Fe^{4+} = O) + HA \longrightarrow CII(Por - Fe^{4+} - OH) + A^{\cdot}} \quad (1.16)$$

$$\mathrm{CII(Por - Fe^{4+} - OH) + H_2O_2 \longrightarrow CIII(Por - Fe^{2+} = OOH) + H_2O} \quad (1.17)$$

The β phase works peroxidatively (reactions 1.18- 1.20), by eliminating H_2O_2 with oxidizing alcohols, formate (RH_2) or nitrate as described in Aksoy et al. (Aksoy et al., 2004), thereby releasing O2- and the natural enzyme.

$$\mathrm{CI + NH_3(or\ RH_2 + H_2O_2) \longrightarrow CII + N_3^{\cdot}(or\ R + 2H_2O)} \quad (1.18)$$

$$\mathrm{CII + H_2O_2 \longrightarrow CIII + H_2O} \quad (1.19)$$

$$\mathrm{CII \longrightarrow CAT - Fe^{3+} + O_2^{\cdot -}} \quad (1.20)$$

1.8.1.2.1.5. Glutathione reductase (GR)

Glutathione reductase (GR, EC 1.6.4.2) is a ubiquitous enzyme, which catalyzes the reduction of oxidized glutathione (GSSG) to glutathione (GSH). GR is essential for the glutathione redox cycle that maintains adequate levels of reduced cellular GSH. GSH serves as an antioxidant, reacting with free radicals and organic peroxides, in amino acid transport, and as a substrate for the glutathione peroxidases and glutathione S transferases in the detoxification of organic peroxides and metabolism of xenobiotics, respectively (Dolphin et al., 1989).

This homodimeric enzyme is a member of the family of flavoprotein disulfide oxidoreductases. Each subunit has four domains; beginning at the N-terminus: a FAD-binding domain, an NADPH-binding domain, a central domain, and an interface domain. The active site of GR is at the dimeric interface. Since the GSSG binding site is composed of residues from both subunits, only the dimeric form is active (Bashir et al., 1995)

$$GSSG + NADPH + H^+ \xrightarrow{\text{Glutahions Reductase}} 2GSH + NADP^+ \qquad (1.21)$$

Oxidized glutathione is reduced by a multi-step reaction in which GR is initially reduced by NADPH forming a semiquinone of FAD, a sulfur radical and a thiol. The reduced GR (GR_{red}) reacts with a molecule of GSSG, resulting in a disulfide interchange, which produces a molecule of GSH and the GR_{red}-SG complex. An electron rearrangement in GR_{red}-SG results in a second disulfide interchange, splitting off the second molecule of GSH and restoring the GR to the oxidized form (Massey and Williams, 1965).

1.8.1.2.2. Non-enzymatic antioxidants

These antioxidants are quite a few, namely vitamins (A, C, E, and K), enzyme cofactors (Q10), minerals (Zn, Se, etc.), organosulfur compounds (allium and allium sulfur), nitrogen compounds (uric acid), peptides (glutathione), and polyphenols (flavonoids and phenolic acid).

1.8.1.2.2.1. Glutathione (GSH)

Tripeptide glutathione (GSH) plays a significant role in protection of the organism against oxidative damage for several reasons: (1) It is a cofactor for some enzymes participating in detoxification mechanisms of oxidative stress, as, for example, glutathione peroxidase, glutathione transferase, and dehydroascorbate reductase. (2) GSH is a direct trapper of $^{\bullet}OH$ radical and $^{1}O_2$, and it detoxifies H_2O_2 and lipoperoxides during catalytical action of GPx. (3) Glutathione can reduce the tocopheryl radical directly or indirectly during the reduction of semidehydroascorbate to ascorbate regenerating these important antioxidants back to their active form. GSH plays an important role as an antioxidant in intracellular space and indirectly affects the antioxidative status Also,in extracellular space and membranes where in cooperation with α-tocopherol, it can inhibit lipoperoxidation (Maxwell and Lip 1997; Ďuračková, 1998; Banhegyi et al., 2003).

Oxidized glutathione is regenerated by GR cooperating with NADPH which is produced in the pentose cycle of glucose degradation. The most important source of GSH is the liver where 90 % of the circulating GSH is synthesized de novo. The intracellular concentration of GSH is 500 times higher than its extracellular concentration; thus, GSH has an important function in detoxification processes in the cell and also, has the function of “redox buffer.” Moreover, the correct ratio of GSH/GSSG significantly contributes to the total redox state of the cell. Since many proteins participating in the signaling pathways such as transcription factors and

receptors have thiols groups in the active sites, their function can be influenced by the redox state of the cell. At increased levels of GSSG, thiol groups of proteins can form mixed disulfides, thus changing their physiological function. In this regard, GSSG appears to act as a nonspecific signaling molecule (Valko et al., 2006).

1.8.1.2.2.2. Melatonin

(N-acetyl-5-methoxytryptamine) is an indoleamine neurohormone derived from tryptophan which is predominantly produced by the pineal gland of vertebrates. The secretion of melatonin occurs in a circadian pattern and its highest release is during night time. Melatonin is a multi-functional and evolutionary conserved molecule that acts as a unique powerful antioxidant fighting against oxidative damage.

There are evidences suggesting that melatonin is able to protect cell membranes against lipid peroxidation two-fold as effective as vitamin E, and it is 5 times superior to GSH in scavenging $^{\bullet}OH$. Moreover, in vivo studies have shown that melatonin has powerful antioxidant properties even more than vitamin C, vitamin E, and β-carotene and is also, superior to garlic oil, which helps reduce oxidative stress via a number of ways in the body. Both melatonin and a number of its metabolites are able to detoxify free radicals and their derivatives. It can help protect antioxidant enzymes against oxidative damage. Additionally, melatonin can stimulate various enzymatic antioxidants such as SOD, GPx, GR, and CAT as well as GCS- the rate-limiting enzyme in GSH synthesis- thereby enhancing the amount of intracellular GSH. It has been shown that melatonin is able to increase the efficiency of mitochondrial ETC and, as a result, to reduce electron leakage and free radical (Aslani and Ghobadi, 2016.).

1.8.1.2.2.3. Urate

At normal plasma concentration, urate cleans hydroxyl, superoxide, peroxide radicals and singlet oxygen. But it has no effect on lipid radicals. It also, has an inhibitory effect on vitamin C oxidation.

1.8.1.2.2.4. Bilirubin

Bilirubin is more than just the final product of heme catabolism. Today it is considered to be a fundamental substance which acts as an antioxidant and anti-inflammatory agent in the serum. It can neutralize free radicals and prevent peroxidation of lipids. In addition, there is evidence that it protects the cardiovascular system, neuronal systems, the hepatobiliary system, the pulmonary system and the immune system. Recently the use of pharmacological agents which augment expression of Heme oxygenase 1 (HO-1) has been investigated. Consequently, its metabolites such as carbon monoxide (CO), biliverdin (BV) and bilirubin (BR) could become parts of a therapeutic strategy for treatment of various inflammatory illnesses (Otero Regino et al., 2009).

1.8.1.2.2.5. Albumin

Albumin is a highly soluble protein present in human plasma at normal concentrations between 35 and 50 g/l. Albumin has several important physiological and pharmacological functions. It transports metals, fatty acids, cholesterol, bile pigments, and drugs. It is a key element in the regulation of osmotic pressure and distribution of fluid between different compartments. In normal conditions, its half-life is about 20 days, and its plasma concentration represents equilibrium not only between its synthesis in the liver and its catabolism, but also, its transcapillary escape. In general, albumin represents the major and predominant antioxidant in plasma, a body compartment known to be exposed to continuous oxidative stress. A large proportion of total serum antioxidant properties can be attributed to albumin (Roche et al., 2008). And it Binds copper, heme, and scavenges HOCI (Gutteridge, 1995).

1.8.1.2.2.6. Ferritin

Ferritin binds the iron in tissue.

1.8.1.2.2.7. Transferrin and lactoferrin

The Iron transport protein transferrin is usually one-third loaded with iron and keeps the concentration of free iron in plasma effectively nil. Iron Bound to transferrin will not participate in radical reactions, and the available iron-binding capacity gives it a powerful antioxidant property towards iron-stimulated radical reactions (Gutteridge et al., 1981). Similar Considerations apply to lactoferrin, which, like transferrin, can bind 2 mol of iron per mol of protein but retain its own iron at pH values as low as 4.0. (Gutteridge, 1995.).

1.8.2. Mechanism of action of antioxidants

Two principle mechanisms of action have been proposed for antioxidants. The first is a chain- breaking mechanism by which the primary antioxidant donates an electron to the free radical present in the systems. The second mechanism involves removal of ROS/reactive nitrogen species initiators (secondary antioxidants) by quenching chain-initiating catalyst. Antioxidants may exert their effect on biological systems by different mechanisms including electron donation, metal ion chelation, co-antioxidants, or by gene expression regulation (Lobo et al., 2010).

1.8.2.1. Levels of antioxidant action

The antioxidants acting in the defense systems act at different levels such as preventive, radical scavenging, repair and de novo, and the fourth line of defense, i.e., the adaptation.

The first line of defense is the preventive antioxidants, which suppress the formation of free radicals. Although the precise mechanism and site of radical formation *in vivo* are not well elucidated yet, the metal-induced decompositions of hydroperoxides and H_2O_2 must be one of the important sources. To suppress such

reactions, some antioxidants reduce hydroperoxides and H_2O_2 beforehand to alcohols and water, respectively, without generation of free radicals and some proteins sequester metal ions. GPx, GST, phospholipid hydroperoxide glutathione peroxidase (PHGPX), and peroxidase are known to decompose lipid hydroperoxides to corresponding alcohols. PHGPX is unique in that it can reduce hydroperoxides of phospholipids integrated into biomembranes. GPx and CAT reduce H_2O_2 to water.

The second line of defense is the antioxidants that scavenge the active radicals to suppress chain initiation and/or break the chain propagation reactions. Various endogenous radical-scavenging antioxidants are known: some are hydrophilic and others are lipophilic. Vitamin C, uric acid, bilirubin, albumin, and thiols are hydrophilic, radical-scavenging antioxidants, while vitamin E and ubiquinol are lipophilic radical-scavenging antioxidants. Vitamin E is accepted as the most potent radical-scavenging lipophilic antioxidant.

The third line of defense is the repair and *de novo* antioxidants. The proteolytic enzymes, proteinases, proteases, and peptidases, present in the cytosol and in the mitochondria of mammalian cells, recognize, degrade, and remove oxidatively modified proteins and prevent the accumulation of oxidized proteins.

The DNA repair systems also, play an important role in the total defense system against oxidative damage. Various kinds of enzymes such as glycosylases and nucleases, which repair the damaged DNA, are known.

There is another important function called adaptation where the signal for the production and reactions of free radicals induces formation and transport of the appropriate antioxidant to the right site (Lobo et al., 2010).

1.9. Serum Enzymes

1.9.1. Transaminases or aminotransferases (ALT, AST)

The aminotransferases (formerly called transaminases) catalyze transfer of the α-amino group of aspartate (aspartate aminotransferase, AST) or alanine (alanine aminotransferase, ALT) to the α-keto group of ketoglutarate, with pyridoxal phosphate (vitamin B6) as a cofactor (Berk and Korenblat, 2012).

Aspartate aminotransferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2) are enzymes found mainly in the liver, but also, found in red blood cells, heart cells, muscle tissue and other organs, such as the pancreas and kidneys (Huang et al., 2006). ALT is a purely cytosolic enzyme. Distinct isoforms of AST are present in cytosol and mitochondria. Expression of the mitochondrial isoform and its physiologic export from the hepatocyte are upregulated by ethanol (Berk and Korenblat, 2012).

AST or ALT levels are a valuable aid primarily in the diagnosis of liver disease. Although not specific for liver disease, it can be used in combination with other enzymes to monitor the course of various liver disorders. The normal concentrations in the blood are from 5 to 40 U l^{-1} for AST and from 5 to 35 U l^{-1} for ALT. However, when body tissue or an organ such as the liver or heart is diseased or damaged, additional AST and ALT are released into the bloodstream, causing levels of the enzyme to rise. Therefore, the amount of AST and ALT in the blood is directly related to the extent of the tissue damage. After severe damage, AST levels rise 10 to 20 times and greater than normal, whereas ALT can reach higher levels (up to 50 times greater than normal). On the other hand, the ratio of AST to ALT (AST/ALT) sometimes can help determine whether the liver or another organ has been damaged.

AST and ALT are Also, biological catalyst. Therefore, the assay of AST and ALT activity all based on the following enzyme reactions included original (Eq. (1.22) and (1.23)) and succeeding (Eq. (1.24)-(1.28)) reactions.

$$\mathrm{L-aspartate+\ \alpha-ketoglutarare\ \xrightarrow{GOT}\ oxalacetate+L-glutamate} \quad (1.22)$$

$$\mathrm{L-alamine+\ \alpha-ketoglutarare\ \xrightarrow{GPT}\ pyruvate+L-glutamate} \quad (1.23)$$

$$\mathrm{oxalacetate\ \xrightarrow{OAC}\ pyruvate+\ CO_2} \quad (1.24)$$

$$\mathrm{pyruvate+\ O_2+phosphate\ \xrightarrow{POP}\ acetylphosphate+\ CO_2+\ H_2O_2} \quad (1.25)$$

$$\mathrm{L-glutamate+\ O_2\ \xrightarrow{GLOX}\ \alpha-oxogluatrate+NH_3+\ H_2O_2} \quad (1.26)$$

$$\mathrm{oxalacetate+NADH+\ H^+\ \xrightarrow{PH7.8}\ MALATE+\ NAD^+} \quad (1.27)$$

$$\mathrm{Pyruvate+NADH+\ H^+\ \xrightarrow{PH7.4}\ L-lactate+\ NAD^+} \quad (1.28)$$

Where GlOX is glutamate oxidase, POP is pyruvate oxidase, and OAC is oxalacetate decarboxylase (Huang et al., 2006).

1.9.2. Lactate dehydrogenase (LDH or LD)

Lactate dehydrogenase (LDH) is an enzyme that catalyzes the conversion of pyruvate to lactate, along with NADH to NAD+, during glycolysis in conditions of hypoxia. The LDH enzyme is present in all cells, but it is concentrated in muscle, liver, and kidney. LDH exists as five isozymes, LDH-1 through LDH-5, each composed of four subunits. Differential LDH isozyme levels are used diagnostically (Markert et al., 1975). Isozyme LDH-1 (4H) has four heart subunits and is the major isozyme in heart. Isozyme LDH-2 (3H1M) has three heart and one muscle subunit and is the major isozyme in the macrophage-monocyte system and serum. The LDH-3 (2H2M) isozyme has two heart and two muscle subunits and is the major isozyme in lungs. Isozyme LDH-4 (1H3M) has one heart and three muscle subunits and is the primary isozyme in kidneys. The LDH-5 (4M) isozyme has four muscle subunits and is the major isozyme in liver and skeletal muscle. The concentration of LDH is elevated in serum as a result of organ infarction and significant cell death that results in loss of cytoplasm.

For example, elevations of LDH result from conditions such as hepatitis, shock, hypoxia, extreme hypothermia, and meningitis, among others. The LDH enzyme has often been used in laboratory animals, along with troponin levels, to detect myocardial damage (Evans, 2009). However, LDH stability is highly susceptible to freezing, and values are affected by storage conditions (Mitruka and Rawnsley, 1981).

$$pyruvate + NADH + H^+ \rightleftharpoons Lactate + NAD^+ \qquad (1.29)$$

1.10. Ethanol or ethyl alcohol

Ethanol has been a part of the human diet for centuries. However, its consumption in excess can result in a number of health problems, most notably liver damage so what is the biochemical basis of these health problems.

Ethanol cannot be excreted and must be metabolized, primarily by the liver. This metabolism occurs by two pathways. The first pathway comprises two steps. The first step, catalyzed by the enzyme alcohol dehydrogenase, takes place in the cytoplasm:

$$\underset{\text{Ethanol}}{CH_3CH_2OH} + NAD^+ \xrightarrow{Alcohol\ dehydrogenase} \underset{\text{Acetaldehyde}}{CH_3CHO} + NADH + H^+ \qquad (1.30)$$

The second step, catalyzed by aldehyde dehydrogenase, takes place in mitochondria:

$$\underset{\text{Acetaldehyde}}{CH_3CHO} + NAD^+ + H_2O \xrightarrow{Aldehyde\ dehydrogenase} \underset{\text{Acetate}}{CH_3COO^-} + NADH + H^+ \qquad (1.31)$$

Note that ethanol consumption leads to an accumulation of NADH. This high concentration of NADH inhibits gluconeogenesis by preventing the oxidation of lactate to pyruvate. In fact, the high concentration of NADH will cause the reverse reaction to predominate, and lactate will accumulate. The consequences may be hypoglycemia and lactic acidosis.

The NADH glut Also, inhibits fatty acid oxidation. The metabolic purpose of fatty acid oxidation is to generate NADH for ATP generation by oxidative phosphorylation, but an alcohol consumer's NADH needs are met by ethanol metabolism. In fact, the excess NADH signals that conditions are right for fatty acid synthesis. Hence, triacylglycerols accumulate in the liver, leading to a condition known as "fatty liver."

The second pathway for ethanol metabolism is called the ethanol inducible microsomal ethanol-oxidizing system (MEOS). This cytochrome P450-dependent

pathway generates acetaldehyde and subsequently acetate while oxidizing biosynthetic reducing power, NADPH, to NADP+. Because it uses oxygen, this pathway generates free radicals that damage tissues. Moreover, because the system consumes NADPH, the antioxidant glutathione cannot be regenerated, exacerbating the oxidative stress.

What are the effects of the other metabolites of ethanol? Liver mitochondria can convert acetate into acetyl CoA in a reaction requiring ATP. The enzyme is the thiokinase that normally activates short-chain fatty acids.

$$\text{Acetate} + \text{coenzyme A} + \text{ATP} \longrightarrow \text{acetyl CoA} + \text{AMP} + \text{PP}_\text{i} \qquad (1.32)$$

$$\text{PP}_\text{i} \longrightarrow 2\ \text{P}_\text{i} \qquad (1.33)$$

However, further processing of the acetyl CoA by the citric acid cycle is blocked, because NADH inhibits two important regulatory enzymes: isocitrate dehydrogenase and a-ketoglutarate dehydrogenase. The accumulation of acetyl CoA has several consequences. First, ketone bodies will form and be released into the blood, exacerbating the acidic condition already resulting from the high lactate concentration. The processing of the acetate in the liver becomes inefficient, leading to a buildup of acetaldehyde. This very reactive compound forms covalent bonds with many important functional groups in proteins, impairing protein function. If ethanol is consistently consumed at high levels, the acetaldehyde can significantly damage the liver, eventually leading to cell death.

Liver damage from excessive ethanol consumption occurs in three stages. The first stage: is the aforementioned development of fatty liver. In the second stage: alcoholic hepatitis groups of cells die and inflammation results. This stage can itself be fatal. In stage three: cirrhosis fibrous structure and scar tissue are produced around the dead cells. Cirrhosis impairs many of the liver's biochemical functions. The cirrhotic liver is unable to convert ammonia into urea, and blood levels of ammonia rise. Ammonia is toxic to the nervous system and can cause coma and death. Cirrhosis of the liver arises in about 25% of alcoholics, and about 75% of all cases of liver cirrhosis are the result of alcoholism. Viral hepatitis is a nonalcoholic cause of liver cirrhosis (Berg, 2002).

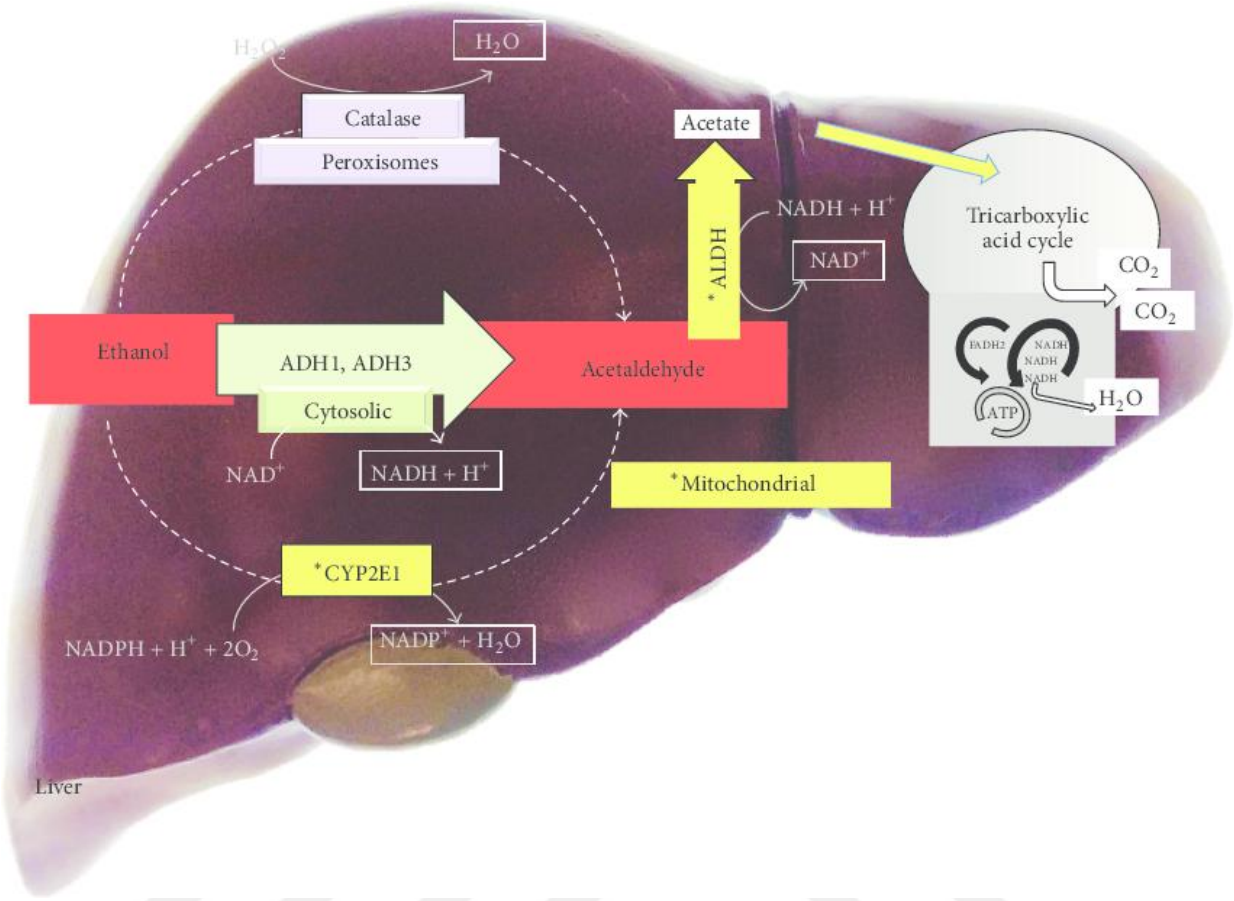


Figure 1. 2. Mechanisms of ethanol metabolism in the liver. Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) are the main enzymes that convert ethanol to acetate in the liver (Hernández et al.,2016.).

1.11. *Origanum onites L.*

Natural antioxidants are considered to be useful agents for the prevention of diseases. Many studies have shown that phenolic compounds in plant essential oils display antioxidant activity as a result of their capacity to scavenge free radicals. It has been shown that many species of *Origanum* have a high amount of phenolic contents in their essential oils.

The genus *Origanum* is a member of the Lamiaceae family and has a complex taxonomy. On the basis of morphological variation, the genus has been divided into 10 sections consisting of 42 species or 49 taxa (species, subspecies, and varieties). Forty-six Origanum taxa out of 49 are very locally distributed in the Mediterranean area and 21 taxa are local Turkish endemics. *O. onites* is one of the most economically important

species; it belongs to the Majorana section in Turkey and its native distribution is in Western and Southern Anatolia and their islands (Tonk et al., 2010).

Turkey is the principal supplier of oregano. Oregano exports from Turkey amounted to 7,400 tons in 2000, for earnings of U.S.$15,400,000. The commercial *Origanum* species include *O. onites*, *O. vulgare* subsp. *hirtum*, *O. majorana*, *O. minutiflorum* (endemic), and *O. syriacum* var. *bevanii*. In addition, *Thymbra*, *Coridothymus*, and *Satureja* species are Also,collected and exported under the name oregano (Kekik in Turkish) (Azcan et al., 2004).

Use of oregano dates back to ancient times in anatolia. The leaves of the plant have been used as a condiment herb for flavoring fish, meat, vegetables, salad dressing, and wine since approximately the seventh century BC. It was also, of critical importance as a traditional remedy for treatment of a variety of ailments. Nowadays it is used as a spasmodic, antimicrobial, expectorant carminative, and aromatic for whooping and convulsive coughs and digestive disorders, and is also, used topically as an antiseptic and astringent. Its vegetative parts and biochemical extracts are commonly used in the food and spice industry.

Industrial use of essential oils for antimicrobial, antiviral, antifungal, insecticidal, medicinal, and cosmetic purposes has, in recent years, increased studies on plants, for example *O. onites*, from which essential oils are extracted. The essential oil of oregano contains carvacrol and/or thymol as the main component(s) and other minor constituents such as c-terpinene, p-cymene, linalool, terpinen-4-ol, and sabinene hydrate. The phenolic compound carvacrol is the main component of the essential oil of *O. onites*, although thymol and linalool chemotypes are encountered, rarely, in wild populations. Carvacrol is an important impact compound of oregano aroma and the largest component of the active extract of the aroma. Thymol and carvacrol are biosynthesized by aromatization of c-terpinene to p-cymene followed by hydroxylation of p-cymene. Eucalyptol, borneol, b-bisabolene, and three currently unknown compounds from minor constituents were identified as aroma-impact compounds of *O. onites* in a recent study.

Generally, the major components determine the biological properties of the essential oils. The biological activity of oregano mainly results from carvacrol and thymol, and carvacrol has multiple pharmacological actions.

Carvacrol is a phenolic monoterpene isomeric with thymol. While thymol is crystalline, carvacrol is a liquid with thymol-like characteristic odor. It is practically insoluble in water but freely soluble in ethanol and ether. It is the major constituent in the essential oils of plants known and used as "Kekik" in Turkey (Oregano in Greece). In Turkey, such plants are used as condiment to impart a spicy flavor to food or as herbal tea. The carvacrol-rich oils are externally used by rubbing on skin to sooth rheumatic pain. Scientific evidence for such a use has recently appeared in the world literature, when carvacrol was found to inhibit prostaglandin-synthesis which is an important mechanism in pain-killing and anti-inflammatory processes. The distillates of *Origanum, Satureja,* etc., after the oil is removed, are taken orally to cure stomach ulcers (Tonk et al., 2010)

Other reported biological effects of carvacrol are as follows: Antibacterial, antifungal, acetylcholine esterase inhibition, lipid peroxidase inhibition, radical scavenging effect, white blood cell macrophage stimulant and cardiac depressant activity. Due to their antioxidant properties carvacrol-rich oils are recommended for the preservation of food. (Kirimer et al., 1995). Classification of *Origanum onites L.*

Kingdom Plantae – Plants
Subkingdom Tracheobionta – Vascular plants
Superdivision Spermatophyta – Seed plants
Division Magnoliophyta – Flowering plants
Class Magnoliopsida – Dicotyledons
Subclass Asteridae
Order Lamiales
Family Lamiaceae – Mint family
Genus *Origanum* L. – origanum
Species *Origanum onites* L. – pot marjoram

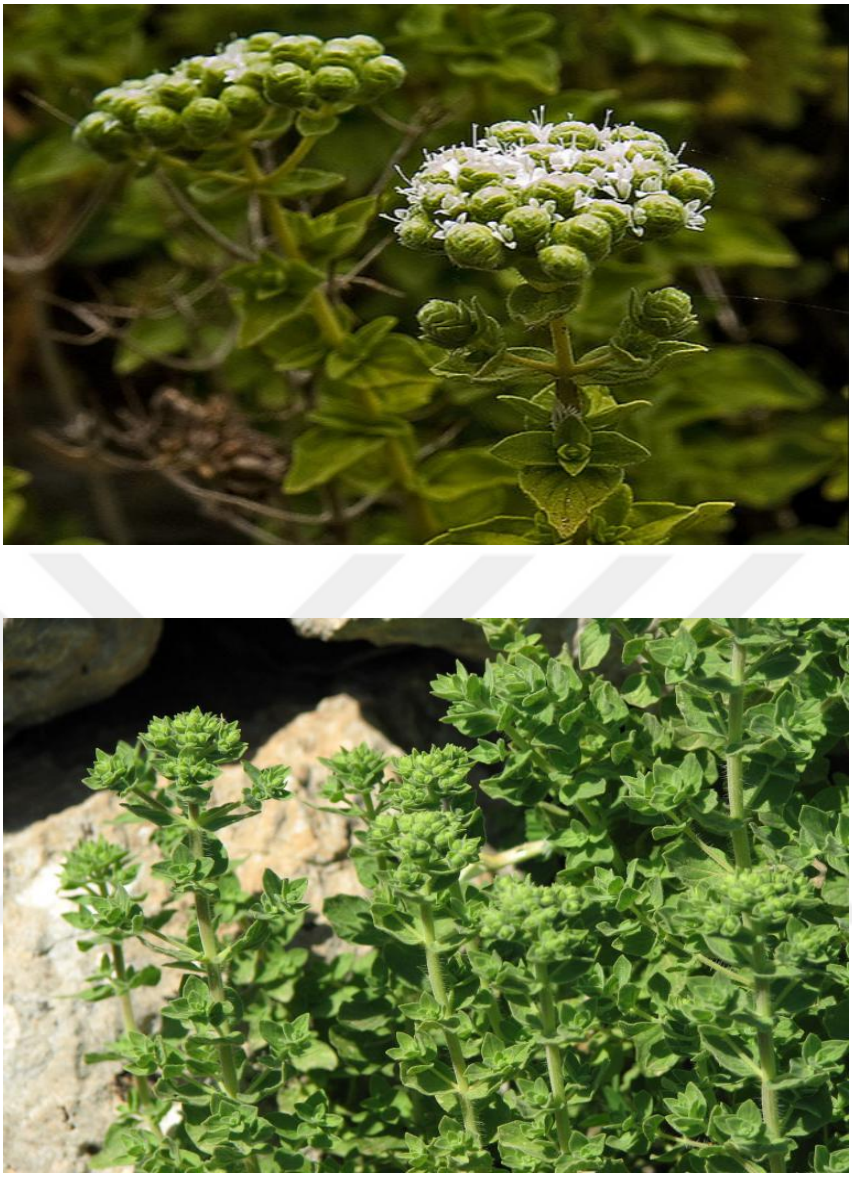


Figure 1. 3. *Origanum onites* L.

1.12. Research Purpose

Because free radicals and other oxygen derived species are produced continuously in biological systems due to stress factors of endogenous and exogenous origin; The cell has developed powerful and complex enzymatic and molecular antioxidant defense systems to limit exposure to these stress factors. Antioxidants are known as agents that protect lipids, carbohydrates, proteins, DNA and other oxidizable substrates from oxidation. Free radicals cause tissue aging and some diseases such as

cancer and cardiovascular diseases. It is therefore essential to protect foods and the human body against these free radicals. The antioxidant defense system in the body forms the natural defense system of the organism. Antioxidants which are taken with nutrition and generally are vitamins. Also, contribute to this defense. If these two groups fail in the defense system or if the presence of free radicals in the body increases above normal this means that the body's antioxidant balance is impaired and this leads to various abnormalities in the body.

O. onites contain important active ingredients in human nutrition and treatment; so, in this study we aim to investigate the hepatoprotective effects of *O. onites* against EtOH induced oxidative stress in rats by measuring the liver damage biomarkers in serum such as ALT, AST enzyme levels and TC and TP content level .

In addition, antioxidant enzymes that can be considered as an indicator of antioxidant capacity activity in liver, kidney and erythrocyte tissue samples include CAT, GST, GPx, GR, SOD activities, GSH level and MDA contents will be determine. The effects of *O. onites* on said biological parameters are important for scientific studies. We believe that the study of thyme in warm-blooded organisms in vivo will fill an important gap in the research field of food biochemistry.

2. LITERATURE REVIEW

Lagouri et al. (1993), tested four samples of essential oils obtained from plant species with a wide distribution in Greece and economic importance for the world-wide spice market and carvacrol and thymol for their possible antioxidant activity. The four-plant species were *Origanum vulgare* subsp, *hirtum, O. onites, Coridothymus capitatus* and *Satureja thymbra.* The essential oils have been chemically characterised by gas chromatography-ion trap detection. All the essential oils examined and also, carvacrol and thymol were found to have antioxidant activity when tested on TLC plates and by measuring peroxide values of lard stored at 35 ~ ^{o}C. and the results indicated that the antioxidant effect may be related to the presence of carvacrol and thymol in essential oils, which may be important in suppressing lipid oxidation.

(Lagouri and Boskou, 1995), screened various essential oils for their antioxidant activity by measuring bleaching of beta-carotene in the coupled oxidation of beta-carotene and linoleic acid. The choice of the plants studied was based on two criteria: a. The plant is known for antioxidant activity. b. There is evidence from the literature that the essential oil contains compounds with a phenolic ring and an electron repelling group. And the results have shown that oils containing carvacrol, thymol and eugenol showed marked antioxidant activity coefficients. Oils from plants of oregano species protected purified lard stored at 35°C. The inhibition of oxidation exhibited was highly carvacrol + thymol content dependent.

Pizzale et al. (2002), tested the antioxidant activity of methanolic extracts of oregano and sage samples. Samples of oregano belonged to *O. onites* and *Origanum indercedens* species, whilst samples of sage belonged to *Salvia officinalis* and *Salvia fruticosa* species. Two methods had been used to evaluate the antioxidant activity of sage and oregano extracts: the crocin test and the Rancimat test. The methanolic extracts had been also analysed by HPLC for the qualitative/quantitative determination of phenolic compounds. The results have shown that the antioxidant activities of sage samples were, on average, higher than those of oregano samples. Some samples of sage

had a very high antioxidant activity, with induction times more than 10-fold higher than that of lard used as the reference sample.

Andoğan et al. (2002), examined the composition and antimicrobial properties of essential oils obtained from *O. onites, Mentha piperita, Juniperus exalsa, Chrysanthemum indicum, Lavandula hybrida, Rosa damascena, Echinophora tenuifolia, Foeniculum vulgare*. To evaluate the *in vitro* antibacterial activities of these eight aromatic extracts; their *in vitro* antimicrobial activities were determined by disk diffusion testing, according to the NCCLS criteria. *Escherichia coli* (ATTC 25922), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATTC 27853 were used as standard test bacterial strains. And our results have shown that *O. onites recorded* antimicrobial activity against all test bacteria and was strongest against *Staphylococcus aureus* and so, they suggested that essential oils have potential use as antimicrobials. Essential oils and main components of some of these oils, such as carvacrol, citronellol, geraniol, and nerol have been previously reported to have antibacterial activity.

Sağdıç (2003) used two thyme (*Thymus vulgaris* L. and *Thymus serpyllum* L.) and three oregano (*Origanum vulgare* L., *O. onites* and *Origanum majorana* L.) hydrosols, which are widely used in food products and as drinks, and tested their inhibitory effects against four pathogenic bacteria (*Escherichia coli* ATCC 25922, E. coli O157:H7 ATCC 33150, *Staphylococcus aureus* ATCC 2392 and *Yersinia enterocolitica* ATCC 1501) as a potential natural antimicrobials because recently there has been an increase in risk in the rate of infections with antibiotic resistant microorganisms. Based on the results of this study, the two thyme and three oregano hydrosols appeared to inhibit the growth of the four pathogens tested. Thyme and oregano hydrosols at 50 and 75mL/100mL concentrations were completely inhibitive on bacterial growth in broth culture and according to the results of this study they suggested the possibility of using thyme and oregano hydrosols in food preservation and drinks.

Zeytinoglu et al. (2003), investigated the effects of carvacrol obtained by fractional distillation of *O. onites* essential oil, on DNA synthesis of *N-ras* transformed myoblast cells, CO25. The results have shown that the incubation of the cells with

different doses of carvacrol prevented DNA synthesis in the growth medium and ras-activating medium, which contains dexamethasone. Therefore, this result demonstrates that carvacrol inhibits growth of myoblast cells even after activation of mutated *N-ras* oncogene, suggesting the possibility that carvacrol may find application in cancer therapy by preventing prenylation of many proteins including Ras.

Baydar et al. (2004), evaluated the chemical composition and the antibacterial properties of the essential oils obtained from the aerial parts of the four Lamiaceae species, *Origanum minutiflorum, O. onites, Thymbra spicata* and *Satureja cuneifolia*, with commercial importance in Turkey. And the results have shown that the major constituent of the oils determined by GC was cavracrol (86.9% in O. onites, 84.6% in O. minutiflorum, 75.5% in T. spicata and 53.3% in S. cuneifolia). Then, the four essential oils were investigated for antibactrial activity against *Aeromonas hydrophila, Bacillus amyloliquefaciens, B. brevis, B. cereus, B. subtilis, Corynebacterium xerosis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Listeria monocytogenes, Micrococcus luteus, Mycobacterium smegmatis, Proteus vulgaris, Staphylococcus aureus* and *Yersinia enterocolitica*, using a paper disc diffusion method. All essential oils inhibited all bacteria at concentrations of <1/100 (v/v). The essential oil of *T. spicata* was the most active. *B. amyloliquefaciens* was the most sensitive. Finally, the results of this study suggested the possibility of using the four essential oils or some of their components as natural food preservatives, because the oils possess strong antibacterial activity.

Ipek et al. (2005), evaluated the genotoxic and antigenotoxic effects of the essential oil of *O. onites* and carvacrol that are used in medicine, flavoring of food and crop protection by Ames Salmonella/microsomal test. The mutagenic activity was initially screened using *Salmonella typhimurium* strains TA98 and TA100, with or without S9 metabolic activation. It was shown that both of them strongly inhibited mutagenicity induced by 4-nitro-o-phenylenediamine and 2-aminofluorene in both strains with or without S9, respectively and these results indicated significant antimutagenicity of the essential oil and carvacrol in vitro, suggesting its pharmacological importance for the prevention of cancer.

Cetin et al. (2006), extracted the essential oils from aerial parts of *O. onites* and fruit peels of *Citrus aurentium* L. were tested at three doses (0.1, 0.5 and 1%) against 4th and 5th instar larvae of the pest (*Thaumetopoea wilkinsoni* Tams). The results showed that the activities were concentration dependent. The LD_{50} and LD_{90} values were 0.288 and 0.926% for *O. onites*, 0.530 and 2.306% for *C. aurentium*, respectively. And they concluded that the plant oils examined in this study offer great potential as new materials against PPM and the results obtained from the study could be useful in the search for new botanical larvicides. The use of the botanical derivatives in PPM control instead of synthetic insecticides could reduce environment effects.

Pari et al. (2008), Performed this study to evaluate the effect of grape leaf extract (GLEt) on antioxidant and lipid peroxidation states in liver and kidney alcohol induced toxicity. The results showed that in vitro studies with DPPH* and ABTS*+ (cation radical) showed that GLEt possesses antioxidant activity and they indicated that GLEt (100 mg/kg body weight) has a protective action against alcohol-induced toxicity as evidenced by the lowered tissue lipid peroxidation and elevated levels of the enzymic and non-enzymatic antioxidants in liver and kidney and hence they suggestd that GLEt play a beneficial role in the treatment of alcohol induced tissue damage, which could be one of its therapeutic values.

Uyanoglu et al. (2008), investigated the possible effects of carvacrol obtained from origanum oil upon the regenerative feature of the liver subsequent to partial hepatectomy in rats. They divided the male Wistar Albino rats, weighing 230730 g, into three experimental groups. Group I (n = 8) were used as sham operation group. Group II (n = 8) were applied saline solution and hepatectomy. Carvacrol and hepatectomy (73 mg/kg) were applied to Group III (n = 8). One dose of test material was injected 1 h before 68% partial hepatectomy and the results have shown that the liver regeneration, mitotic index and PCNA index increased significantly in rats of Group III over the Group II at the 72nd hour after partial hepatectomy. Histological evaluations were also similar with these results of PCNA and mitotic indexes. In AST, ALT, TNF-a and IL-6 levels, there was no statistically significant difference. According to these results, we can conclude that carvacrol increases the liver regeneration rate.

Coskun et al. (2008), studied the essential oils that were extracted by steam distillation from *O. onites* from northwest Turkey were analyzed using an GC-MSD system. Carvacrol was the major component (64.3%) of the oil. Both pure commercial carvacrol and essential oil at concentrations of 1.5%, 3.2%, 6.25%, 12.5%, 25%, 50%, and 100% (v/v) were tested for their effects against engorging *Rhipicephalus turanicus*, which were collected from cattle breeding in the region. And the results have shown that pure carvacrol killed all the ticks following 6 h of exposure, while 25% and higher concentrations of the oil were completely effective in killing the ticks by the 24^{th} hour post-treatment ($p<0.05$). and these findings indicated that the essential oil of *O. Onites* has potential to be utilized at reasonable concentrations to control tick infestations.

Özdemir et al. (2008), investigated the effects of *O. onites* on endothelial function and antioxidative status in 48 patients with mild hyperlipidaemia who required no drug therapy. And the results have shown a significantly greater increase in high density lipoprotein-cholesterol and significantly greater decreases in low density lipoprotein-cholesterol, apolipoprotein B, lipoprotein(a) and highsensitivity C-reactive protein occurred in the study group compared with the control group over the 3-month study period and they concluded tha the consumption of *O. onites* distillate had beneficial effects on lipid profiles, antioxidant status and and flow- and nitroglycerine-mediated dilatation of the brachial artery in patients with mild hyperlipidaemia.

Korukluoglu et al. (2009), essential oils (EOs) and extracts (methanol, acetone and diethyl ether) of fresh and dried *O. Onites* were used to determine the antifungal effect on *Alternaria alternata*, *Aspergillus flavus* (two strains), *Aspergillus niger* (two strains), *Aspergillus parasiticus*, *Fusarium semitectum*, *Fusarium oxysporum*, *Mucor racemosus* and *Penicillium roqueforti* by disk diffusion methods. The results have shown that the antifungal activity of the fresh herb was greater than that of the dried herb. Also, the results suggested that the constituents of *O. onites* have definite potential against fungi. Phenolic, alcoholic and terpenoid constituents could be used for the preservation of raw and processed foods. Therefore, oregano could be an important source of naturally active compounds useful for developing effective antifungal drugs.

De Martino et al. (2009), analysed the essential oils obtained from inflorescences of three *Origanum vulgare* L. ssp. *hirtum* (Link) Ietswaart samples, growing wild in

different locations in Campania (Southern Italy). Three chemotypes had been found: the first, with a prevalence of carvacrol/thymol; the second, characterized by the prevalence of thymol/α-terpineol; the third, featuring a prevalence of linalyl acetate and linalool. This chemical study attempts to provide a contribution in shedding light on the relationship between chemical composition and biotypes and/or chemotypes in *Origanum vulgare* ssp. *hirtum*. The essential oils have been also evaluated for their antibacterial activity against 10 selected microorganisms. The data obtained contribute to the future view to use the essential oils as natural preservatives for food products, due to their positive effect on their safety and shelf life.

Tonk et al. (2010), characterized fourteen Turkish oregano clones chemically and genetically. Essential oil obtained by hydro-distillation was characterized by gas chromatography. Genetic variation was determined by use of random amplified polymorphic DNA (RAPD) markers. Variation of essential oils in the selected clones was subjected to cluster analysis, and the results have shown that two chemotypes, carvacrol and thymol, were identified. Carvacrol was found to be the main component in all clones except clone-661, the main component of which was thymol and they indicated that there is no relationship between genetic structure of the selected clones and essential oil composition.

Özkan et al. (2011), determined the effects of essential oil from Turkish oregano (*O. onites*) and its 2 major phenolic components, carvacrol and thymol, on cell viability, and to investigate their antioxidant effects against hydrogen peroxide-induced cytotoxicity and membrane damage in hepatoma G2 (Hep G2) cells. Their antioxidant properties were also evaluated with 2 in vitro complementary test systems: DPPH radical scavenging activity and linoleic acid oxidation inhibition. The results have shown that the essential oil of *O. onites* decreased cancer cell viability at higher concentrations. The essential oil was found to be less cytototoxic than carvacrol and thymol for Hep G2 cells. On the other hand, with the *O. onites* essential oil, carvacrol and thymol protected the cells against H_2O_2-induced cytotoxicity when the cells were preincubated with the oil and its components at a lower concentration (<IC50) before H_2O_2 incubation.

Economou et al. (2011), studied the variability in essential oil content and composition of *Origanum hirtum* L., *O. onites, Coridothymus capitatus* (L.) and *Satureja thymbra* L. populations from the Greek island Ikaria. All of these four species exhibited high essential oil content, namely *O. onites* 3–4.3%, *S. thymbra* 4–6.5%, *C.* capitatus 3.7–5.6% and *O. hirtum* 5.5–10.0% (v/w). Carvacrol was the main constituent of the essential oils of all species, followed by γ-terpinene, p-cymene and caryophyllene, while thymol was not detected. All constituents varied remarkably among the four species, with carvacrol exhibiting the lowest variation. Carvacrol content varied between 72.3 and 89.2% in *O. onites*; 46.5 and 58.0% in *S. thymbra*; 82.9 and 90.9% in *C. capitatus*; and 84.4 and 93.8% in *O. hirtum*.

Sivas et al. (2011), studied the essential oil of *O. onites* and its phenolic constituent carvacrol were examined for their cytotoxic and apoptotic effects in a human hepatocellular carcinoma cells Hep-G2. And the WST-1 and neutral red uptake assays were performed to determine the inhibitory effects of the oil and carvacrol on the growth of the cells. Possible induction of apoptosis by *Origanum* oil and carvacrol was further investigated by acridine orange/ethidium bromide (AO/EB) staining. And according to the results obtained the *Origanum* oil and carvacrol was significantly cytotoxic and induced apoptosis in Hep-G2 cells. And they concluded that both essential oil and its major constituent carvacrol significantly exhibited cytotoxic and apoptotic activities in hepatocellular carcinoma cells, indicating its potential for use as an anticancer agent.

Bostancıoğlu et al. (2012), investigated the essential oil of *O. onites* (OOEO) for a possible anti-angiogenic activity and their experimental results revealed that three concentrations (125, 250 and 500 µg/ml) of OOEO could markedly inhibit cell viability and induced apoptosis of 5RP7 cells. Also, could block in vitro tube formation and migration of RATEC and because this essential oil possesses much weaker cytotoxicity against endothelial cells while very strong effect against cancer cells, and they concluded that these biological effects are attractive for pursuits of anti-angiogenesis and conventional anticancer activities.

Kotan et al. (2014), made an experiment to determine the antibacterial activity of the essential oil, the extracts and pure metabolites of *O. onites* against plant pathogenic

bacteria and potential use of the extracts against the diseases caused by some phytopathogenic bacteria in vitro and in vivo conditions. The essential oil and the extracts of *O. onites*, and its pure compounds isolated from the acetone extract were individually tested against a total of 14 phytopathogenic bacterial strains. The essential oil and hexane extract contained mainly carvacrol (70.50% and 80.50%), p-cymene (13.97% and 0.96%), thymol (2.19%and 7.53%, respectively). And they concluded that hexane, chloroform and acetone extracts applications of *O. onites* significantly reduced the disease symptoms. And these antibacterial effects of the extracts can be attributed to their phenolic content, carvacrol.

Baranauskaitė et al. (2016), tried to increase the extraction efficiency of carvacrol, rosmarinic, oleanolic and ursolic acid from the different species of oregano herbs (*O. onites*, *Origanum vulgare* spp. hirtum and *Origanum vulgare* L.). Various extraction methods (ultrasound-assisted, heat-reflux, continuous stirring, maceration, percolation) and extraction conditions (different solvent, material: solvent ratio, extraction temperature, extraction time) were used, and the active substances were determined by HPLC and the results have shown that the carvacrol, rosmarinic, oleanolic and ursolic acid extraction from oregano herbs depended not only on the oregano species (*O. vulgare*, *O. onites* and *O. vulgare* spp. hirtum), but also, on the extraction method used for the study.

Diler et al. (2017), investigated the effects of different levels of *O. onites* essential oil as feed additives on the growth performance, antioxidant activity and disease resistance of rainbow trout. Fish (26.05 ± 0.15 g) were fed the experimental diets supplemented with four different concentrations (0.125, 1.5, 2.5 and 3.0 mL kg^{-1}) of *O. onites* essential oil for 90 days and the results have shown that the fish fed diets containing essential oil of *O. onites* had significantly higher final weight than the control group and suggested that the essential oil of *O. onites* could be applied as growth promoter and also, it improved disease resistance when added to rainbow trout feed.

3. MATERIALS AND METHODS

3.1. Experimental Plant

Our plant material *Origanum onites* L. were taken from medical and aromatic plants garden of Van Yüzüncü Yıl University Faculty of Agriculture Field Crops Department.

3.2. Preparation of Ethyl Alcohol and *Origanum onites* Infusion

Plant material *O. onites* that were taken from medical and aromatic plants garden of Yüzüncü Yıl University Faculty of Agriculture Field Crops Department were left at room temperature in the dark until dried. Subsequently, the samples were ground to small pieces and stored at room temperature until used. For the *O. onites* infusion we weighted 3 grams of *O. onites* for each 100 mL of tap water and then we poured the boiling water to the plant and let it brew for 15-20 minutes after that the mixture was filtered and putted aside to cool. For the 20% Ethyl alchol mixture we measured 20 mL of Ethyl alchol and added 80 mL of tab water to it and then we shaked it gently and putted it aside. For the *O. onites* + EtOH mixture we poured 80 mL of boiling tap water to 3 grams of *O. onites* and let it brew for 15-20 minutes after that the mixture was filtered and 20 mL of EtOH was added to it and putted aside to cool. All of these mixtures were prepared, brewed daily and freshly and cooled to drink each morning and then they were given to the determined groups according to the specified rates.

3.3. Experimental Animals

This study was carried out with the (27552122-604.01.02-E.32257) Numbered permission of the Local Ethics Committee of Experimental Animal of Van Yuzuncu Yil University dated 30.04.2018. In our study, 28 *Wistar albino* female rats aged 3-4 months and weighing between 150-250gr provided by the Experimental Animal

Research Centre of Van Yuzuncu Yil University were used. The rats were placed in standard plastic rat cages and were adapted to the laboratory conditions and kept at 22 ± 2 °C in a 12-hour photoperiod. In the study, all necessary measures were taken to minimize the factors that would negatively affect the parameters. For this purpose, the subjects in the control and other experimental groups were kept under the same conditions. The experiment lasted for 30 days.

3.4. Establishment of experimental groups

The 28 rats used in this study were divided into 4 groups randomly selected 7 rats in each group. The groups were designed in accordance with the purpose of the study as follows:

1st Group : Normal Control (NC) Group (n=7) : Nothing was applied to this group; it was fed only with standard rat food and water.
2nd Group : 20% Ethyl alcohol (EtOH) Group (n=7) : This group was given standard rat feed and 20% EtOH.
3rd Group : %3 *Origanum onites* infusion (ORI) Group (n=7) : This group was given standard rat feed and freshly brewed and chilled 3% *O. onites* infusion.
4th Group : %3 *Origanum onites* + 20% Ethyl alcohol (ALC+ORI) Group (n=7) : This group was given standard feed and a mixture of 3% *O. onites* and 20% EtOH.

During the first day of our experiment, normal rat feed and 750 mL of water or alcohol were given to the rats of each group. The daily water, alcohol, thyme infusion and consumption amounts of each group were recorded daily by measuring the water and mixtures that they did not drink from the previous day. Furthermore, the feed consumption ratio of the rats was weighed and recorded every day for 30 days. During the experiment, no restrictions on eating and drinking of rats have been made by paying attention to cleanliness. We controllably finished our experiment after 30 days.

3.5. Taking Blood and Tissue Samples

By the end of experimental period, all rats were anesthetized with 10% ketamine and sacrificed. Intracardiac blood samples were taken from their hearts with the help of injectors. Blood were taken into EDTA and biochemistry tubes. The EDTA blood was used for erythrocyte pellet, and the blood in the biochemistry tubes were used for the parameters to be looked at in serum. MDA and GSH levels of erythrocyte were measured on the same day. On the other hand; at the end of the 30-day trial, the rats' kidney, erythrocyte and liver tissues were taken and washed with physiological water and dried with blotting paper. And after that they were kept in the freezer (-80 ^{o}C) until the time of analysis. Antioxidant defense system elements and MDA measurement were performed in erythrocyte pellet and tissue homogenates obtained (Xia et al., 1994).

3.6. Erythrocyte Pellet Preparation

For preparation of erythrocyte pellet; 1 mL of blood from EDTA tubes was taken to another test tube and 2 mL saline (0.9% NaCl) was added. Then they were centrifuged at 3000 rpm at $^{+}$4 °C in a cooled centrifuge for 15 minutes to separate the plasma, therefore the erythrocytes washing process was started. After centrifugation, the resulted plasma was discarded from the blood samples that were separated, and each time an equal amount of saline (0.9% NaCl) was added to the remaining volume and this process was repeated three times. The prepared erythrocyte pellet was stored in the deep freeze at -80 °C until analysis. GSH determination with MDA was performed immediately in the erythrocyte pellet. The prepared erythrocyte pellet were stored in the deep freeze at -80 °C until the determination of SOD, GSH-Px, GST, GR, and CAT enzyme activities from antioxidant enzymes (Anonim, 2010).

3.7. Preparation of Tissue homogenates

The kidney, erythrocyte and liver tissues of the rats that were kept in the freezer (-80 ^{o}C) Following the previous treatment, were slowly dissolved until they reached room temperature. Tissue homogenates for the determination of the antioxidant enzyme and MDA in the tissues was performed as follows. For the extraction, buffer containing 0.32 mol/L sucrose, 1mmol/L EDTA, 10 nm/L Tris HCl (pH 7.4) was prepared and 500 mg tissues were weighed in 25 mL glass beakers on a digital scale (Chyo Jl-180) and 5 mL cold buffer was added. Tissues were thoroughly crushed with glass baguette and homogenized for 3 minutes in Ultrasonic homogenizer. The extract was immediately centrifuged for 30 minutes at 9500 rpm in a cooled centrifuge (BHG Hermle) at $^{+}4$ $^{\circ}$C. clear supernatants from kidney and liver tissue were prepared for analysis (Marklund, 1990; Xia et al., 1994).

3.8. Devices and Laboratory Materials Used in Analysis

1. Spectrophotometer (Shimadzu UV / VIS-1201)
2. Spectrophotometer (Shimadzu UV-1800)
3. Magnetic stirrer (ARE Stirrer / WELP Scientifica)
4. Shaker (Gallenkamp)
5. Digital balance (Chyo Jl azi 180)
6. Cooled centrifuge (Hettich® Universal 320/320R)
7. Refrigerator (arcelik)
8. Water bath (BM 101 Nüve)
9. Ultrasonic homogenizer (20KHz Jencons)
10. Yellowline by IKA * DI 25 Basic Homogenizer
11. pH Meter (Mettler Toledo Inlab Expert Pro-ISM Seven Excellence)
12. Vortex (Nüve NM 110)
13. Precision balance (Precisa XR-205SM-DR)
14. Water bath (Nickel Weston-S-Mare Avon / Clifton)
15. Ultra-Low Temperature Freezer (Wisecryo)
16. Pure water device (Kross)

17. Eppendorf plus micro pipette
18. Whatman filter paper
19. Falcon 50 mL plastic tubes
20. Sterile injector (5, 10 mL) (Hayat)
21. Sterile insulin injector (1 mL) (Hayat)
22. EDTA tubes
23. Biochemistry tubes
24. Automatic pipette tips (10, 100 and 1000 µl) (Isolab)
25. Scissors
26. Plastic strainer
27. Scalpel
28. Filter paper

3.9. Chemical Substances Used in Analyzes

1. Tris (Hydroxymethyl aminomethane hydrochloride tris HCl)
2. Sodium sulfate (Sigma)
3. Sodium citrate
4. Beta Nicotinamide Adenine Dinucleotide Phosphate (NADPH)
5. Metaphosphoric acid
6. Trichloroacetic acid (TCA)
7. SOD and GSH-Px enzyme kit (RANSOD)
8. Reduced glutathione (GSH)
9. Sodium chloride (NaCl)
10. Ethylene diamine tetra acetic acid (EDTA)
11. Oxidized glutathione (GSSG)
12. Thiobarbituric acid (TBA)
13. Butylated hydroxytoluene (BHT)
14. 1,1,3,3-Tetraethoxypropane (MDA)
15. 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB)
16. Ethanol
17. Ketamine (10%)

18. Disodium carbonate (Na_2CO_3)
19. Sodium hydroxide (NaOH),
20. Disodium hydrogen phosphate (Na_2HPO_4)
21. Sodium dihydrogen phosphate (NaH_2PO_4)
22. Sulfosalicylic acid (SSA)
23. Ascorbic acid
24. Sucrose
25. Glyoxylic acid

3.10. Experimental Protocol

3.10.1. Determination of serum enzyme levels

Serum enzyme activities AST (EC2.6.1.1), ALT (EC2.6.1.2) and TP, TC levels were determined via commercial kits (DPC; Diagnostic Products Corporation, USA) using autoanalyzer (INTEGRA-800 ROCHE) kits.

3. 10.2. SOD enzyme assay

Assay Principle: The role of SOD is to accelerate the dismutation of the toxic $O_2^{\bullet-}$, produced during oxidative energy processes, to H_2O_2 and O_2. This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (I.N.T) to form a red formazan dye. The SOD activity is then measured by the degree of inhibition of this reaction. (McCord and Fridovich, 1969).

$$\text{Xanthine} \xrightarrow{\text{Xanthine Oxidase}} \text{uric acid} + O_2^{\cdot-} \quad (3.1)$$

$$\text{I. N. T} \xrightarrow{O_2^{\cdot-}} \text{Formazan dye (colorful compound)} \quad (3.2)$$

OR

$$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \xrightarrow{\text{SOD}} O_2 + H_2O_2 \quad (3.3)$$

Table 3. 1. Reagent Composition

Contents	Initial Concentration of solutions
R1a. Mixed Substrate	
Xanthine	0.05 mmol/L
I.N.T.	0.025 mmol/L
R1b. Reagent	
CAPS	40 mmol/L pH 10.2
EDTA	0.94 mmol/L
R2. Xanthine Oxidase	80 U/L
CAL Standard	5.70/mL

Experimental Procedure: SOD enzyme activity was measured with the Randox-Ransod enzyme kit on a Shimadzu UV / VIS-1201 spectrophotometer at 505 nm at 37^{o}C (Randox Lab., 1996). The erythrocyte pellet and tissue supernatants, which were prepared before and kept in the freezer (-80ºC) until the time of analysis, were used as the analysis material. The following reagents were pipetted into the cuvette.

Table 3. 2. Pipetting of SOD reagents into the cuvette

	Standard S1 (µL)	Standard S2 (µL)	Samples (µL)	Control (µL)
Ransod Sample Diluent	15	-------	.....	.....
Standard	.....	15	.....	.....
Diluted Sample	.....	.....	15	.....
Diluted Control	.....	.....	.....	15
Mixed Substrate (R1)	500	500	500	500
Xanthine Oxsidase (R2)	75	75	75	75

Mix, the initial absorbance A_1 was read after 30 seconds and the time was started simultaneously. The final absorbance A_2 was read after 3 minutes.

Calculation: The optical density results obtained from the spectrophotometer were replaced by the following equation and SOD enzyme % inhibitions were calculated.

$$100 - \frac{(\Delta A_{Std/min} x 100)}{(\Delta A_{Blank/min})} = \%\dot{I}nhibition \qquad (3.4)$$

$$100 - \frac{(\Delta A_{sample/min} x 100)}{(\Delta A_{Blank/min})} = \%\dot{I}nhibition \quad (3.5)$$

Standard graph for the calculation of SOD enzyme activity was obtained. The SOD activity U/g was calculated as the whole tissue by taking into account the dilution factor with the formula y = 48.85Ln (x) - 12.218 obtained from the standard graph.

3. 10.3. CAT enzyme assay

Assay Principle: Enzyme activities were measured in erythrocyte pellet and supernatants. The activity of CAT was determined according to the spectrophotometric method based on the consumption of H_2O_2 at 37 °C and 240 nm (Aebi, 1974). Catalase catalyzes the separation of H_2O_2 to water and oxygen according to the following reaction.

$$H_2O_2 \xrightarrow{\text{catalase}} H_2O + O_2 \quad (3.6)$$

The decomposition rate of H_2O_2 by catalase enzyme was read at a wavelength of 240 nm on a spectrophotometer. Because H_2O_2 absorbs light at this wavelength. The solutions for the preparation of phosphate buffers will be prepared as described in the table below

Table 3. 3. Preparation of CAT phosphate buffer (pH: 7.5)

Chemicals	MW. (g/mol)	50 mM (g)	Final volume dH_2O (mL)	Solution Name
KH_2PO_4	136,09	6.805	1000	A
$Na_2HPO_4.12H_2O$	358,14	17.907	1000	B1
$Na_2HPO_4.2H_2O$	178,14	8.907	1000	B2
Na_2HPO_4 (pure)	141,96	7.098	1000	B3

Table 3. 4. Mixing of CAT phosphate buffer solutions

50 mM (L) Phosphate buffer	A Solution (mL)	B Solution (mL)
For pH 7.5	160	840
For PH 7	413	587
For PH 6.5	685	315

H_2O_2 solution is the phosphate buffer adjusted at the absorbance 0.500 nm with H_2O_2. Approximately 300 mL of a phosphate buffer with pH 7.50 is transfered to a colored container (plastic, glass, mica). Zero the spectrophotometer with phosphate buffer at 240 nm and then H_2O_2 is added to the buffer in the colored container with 10-20 µL volumes. This is is continued until the Optical Density (OD) is adjusted at a wavelength of 0.500 nm.

Experimental Procedure: After the addition of the sample, the quartz cuvette is closed without waiting, and immediately the cuvette is upside downed and the absorbance is read. Absorbance reduction is recorded for 5 minutes, once every 15 seconds. The calculation is based on the highest (OD1) and lowest (OD2) values of 1-minute linear absorbance reduction.

Table 3. 5: Pipetting the CAT buffer and samples into the cuvette

	Blank (mL)	Sample (mL)
Phosphate Buffer	2.99	-
H_2O_2 Solution	0.01	-
Phosphate Buffer with H_2O_2	-	2.99
Supernatant	-	0.01

Calculation: $K = \{(2.3 \times \log (OD_1/ OD_2)) / \Delta t\ (sn)\}$
The catalase activity was calculated as U/g tissue for tissues and U/mL for erythrocytes.

3. 10.4. GPx enzyme assay

Assay Principle: GPX catalyses the oxidation of GSH by Cumene Hydroperoxide. In the presence of GR and NADPH, the GSSG is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured (Paglia and Valentine, 1967).

$$2GSH + ROOH \xrightarrow{GSH-Px} ROH + GSSG + H_2O \quad (3.7)$$

$$GSSG + NADPH + H^+ \xrightarrow{GR} NADP^+ + 2GSH \quad (3.8)$$

Table 3. 6. Reagent Composition

Contents	Concentration in the Test
R1a. Reagent	
Glutathione	4.0 mmol/L
Glutathione Reductase	≥ 0.5 U/L
NADPH	0.34 mmol/L
R1b. Reagent	
Phosphate Buffer	0.05 mol/L pH 7.2
EDTA	4.3 mmol/L
R2. Cumene Hydroperoxide	0.18 mmol/L
R3. Diluting Agent	

Experimental Procedure: GPx activity was measured by Randox-Ransel enzyme kits and Shimadzu UV / VIS-1201 spectrophotometer at 340 nm, 37 ºC with ultraviolet method (Randox Lab., 2013). The erythrocyte pellet and tissue supernatants, which were prepared before and kept in the freezer (-80ºC) until the time of analysis, were used as the analysis material.

Table 3. 7. Pipetting of GSH-Px reagents into the cuvette

	Diluted Sample (µL)	Reagent Blank dH_2O (µL)
Sample	10	10
Reagent (R1)	500	500
Cumene (R2)	20	20

The contents of cuvette was mixed and the initial absorbance of sample and reagent blank was read after one minute and the timer was started simultaneously. We read it again after 1 and 2 minutes.

Calculation: U/g tissue or mL erythrocyte pellet = 8412 x 75 µl ΔA 340 nm/min. For all tissue results of the sample and the blank U/g, the blank value (U/g) was removed from the sample value (U/g). Considering the dilution factor, the results were calculated as U/g tissue for whole tissue and U/mL for erythrocytes.

3.10.5. GST enzyme assay

Assay Principle: Glutathione S-transferase catalyzes the reaction between 1-chloro-2,4-dinitrobenzene (CDNB) and the -SH group of glutathione. Enzyme activity is determined by measuring the intensity of glutathione conjugation with CDNB at 37 °C at 340 nm (Mannervik and Guthenberg, 1981).

$$ROOH + 2GSH \xrightarrow{GSH} GSSG + ROH + H_2O \quad (3.9)$$

Experimental Procedure: The erythrocyte pellet and tissue supernatants, which were prepared before and kept in the freezer (-80ºC) until the time of analysis, were used as the analysis material. Absorbance measurement was carried out in the quartz cuvette at 340 nm. Absorbances were recorded every 15 seconds for 3 minutes. Calculations were made by taking into account the dilution factor from the formula (EU = 3 (A /9.6)) by determining the absorbance change per minute from the linear part of the change in absorbance range. The results were calculated as U/g tissue for tissues and U/mL for erythrocytes. The liver, kidney, and erythrocyte supernatants were diluted in proportion as 1/10 in GST

Table 3. 8: GST measurement by taking the mixture into the cuvette

	Blank (mL)	Sample (mL)	Final Concentration (mM)
PBS	2.7	2.7	100 mM
Distil water	0.1	-	-
CDNB	0.1	0.1	1 mM
GSH	0.1	0.1	1 mM
Supernatant	-	0.1	-

3.10.6. GR enzyme assay

Assay Principle: GR catalyzes the reduction of GSSG to GSH by NADPH. GR activity (EU) was calculated from the amount of NADPH spent per minute at 340 nm at 37 ºC. The linear portion of the absorbance measured for 5 minutes was calculated per minute (A = ε x b x c), (Carlberg and Mannervik, 1985). The results were calculated as U/g tissue for tissues and U/mL for erythrocytes.

$$GSSG + NADPH + H^+ \xrightarrow{GR} 2GSH + NADP^+ \quad (3.10)$$

Previously stored erythrocyte pellet and tissue supernatants were used as analysis material. Enzyme measurements were made by mixing the amounts in the following table in an appropriate manner. The liver, kidney, and erythrocyte supernatants were Diluted in proportion as 1/20 in GR

Table 3. 9. GR measurement with mixtures in the table

Measurement Procedure	Blank (µL)	Sample (µL)
Phosphate Buffer	1500 µL	1500 µL
Homogenization Buffer	1200 µL	-----
β-NADPH 2 Mm	150 µL	150 µL
GSSG 20 mM	150 µL	150 µL
Sample (Supernatant)	------	1200 µL

3.10.7. Determination of GSH

Assay Prıncıple: During the erythrocyte GSH reading all the proteins that did not carry sulfhydryl (-SH) group were precipitated by precipitating solution. In tissue supernetants using the phosphate buffer, The sulfhydryl group of GSH react with DTNB (5,5-dithio-*bis*-(2-nitrobenzoic acid), Ellman's Reagent) and produces a yellow colored 5-thio-2-nitrobenzoic acid(TNB) and this compound absorbs light at 412 nm. Against any chance of reduction in the GSH level the GSH level in erythrocyte tissue was observed within 24 hours.

Reagent Preparation:

1. Precipitator Content: 1.67 g of metaphosphoric acid, 0.2 g of EDTA, 30 g of NaCl was dissolved in 100 mL of dH_2O.
2. Phosphate Buffer: Prepared with 0.3 M disodium phosphate, dH_2O.
3. DTNB (Ellman's Reagent): 40 mg DTNB, 1g sodium citrate, complete with 100 mL of dH_2O.

Experimental Procedure: 200 µL of whole blood in EDTA tubes was transferred to another tube. After that the Hemolysis process was performed by adding 1.8 mL of dH_2O. Then the hemolysate was mixed with 3 mL of precipitant solution and putted aside for 5 minutes. The mixture was then filtered through whatman filter paper (No 42). 2 mL of the supernatant from the sample was transferred to another tube. After adding 8 mL of phosphate buffer to the tube, the tube was vortexed and then the mixture was kept in a hot water bath at 60 ºC for 10 minutes. After that the tubes we pulled out of the water bath and putted aside until they come to room temperature and then 1 mL of DTNB was added to the tube. For the standard, 40 mg of GSH solution was prepared freshly. Using Shimadzu UV / VIS-1201 spectrophotometer the optical densities of standard samples were read at 412 nm against the blank. In other tissues (ex. kidney and liver) 1 mL of supernatant were taken into a tube and then 5 mL of phosphate buffer was added to the tube, after that the tube was vortexed and then the mixture was kept in a hot water bath at 60 ºC for 10 minutes. After that the tubes we pulled out of the water bath and putted aside until they come to room temperature and then 1 mL of DTNB was added to the tube. Then using Shimadzu UV / VIS-1201 spectrophotometer the optical densities of standard samples were read at 412 nm against the blank. Results were calculated as mg/g tissue for tissues, mg/mL for erythrocytes (Beutler et al., 1963; Rizzi et al., 1988).

3.10.8. Determination of MDA

Assay Principle: This assay is based on the reaction of one of the Lipid peroxidation products resulted from the reaction of fatty acids with free radicals, MDA with thiobarbituric acid (TBA) and forming an MDA-TBA2 adduct that absorbs light strongly at 532 nm (Jain et al., 1989).

Reagent Preparation:

1. 0.1 M EDTA Solution: 37.224 g of EDTA-Na_2H_2O is dissolved in 1 liter of distilled water.
2. BHT Solution: 0.202 g BHT is dissolved in 25 mL of pure ethyl alcohol.
3. NaOH Solution (0.05 N): 2 g of NaOH is dissolved in 1 liter of dH_2O.
4. TBA Solution (1%): take 1 g of TBA and add 0.05 N NaOH until it is completed to 100 mL.
5. TCA (30%): 30 g of TCA is dissolved in 100 mL of dH_2O.
6. Phosphate Buffer: 8.1 g of NaCl, 2.302 g of Na_2HPO_4 OR 0.194 g of NaH_2PO_4 dH_2O is dissolved in 1 liter (pH 7.4).

Experimental Procedure**:** The colored reaction of Lipid peroxidation product MDA level with TBA reagent was measured on the Shimadzu UV / VIS-1201 spectrophotometer at maximum absorbance of 532 nm. 200 µL of the erythrocyte pellet or tissue supernatants were taken into a tube and then suspended with 800 µL phosphate buffer and 25 µL BHT. Then 500 µL of 30% TCA was added. After that the tubes were vortexed and kept in the refrigerator for 2 hours at -20 °C. Then they were centrifuged at 2000 rpm for 15 minutes. 1 mL of supernatant was taken and transferred to another tube. 75 µL of EDTA Na_2H_2O and 250 µL of TBA were added to the tubes. The tubes were vortexed and kept in a hot water bath at 90 ºC for 15 minutes. Then they were brought to room temperature and the optical densities were read at 532 nm (the amount of MDA in the hemoglobin in erythrocytes was determined by subtracting the 600 nm OD from 532 nm OD) (Jain et al., 1989).

A = a x b x c

A = Absorbance a = Extinction coefficient

b = Light path c = Concentration

1. Dilution: 0.2 + 0.8 + 0.025 + 0.5 = 1.525 / 0.2 = 7.625

2. Dilution: 1 + 0.075 + 0.25 = 1.325 / 1 = 1.325

Result = 7.625 x 1.325 = 10.103 125 = F

c = A/a x b = (A/mol x cm)/1.56 x 10^5 x L) x (1/cm) x (10^9 nM/mol) x (L/10^3x mL)

c = A x 1 x F x 10 / 1.56 = nmol/g tissue or nmol/g erythrocytes.

3.11. Statistical Analysis

Mean and standard deviation (X ± SD) were calculated using Minitab ready program according to the standard methods; The difference between the group averages was calculated using the One-Way ANOVA-Turkey test. $p \leq 0.05$ was considered statistically significant (İkiz et al., 1996).

4. RESULTS

4 groups of 7 rats in each were formed from 28 *Wistar albino* rats. The groups were as follows; control, 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH groups respectively. The control group was fed only with standard rat feed and water, the 20% EtOH group was given standard rat feed and 20% EtOH, the 3% *O.onites* infusion group was given standard rat feed and freshly brewed and chilled 3% *O. onites* infusion, the 3% *O. onites* + 20% EtOH group was given standard feed and a mixture of 3% *O. onites* infusion and 20% EtOH mixture infusion.

At the end of 30-day experiment, liver damage biomarkers in serum such as ALT, AST and TC, TP levels were determined as an indicator of hepatoprotective effects of *O. onites.* In addition, antioxidant enzymes that can be considered as an indicator of antioxidant activity in liver, kidney and erythrocyte tissue samples include GPx, CAT, GST, SOD, GR activities, MDA content and GSH levels were determined.

In this study, the results obtained at the end of 30 days are shown with tables and graphs as follows:

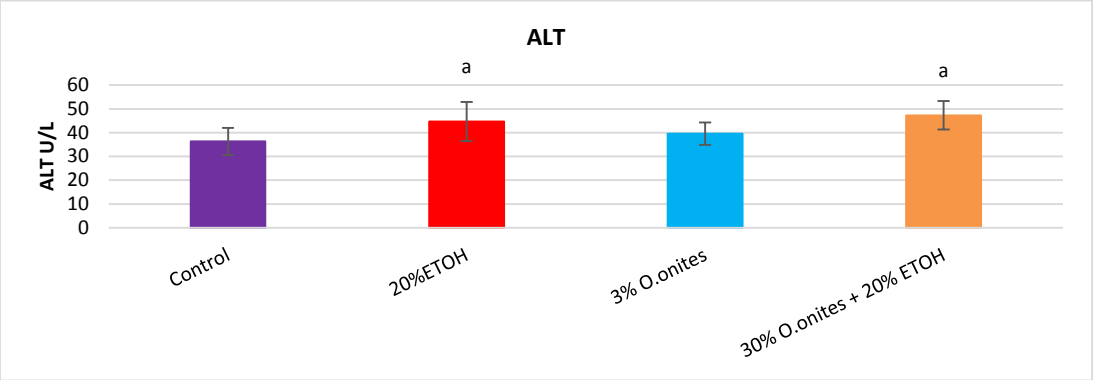
Table 4.1.Serum parameter levels of experimental groups as biomarkers of liver damage

Parameters	Experimental Groups			
	Conrtol	20% EtOH	3% *O. onites*	3% *O. onites* +20% EtOH
	X ± SD	X ± SD	X ± SD	X ± SD
ALT U/L	36.3±5.7	44.7±8.2[a]	39.6±4.7	47.3±6.0 [a.]
AST U/L	128.7±7.5	140.1±11.5[a]	108.0±12.9 [a,]	113.0±9.5 [a,b]
TC mg/dL	69.1±6.0	44.9±4.9 [a]	48.9±6.2 [a]	48.7±5.6 [a]
TP mg/dL	72.1±2.5	64.4±3.4 [a]	69.9±3.2	68.3±3.8 [a]

a: The difference between control group with 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* +20% EtOH groups is significant ($p < 0.05$). b: The difference between the 20% EtOH with the 3% *O. onites* + 20% EtOH group is significant ($p < 0.05$).

The hepatoprotective effects of *O. onites* is shown in Table 4.1. the control, 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH groups were measured using the liver damage biomarkers in serum such as ALT level values (U/L) respectively: 36.3±5.7, 44.7±8.2, 39.6±4.7, 47.3±6.0, AST enzyme level values (U/L)

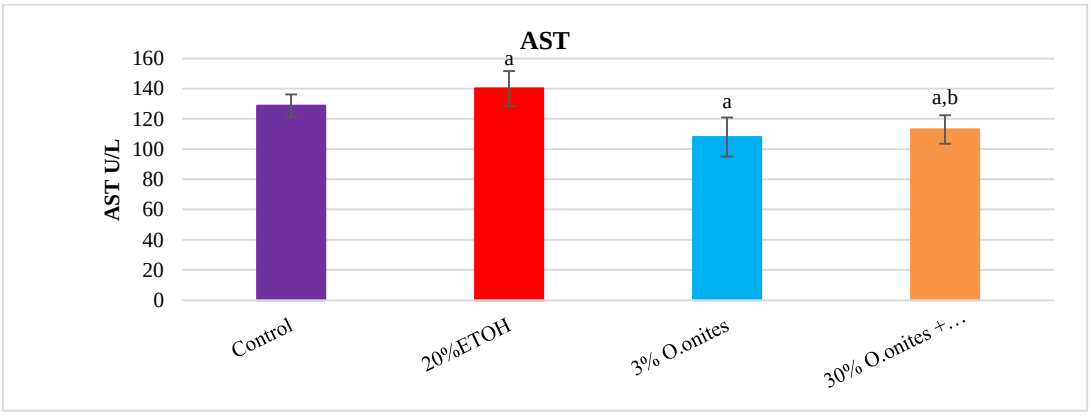
respectively: 128.7±7.5, 140.1±11.5, 108.0±12.9, 113.0±9.5, and TC level values (mg/dL) respectively: 69.1±6.0, 44.9±4.9, 48.9±6.2, 48.7±5.6, TP level values (mg/dL) respectively: 72.1±2.5,64.4±3.4,69.9±3.2, 68.3±3.8, were measured respectively.



a: The difference between Control group with 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* +20% EtOH groups is significant ($p < 0.05$).

Figure 4. 1. Comparison of ALT levels in experimental groups.

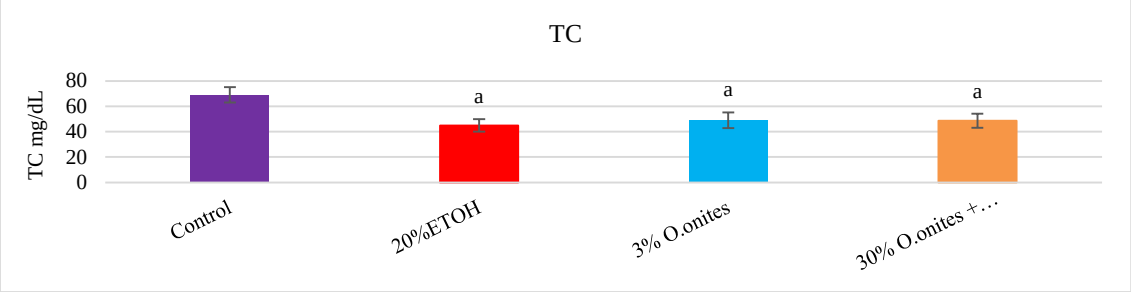
According to the results in Table 4.1; The ALT enzyme level values of the 20% EtOH and 3% *O. onites* + 20% EtOH groups were statistically significantly higher ($p < 0.05$) when we compared it to the control group. The comparison of other treatment groups were insignificant statistically.



a: The difference between control group with 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* +20% EtOH groups is significant ($p < 0.05$). b: The difference between the 20% EtOH with the 3% *O. onites* + 20% EtOH group is significant ($p < 0.05$).

Figure 4. 2. Comparison of AST levels in experimental groups.

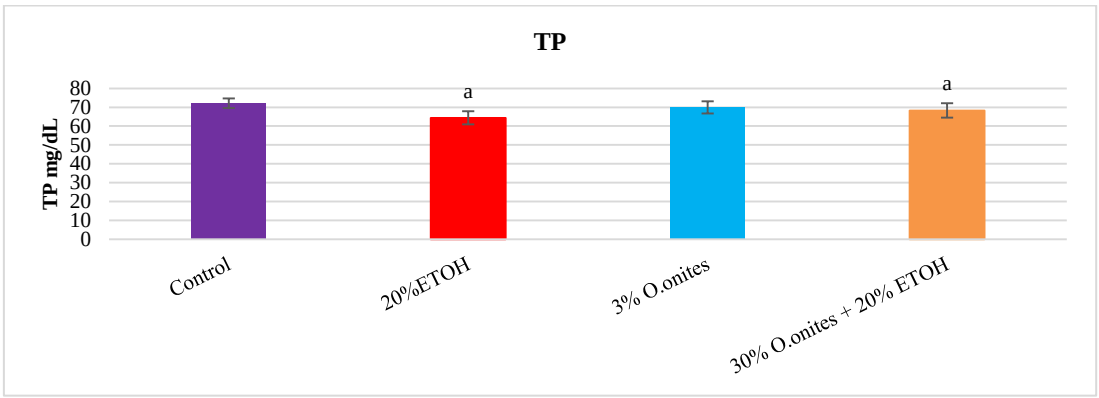
According to the results in Table 4.1; The AST enzyme level values of the 20% EtOH group were statistically significantly higher ($p<0.05$) in comparison to the control group. On the other hand, there was a significant reduction ($p<0,05$) in AST level values of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH groups in comparison with the control group. Also, there was a significant reduction ($p<0,05$) in AST level values of 3% *O. onites* + 20% EtOH group in comparison with the 20% EtOH group. The comparison of other treatment groups were insignificant statistically.



a: The difference between control group with 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* +20% EtOH groups is significant ($p <0.05$).

Figure 4. 3. Comparison of TC levels in experimental groups.

According to the results in Table 4.1; There is a statistically valuable decrease ($p<0,05$) in the TC level values of 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH groups in comparison with control.



a: The difference between Control group with 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* +20% EtOH groups is significant ($p <0.05$).

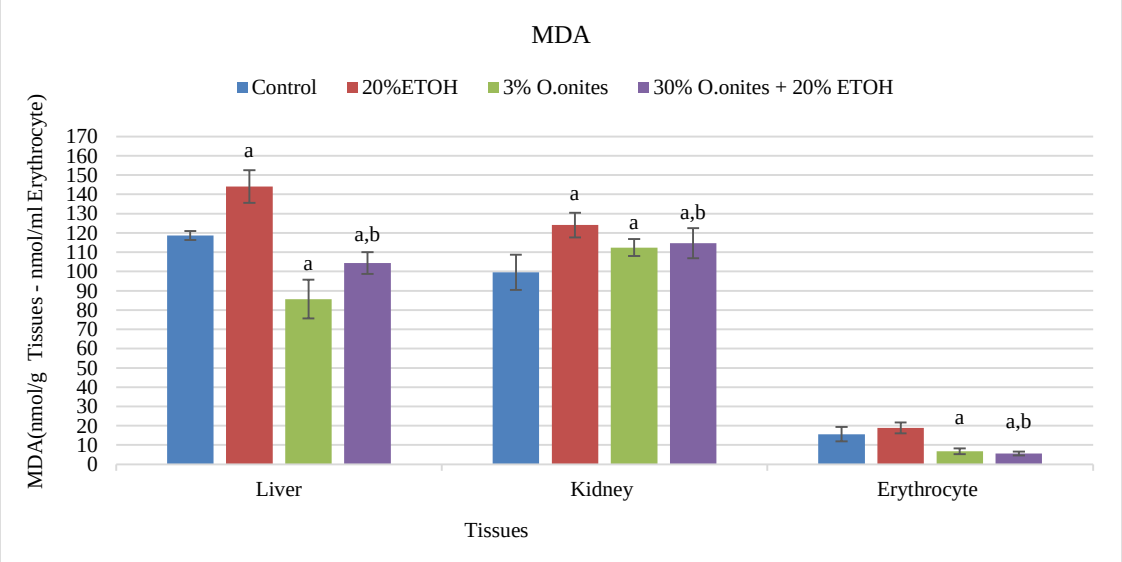
Figure 4. 4. Comparison of TP levels in experimental groups.

According to the results in Table 4.1; There is a statistically valuable decrease ($p<0,05$) in TP level values of 20% EtOH and 3% *O. onites* + 20% EtOH groups in comparison to the control group, on the other side, The TP level values of the 3% *O. onites* infusion group were statistically significantly higher in comparison with the 20% EtOH group. The comparison of other treatment groups were insignificant statistically.

Table 4. 2. MDA levels in various tissues of rats in experimental groups

Tissues	Experimental Groups			
	Control	20% EtOH	3% *O. onites*	3% *O. onites* +20% EtOH
	X ± SD	X ± SD	X ± SD	X ± SD
Liver nmol/g	118.60±2.34	144.05±8.48[a]	85.67±10.03[a]	104.36±5.70[a,b]
Kidney nmol/g	99.55±9.09	124.06±6.32[a]	112.41±4.35[a]	114.72±7.79[a,b]
Erythrocyte nmol/mL	15.63±3.7	18.87±2.83	6.75±1.44[a]	5.55±0.97[a,b]

a: The difference between Control group with 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* +20% EtOH groups is significant ($p<0.05$). b: The difference between the 20% EtOH with the 3% *O. onites* + 20% EtOH group is significant ($p<0.05$).



a: The difference between Control group with 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* +20% EtOH groups is significant ($p<0.05$). b: The difference between the 20% EtOH with the 3% *O. onites* + 20% EtOH group is significant ($p<0.05$).

Figure 4.5. Comparison of MDA levels in various tissues of rats in experimental groups.

As indicated in Table 4.2, MDA (nmol/g) values found in various tissues in the control, 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH groups

respectively are; liver tissue (nmol/g): 118.60±2.34, 144.05±8.48, 85.67±10.03, 104.36±5.70, kidney tissue (nmol/g) : 99.55±9.09, 124.06±6.32, 112.41±4.35, 114.72±7.79, erythrocyte tissue (nmol/mL): 15.63±3.7, 18.87±2.83, 6.75±1.44, 5.55±0.97.

According to these results; In the liver tissue a statistically valuable reduction ($p < 0.05$) was found in MDA content of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group in comparison with the control group. On the other side, there was a statistically valuable increase ($p < 0.05$) in MDA values of 20% EtOH in comparison to the control group. Also, there is a statistically valuable reduction ($p < 0.05$) in MDA valuess of 3% *O. onites* + 20% EtOH in comparison to the 20% EtOH group.

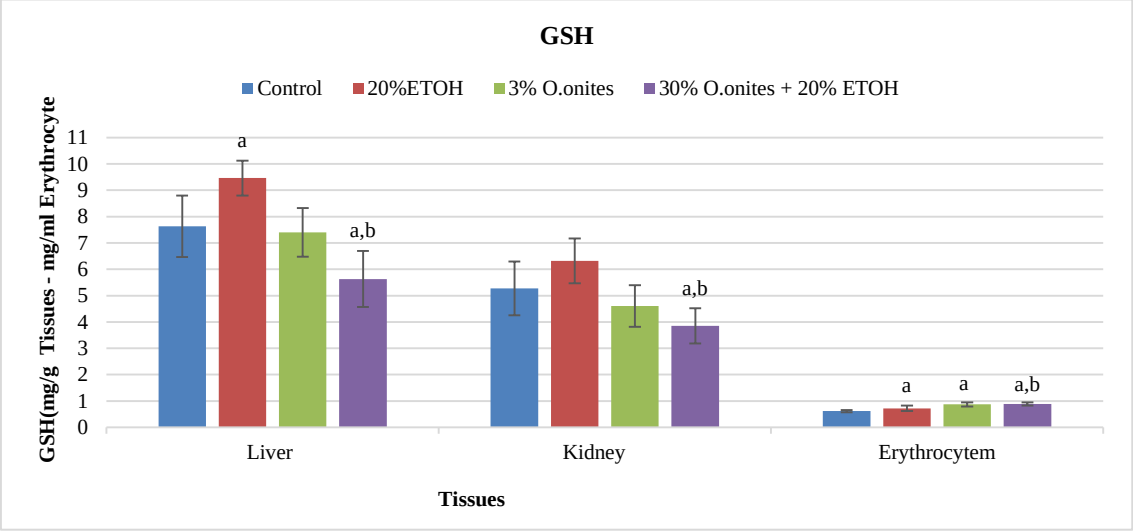
In the kidney tissue a statistically valuable increase ($p < 0.05$) in the MDA values of 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group was found in comparison with the control group. Also, there is a statistically valuable reduction ($p < 0.05$) in MDA valuess of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group in comparison with the 20% EtOH group.

In erythrocyte tissue a statistically valuable reduction ($p < 0.05$) was found in MDA values of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group in comparison with the control group. Although there was an increase in the MDA values of the 20% EtOH group in comparison with to the control group, it was not statistically significant ($p < 0.05$). Also, when we compared the MDA valuess of 3% *O. onites* + 20% EtOH group to that of 20% EtOH group there was a valuable reduction ($p < 0.05$). The comparison of other treatment groups were statistically insignificant.

Table 4. 3. GSH levels in various tissues of rats in experimental groups

Tissues	Experimental Groups			
	Control	20% EtOH	3% *O. onites*	3% *O. onites* +20% EtOH
	X ± SD	X ± SD	X ± SD	X ± SD
Liver mg/g	7.63±1.17	9.46±0.66[a]	7.40±0.92	5.63±1.06[a,b]
Kidney mg/g	5.27±1.02	6.32±0.85	4.61±0.79	3.85±0.67[a,b]
Erythrocyte mg/mL	0.616±0.04	0.720±0.10[a]	0.871±0.08[a]	0.885±0.06[a,b]

a: The difference between Control group with 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* +20% EtOH groups is significant ($p < 0.05$). b: The difference between the 20% EtOH with the 3% *O. onites* + 20% EtOH group is significant ($p < 0.05$).



a: The difference between Control group with 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* +20% EtOH groups is significant ($p < 0.05$). b: The difference between the 20% EtOH with the 3% *O. onites* + 20% EtOH group is significant ($p < 0.05$).

Figure 4. 6. Comparison of GSH levels in various tissues of rats in experimental groups.

As indicated in Table 4.3, GSH (mg/g) values found in various tissues in the control, 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH groups respectively are; liver tissue (mg/g): 7.63±1.17, 9.46±0.66, 7.40±0.92, 5.63±1.06, kidney tissue (mg/g) : 5.27±1.02, 6.32±0.85, 4.61±0.79, 3.85±0.67, erythrocyte tissue (mg/mL): 0.616±0.04, 0.720±0.10, 0.871±0.08, 0.885±0.06.

A valuable increase ($p < 0.05$) was found in GSH values of liver tissue in 20% EtOH group insignificant control group. On the other hand, there was a statistically valuable reduction ($p < 0.05$) when we compared the 3% *O. onites* + 20% EtOH group to the control group. Although there was a decrease within the GSH value of 3% *O. Onites* infusion group compared with the control group, it was statistically insignificant ($p<0.05$). Also, there was a statistically valuable reduction ($p<0,05$) within the GSH level values 3% *O. onites* + 20% EtOH group compared with the 20% EtOH group.

In kidney tissue, a statistically valuable reduction ($p < 0.05$) was shown in the GSH levels of 3% *O. onites* + 20% EtOH group when compared to the control group. Although there was an increase within the GSH values of 20% EtOH group when compared to the control group, it was statistically insignificant ($p < 0.05$). On the other hand, the *O. onites* + 20% EtOH group showed a valuable decrease ($p < 0.05$) in

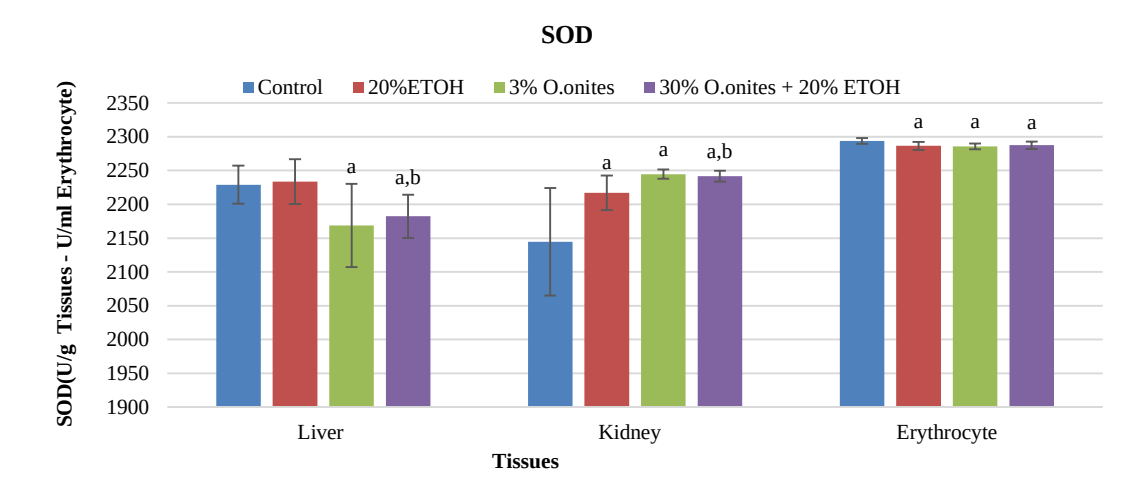
comparison with the 20% EtOH group. The comparison of GSH values of other treatment groups was statistically insignificant.

GSH values of Erythrocyte tissue were statistically significantly higher in 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH groups in comparison with the control group (p <0.05). Also, there was a statistically valuable increase (p<0,05) in the GSH level values of 3% *O. onites* + 20% EtOH group in comparison with the 20% EtOH group.

Table 4. 4. SOD enzyme levels in various tissues of rats in experimental groups

Tissues	Experimental Groups			
	Control X ± SD	20% EtOH X ± SD	3% *O. onites* X ± SD	3% *O. onites* + 20% EtOH X ± SD
Liver U/g	2229.05±28.27	2233.72±33.22	2168.82±61.69[a]	2182.21±31.86[a,b]
Kidney U/g	2144,44±79,56	2217.02±25.51[a]	2244,70±6,66[a]	2241,83±8,00[a,b]
ErythrocyteU/mL	2293.71±4.34	2286,48±5,85[a]	2285,78±4,30[a]	2287,39±5,33[a]

a: The difference between control group with 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* +20% EtOH groups is significant (p <0.05). b: The difference between the 20% EtOH with the 3% *O. onites* + 20% EtOH group is significant (p <0.05).



a: The difference between control group with 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* +20% EtOH groups is significant (p <0.05). b: The difference between the 20% EtOH with the 3% *O. onites* + 20% EtOH group is significant (p <0.05).

Figure 4.7. Comparison of SOD enzyme levels in various tissues of rats in experimental groups.

As indicated inTable 4.4, SOD enzyme (U/g) values found in various tissues in the control, 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH groups respectively are; liver tissue (U/g): 2229.05±28.27, 2233.72±33.22, 2168.82±61.69, 2182.21±31.86, kidney tissue (U/g) : 2144,44±79,56, 2217.02±25.51, 2244,70±6,66, 2241,83±8,00, erythrocyte tissue (U/mL): 2293.71±4.34, 2286,48±5,85, 2285,78±4,30, 2287,39±5,33.

According to these results; In the liver tissue, there was a statistically valuable reduction ($p < 0.05$) in SOD values of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group in comparison with the control group. Although there has been an increase in the SOD values of the 20% EtOH group in comparison with the control group, it was statistically insignificant ($p < 0.05$). Also, there was a statistically valuable reduction ($p < 0.05$) in SOD values of 3% *O. onites* + 20% EtOH group in comparison with the 20% EtOH group.

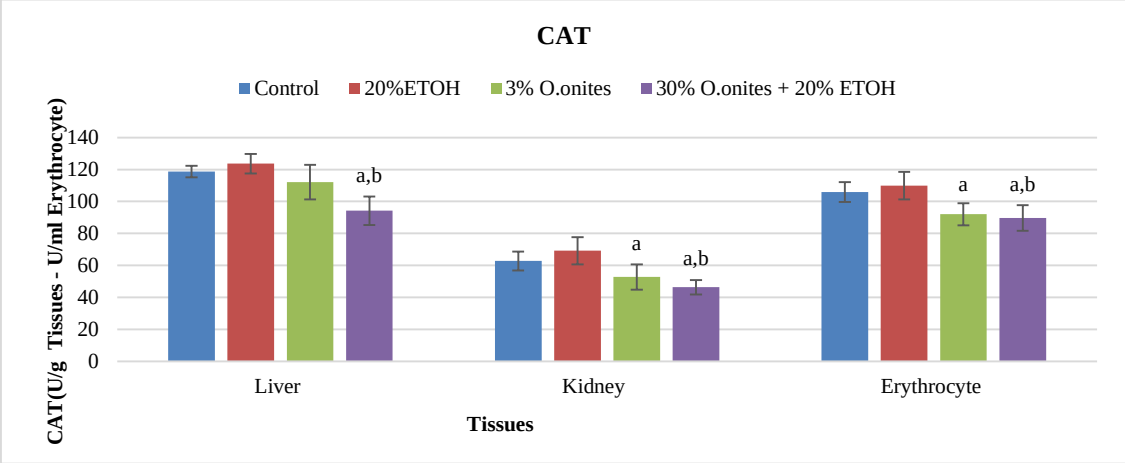
In kidney tissue; a statistically valuable increase ($p < 0.05$) was shown in the SOD levels of 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group in comparison with the control group. Also, a statistically valuable increase ($p < 0.05$) was shown in the SOD levels of 3% *O. onites* + 20% EtOH group when we compaired it with the 20% EtOH group.

In erythrocyte tissue a statistically valuable reduction ($p < 0.05$) was shown in the SOD levels of 20% EtOH , 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH groups in comparison with the control group. The comparison of SOD values of other treatment groups was statistically insignificant.

Table 4. 5. CAT enzyme levels in various tissues of rats in experimental groups

Tissues	Experimental Groups			
	Control	20% EtOH	3% *O. onites*	3% *O. onites* +20% EtOH
	X ± SD	X ± SD	X ± SD	X ± SD
Liver U/g	118.81±3.60	123.66±6.08	112.17±10.78	94.22±8.91[a,b]
Kidney U/g	62.75±5.94	69.15±8.58	52.76±7.92[a]	46.36±4.56[a,b]
Erythrocyte U/mL	105.89±6.21	109.90±8.69	92.01±6.98[a]	89.61±8.04[a,b]

a: The difference between Control group with 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* +20% EtOH groups is significant ($p < 0.05$). b: The difference between the 20% EtOH with the 3% *O. onites* + 20% EtOH group is significant ($p < 0.05$).



a: The difference between control group with 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* +20% EtOH groups is significant ($p < 0.05$). b: The difference between the 20% EtOH with the 3% *O. onites* + 20% EtOH group is significant ($p < 0.05$).

Figure 4.8. Comparison of CAT enzyme levels in various tissues of rats in experimental groups.

As indicated in Table 4.5, CAT enzyme (U/g) values found in various tissues in the Control, 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH groups respectively are; liver tissue (U/g): 2229.05±28.27, 2233.72±33.22, 2168.82±61.69, 2182.21±31.86, kidney tissue (U/g) : 2224,36±12,74, 2228,35±10,74, 2243,82±6,97, 2240,95±8,6 erythrocyte tissue (U/mL): 2295,19±6,30, 2281,90±7,00, 2285,78±4,30, 2287,39±5,33.

According to these results; In the liver tissue a statistically valuable reduction ($p < 0.05$) was shown in the CAT values of 3% *O. onites* + 20% EtOH group when compared to the control group. Although there has been an increase in the CAT values of the 20% EtOH group compared to the control group, it was statistically insignificant ($p < 0.05$). Also, there has been a statistically valuable reduction ($p < 0.05$) in the CAT valuess of 3% *O. onites* + 20% EtOH group in comparison with the 20% EtOH group .

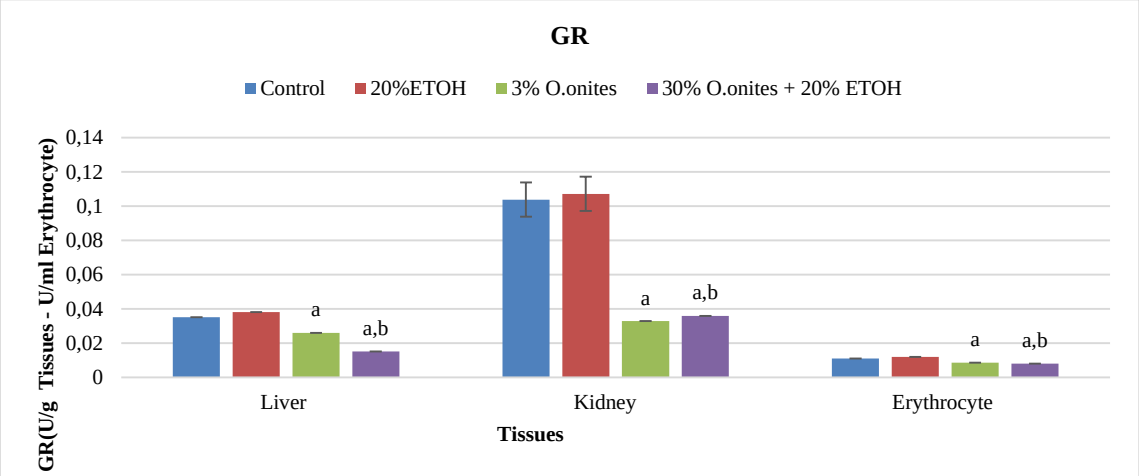
In the kidney tissue there is a statistically valuable reduction ($p < 0.05$) in the CAT values of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group in comparison with the control group. Although an increase was show in the CAT values of the 20% EtOH group compared to the control group, it was statistically insignificant ($p < 0.05$). Also, there was a statistically valuable reduction ($p < 0.05$) in the CAT values of 3% *O. onites* + 20% EtOH group in comparison with the 20% EtOH group.

In the Erythrocyte tissue; a statistically valuable reduction ($p < 0.05$) was shown in the CAT values of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group in comparison with the control group. Although an increase was seen in the CAT values of the 20% EtOH group when compared to the control group, it was statistically insignificant ($p < 0.05$). Also, a statistically valuable reduction ($p < 0.05$) was seen in the CAT valuess of 3% *O. onites* + 20% EtOH group in comparison with the 20% EtOH group. The comparison of CAT values of other treatment groups was statistically insignificant.

Table 4. 6. GR enzyme levels in various tissues of rats in experimental groups

Tissues	Experimental Groups			
	Control X ± SD	20% EtOH X ± SD	3% *O. onites* X ± SD	3% *O. onites* +20% EtOH X ± SD
Liver U/g	0.035±0.001	0.038±0.001	0.026±0.001[a]	0.015±0.001[a,b]
Kidney U/g	0,1037±0,01	0,1071±0,01	0,0328±0,001[a]	0,0359±0,001[a,b]
Erythrocyte U/mL	0,011±0,001	0,012±0,001	0,0085±0,001[a]	0,0080±0,001[a,b]

a: The difference between control group with 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* +20% EtOH groups is significant ($p < 0.05$). b: The difference between the 20% EtOH with the 3% *O. onites* + 20% EtOH group is significant ($p < 0.05$).



a: The difference between control group with 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* +20% EtOH groups is significant ($p < 0.05$). b: The difference between the 20% EtOH with the 3% *O. onites* + 20% EtOH group is significant ($p < 0.05$).

Figure 4. 9. Comparison of GR enzyme levels in various tissues of rats in experimental groups.

As indicated in Table 4.6, GR enzyme (U/g) values found in various tissues in the Control, 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH groups respectively are; liver tissue (U/g): 0.035±0.00, 0.038±0.00, 0.026±0.00, 0.015±0.00, kidney tissue (U/g) : 0,1037±0,01, 0,1071±0,01, 0,0328±0,00, 0,0359±0,00, erythrocyte tissue (U/mL): 0,011±0,00, 0,012±0,00, 0,0085±0,00, 0,0080±0,00.

According to these results; In the liver tissue a statistically valuable reduction ($p < 0.05$) was seen in the GR values of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group in comparison with the control group. Although an increase was seen in the GR values of the 20% EtOH group when compared to the Control, it was statistically insignificant ($p < 0.05$). Also, there was a statistically valuable reduction ($p < 0.05$) in the GR valuess of 3% *O. onites* + 20% EtOH group in comparison with the 20% EtOH group.

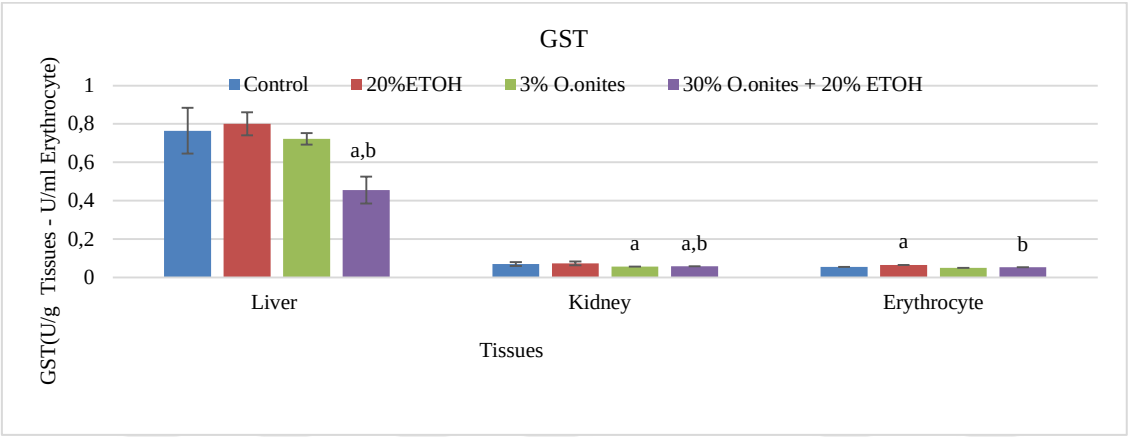
In the kidney tissue; a statistically valuable reduction ($p < 0.05$) was seen in the GR values of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group in comparison with the control group. Although an increase was seen in the GR values of the 20% EtOH group when compared to the control group, it was statistically insignificant ($p < 0.05$). Also, there was a statistically valuable reduction ($p < 0.05$) in the GR valuess of 3% *O. onites* + 20% EtOH group in comparison with the 20% EtOH group.

In the erythrocyte tissue; a statistically valuable reduction ($p < 0.05$) was seen in the GR values of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group when we compaired them to the control group. Although an increase was seen in the GR values of the 20% EtOH group when compared to the control group, it was statistically insignificant ($p < 0.05$). Also, there was a statistically valuable reduction ($p < 0.05$) in the GR valuess of 3% *O. onites* + 20% EtOH group in comparison with the 20% EtOH group. The comparison of GR values of other treatment groups was statistically insignificant.

Table 4. 7. GST enzyme levels in various tissues of rats in experimental groups

Tissues	Experimental Groups			
	Control	20% EtOH	3% *O. onites*	3% *O. onites* +20% EtOH
	X ± SD	X ± SD	X ± SD	X ± SD
Liver U/g	0,7647±0,12	0,8003±0,06	0,7222±0,03	0,4553±0,07[a,b]
Kidney U/g	0,0700±0,01	0,0728±0,01	0,0563±0,00[a]	0,0572±0,00[a,b]
Erythrocyte U/mL	0,055±0,00	0,064±0,00[a]	0,050±0,00	0,052±0,00[b]

a: The difference between control group with 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* +20% EtOH groups is significant (p <0.05). b: The difference between the 20% EtOH with the 3% *O. onites* + 20% EtOH group is significant (p <0.05).



a: The difference between control group with 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* +20% EtOH groups is significant (p <0.05). b: The difference between the 20% EtOH with the 3% *O. onites* + 20% EtOH group is significant (p <0.05).

Figure 4.10. Comparison of GST enzyme levels in various tissues of rats in experimental groups.

As indicated in Table 4.7, GST enzyme (U/g) values found in various tissues in the Control, 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH groups respectively are; liver tissue (U/g): 0,7647±0,12, 0,8003±0,06, 0,7222±0,03, 0,4553±0,07, kidney tissue (U/g) : 0,0700±0,01, 0,0728±0,01, 0,0563±0,00, 0,0572±0,00, erythrocyte tissue (U/mL): 0,055±0,00, 0,064±0,00, 0,050±0,00, 0,052±0,00.

According to these results; In the liver tissue a statistically valuable reduction (p <0.05) was seen in the GST values of 3% *O. onites* + 20% EtOH group in comparison with the control group. Although an increase was seen in the GST values of the 20% EtOH group when compared to the control, it was statistically insignificant (p <0.05).

Also, there was a statistically valuable reduction ($p < 0.05$) in the GST valuess of 3% *O. onites* + 20% EtOH group when compared to the 20% EtOH group.

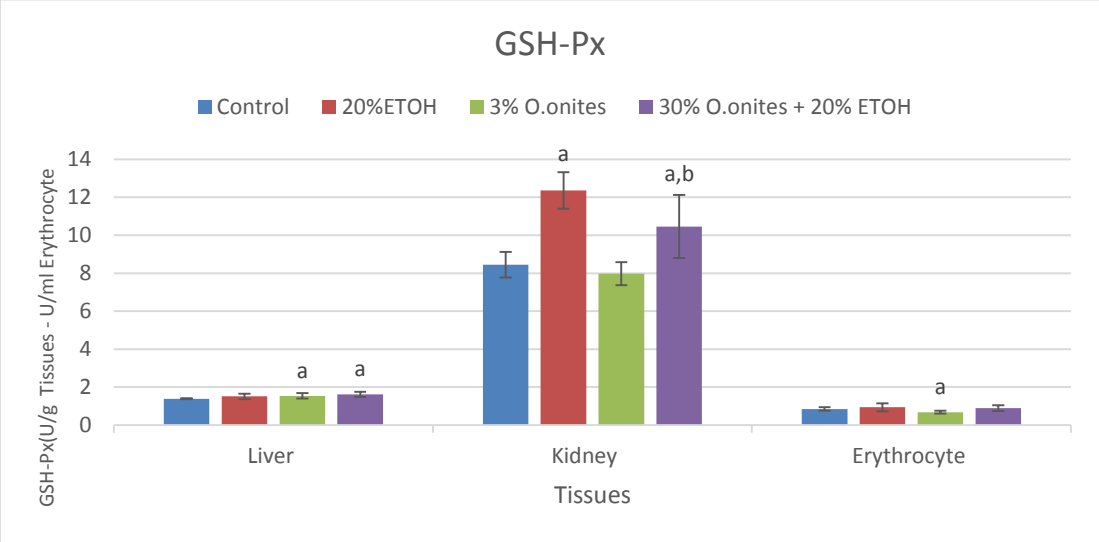
In the kidney tissue; a statistically valuable reduction ($p < 0.05$) was seen in the GST values of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group in comparison with the control group. Although an increase was seen in the GST values of the 20% EtOH group when compared to the control, it was statistically insignificant ($p < 0.05$). Also, there was a statistically valuable reduction ($p < 0.05$) in the GST valuess of 3% *O. onites* + 20% EtOH group when compared to the 20% EtOH group.

In the erythrocyte tissue; a statistically valuable increase ($p < 0.05$) was seen in the GST values of 20% EtOH group when compared to the control group. On the other hand, a statistically valuable reduction ($p < 0.05$) was seen in the GST valuess of 3% *O. onites* + 20% EtOH group when compared to the 20% EtOH group. The comparison of GR values of other treatment groups was statistically insignificant.

Table 4. 8. GSH-Px enzyme levels in various tissues of rats in experimental groups

Tissues	Experimental Groups			
	Control	20% EtOH	3% *O. onites*	3% *O. onites* +20% EtOH
	X ± SD	X ± SD	X ± SD	X ± SD
Liver U/g	1,39±0,03	1,51±0,15	1,54±0,14[a]	1,62±0,14[a]
Kidney U/g	8,45±0,68	12,36±0,96[a]	7,98±0,61	10,46±1,66[a,b]
Erythrocyte U/mL	0,85±0,09	0,94±0,21	0,68±0,08[a]	0,89±0,15

a: The difference between control group with 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* +20% EtOH groups is significant ($p < 0.05$). b: The difference between the 20% EtOH with the 3% *O. onites* + 20% EtOH group is significant ($p < 0.05$).



a: The difference between control group with 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* +20% EtOH groups is significant ($p < 0.05$). b: The difference between the 20% EtOH with the 3% *O. onites* + 20% EtOH group is significant ($p < 0.05$).

Figure 4.11. Comparison of GSH-Px enzyme levels in various tissues of rats in experimental groups.

As indicated in Table 4.8, GSH-Px enzyme (U/g) values found in various tissues in the control, 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH groups respectively are; liver tissue (U/g): 1,39±0,03 , 1,51±0,15 , 1,54±0,14 , 1,62±0,14. kidney tissue (U/g) : 8,45±0,68, 12,36±0,96, 7,98±0,61, 10,46±1,66, Erythrocyte tissue (U/mL): 0,85±0,09, 0,94±0,21 , 0,68±0,08 , 0,89±0,15.

According to these results; In the liver tissue a statistically valuable increase ($p < 0.05$) was seen in the GSH-Px values of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group in comparison with the control group. Although an increase in the GSH-Px values of the 20% EtOH group was seen when we compared it to the control group, it was statistically insignificant ($p < 0.05$).

GSH-Px values of kidney tissue were statistically significantly higher ($p < 0.05$) in 20% EtOH and 3% *O. onites* + 20% EtOH groups in comparison to the control group. Although a reduction was seen in the GSH-Px value of 3% *O. onites* infusion group when compared to the control group, it was statistically insignificant ($p < 0.05$). Also, a statistically valuable reduction ($p<0,05$) was seen in the GSH-Px level values of 3% *O. onites* + 20% EtOH group in comparison with the 20% EtOH group.

In the erythrocyte tissue; a statistically valuable reduction ($p < 0.05$) was seen in the GSH-Px values of 3% *O. onites* infusion group when compared to the control group. Although an increase in the GSH-Px values of the 20% EtOH group was seen when compared to the control group, it was statistically insignificant ($p < 0.05$).

5. DISCUSSION AND CONCLUSION

The biomedical writing is full of claims that free radicals along with the other RS are involved in human maladies. They have been concerned in over two hundred disorders, extending from rheumatoid joint pain, haemorrhagic shock, and Rett disorder, through heart disease and pancreatic fibrosis to intestinal anaemia, AIDS, and indeed male-pattern hair loss (Halliwell and Gutteridge, 2015).

When there is a disturbance between the production of reactive oxygen species such as hypochlorous acid; hydrogen peroxide; alcoxyl; hydroxyl, superoxide and peroxyl radicals; and antioxidant defences against them oxidative stress is produceed, and this increases tissue harm by discharging prooxidative shapes of reactive iron that are ready to drive lipid peroxidation and Fenton chemistry by eroding away protective sacrificial antioxidants. The body encompasses a chain of command of protective methodologies to bargain with oxidative stress inside distinctive cellular compartments, and superimposed on these are gene-regulated guards including the oxidant stress and heat-shock proteins (Gutteridge, 1995).

Does oxidative stress matter? Yes, it does because a few disorders are likely caused by oxidative stress; cancer can in some cases be, for example. Many biological consequences of the excessive radiation introduction are due to oxidative harm. The side effects created by incessant dietary insufficiency of vitamin E are likely mediated by oxidative stress. However, in most maladies, oxidative stress could be a result and not a cause of the malady. Tissue harm leads to discharge of DAMPs and formation of expanded sums of putative ‘injury mediators’, such as prostaglandins, leukotrienes, cytokines and, of course, RS. At different times, all of these have are thought to play a critical role in tissue harm (Halliwell and Gutteridge, 2015).

The methods that are used to determine free radicals directly *in vivo* are novel. Direct determination of free radicals is carried out by electron spin resonance spectrometry (ESR) device, but the analyzes with this device are still in the trial phase and they require detailed medical equipment’s for the procedures. So, the measurement

of free radicals is indirectly done by antioxidant defense system biomarkers and lipid peroxidation products (Akkuş, 1995).

In this study, the reasons for choosing EtOH and functional food (*O. onites*) whose effect has been investigated can be explained as follows briefly;

As specified at the starting of the study, alcohol is the foremost acknowledged addictive substance around the world and its consumption is related to numerous wellbeing, financial, and social issues (Galicia-Moreno and Gutiérrez-Reyes, 2014).

And it can effectively enter into the body by mouth, breathing, skin contact and nourishment chain ways and apply its poisonous impact on living beings (Precious stone and Messing, 1994; Rauch et al., 1994). Here, as a result of the toxic effect of the EtOH; our aim is investigattion of the hepatoprotective and antioxidant role of *O. onites* against the adverse effects that may be seen in the serum enzymes and oxidative stress parameters, which have significant clinical importance and can be used as liver injury biomarkes.

In this study, the reason for choosing EtOH to generate oxidative stress; is because alcohol can easily enter the body through the mouth and it is a peroxidant which is commonly used to produce oxidative stress and experimental liver damage in living organisms (Aykaç et al., 1985; Sonde, et al., 2000; Kolankaya, et al., 2002; Doğan and Çelik, 2010; Yurt and Çelik , 2010).

It was Also, reported that excessive alcohol consumption caused damage to liver tissue and many negative metabolic changes, and these adverse effects varied depending on dose and duration, individual strength, diet, and other factors (Murray et al., 1988). In addition the rate of ethanol disposed of through the lungs and kidneys is only 2-10% of the EtOH ingested. The rest should be oxidized inside the body, primarily inside the liver because it contains the majority of the body's enzymes capable of alcohol oxidation. The specifity of this organ to ethanol my explain why ethanol metabolism cause such a big imbalances in the liver

These effects are increased by the absence of feedback mechanism to balance the ethanol oxidation rate to the normal metabolic state of hepatocyte, and unlike other main sources of calories, the inability of ethanol to be stored or metabolized to a valuable degree in peripheral tissues. The accessible Ethanol at this point turns into the

favored fuel for liver. By evacuating nearly 90% of every single other substrate frequently utilized by the liver (Lundquist et al., 1962) so the intermediary metabolism of the liver is taken over by alcohol. Alcohol dehydrogenase is the major pathway for ethanol digestion within the liver. When a hydrogen is substracted from ethanol it is oxidized to acetaldehyde and each one of these two items is clearly in charge of a combination of metabolic alterations, counting protein metabolism abnormalities and liver injury development (Lieber, 1980).

It is additionally recommended that alcohol utilization leads to lipid peroxidation by inciting oxidative stress in liver and other tissues and this can be a complex and interactive process (Nordmann et al., 1992; Ishii et al., 1997).

In the samples taken from serum and various tissues of rats exposed to subchronic applications of *O. onites* infusion and EtOH; we investigated the antioxidant and hepatoprotective role of *O. onites* against EtOH induced oxidative stress, alcoholic liver damage and any other negative effect EtOH may have by measuring serum ALT, AST, TC and TP values. In addition, we measured CAT, GST, GSH-Px, GR and SOD activities, MDA content and GSH levels of antioxidant enzymes that can be considered as an indicator of antioxidant capacity of *O. onites* in liver, kidney, and blood tissue samples.

The reason for the use of Rat in the study; it is because they are the most widely used vertebrate animal after mice in researches. Rats are used in basic medicine, pharmacology, food, behavior and toxicity studies (Van Zutphen et al., 2003).

In our study, all necessary measures were taken to minimize the factors that would negatively affect the parameters analyzed. Therefore, all subjects in control and other experimental groups were kept under the same conditions. The reason for the selection of these parameters is that it is important to demonstrate the molecular toxicity of EtOH and the healing properties of these functional foods on warm-blooded organisms in terms of food biochemistry.

The reason for choosing the methods we used in our study is that they requires fewer chemicals and is more convenient and easier when you compare it with the existing possibilities of our laboratories. Likewise, because the studies are done in many tissues, we believe that the effect of the functional substances used has a healthy result.

Serum enzyme levels which are indicative of liver damage are shown in Table 4.1 .; changes in MDA content of treatment groups in liver, kidney and erythrocyte tissues in Table 4.2.; GSH level changes in Table 4.3.; SOD enzyme activity changes Table 4.4.; CAT enzyme activity changes Table in 4.5.; changes in GR activity changes in Table 4.6.; changes in GST activity Table 4.7. and GSH-Px enzyme level changes in Table 4.8. as it will be seen, after treatment with 20% EtOH and 3% *O. onites* infusion; significant changes in the serum enzyme levels have been observed and also, in the antioxidant enzyme activities such as GSH-Px, CAT, GR, GST, SOD, in MDA content and GSH levels in liver, kidney, and erythrocyte tissues according to the results obtained at the end of the experiment period.

During the inspection of serum enzyme levels in Table 4.1; The ALT enzyme level values of the 20% EtOH and 3% *O. onites* + 20% EtOH groups were statistically significantly higher when we compared it to the control group. The comparison of other treatment groups were insignificant statistically.

The AST enzyme level values of the 20% EtOH group were statistically significantly higher in comparison to the control group. On the other hand, there was a significant reduction in AST level values of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH groups in comparison with the control group. Also, there was a significant reduction in AST level values of 3% *O. onites* + 20% EtOH group in comparison with the 20% EtOH group. The comparison of other treatment groups were insignificant statistically.

There is a statistically valuable decrease in the TC level values of 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH groups in comparison with control. The comparison of other treatment groups were insignificant statistically.

There is a statistically valuable decrease in TP level values of 20% EtOH and 3% *O. onites* + 20% EtOH groups in comparison to the control group, on the other side, The TP level values of the 3% *O. onites* infusion group were statistically significantly higher in comparison with the 20% EtOH group. The comparison of other treatment groups were insignificant statistically.

In both ALT and AST serum enzyme levels ; a statistically valuable increase was shown in 20% EtOH group values when compared to control group. The increase in

the levels of these enzymes in our study may be the result of changes in the metabolism and detoxification activity of liver as a result of the toxic effects of EtOH. Because some researchers stated that the liver-derived Serum enzyme concentration such as , serum glutamic pyruvic transaminase , glutamic oxaloacetic transaminase, are both often multiplied in patients with alcoholism (Rosman and Lieber, 1994; Niemelä, 2002). There is a large amounts of these enzymes in the liver tissues and it is thought that rather than consumption of alcohol they indicate hepatocyte damage per se. 39–47% of alcohol-dependent humans has shown elevated AST degrees (Helander and Tabakoff ,1997; Hietala et al., 2006). The reductive transfer of an amino group from alanine or aspartate, is catalyzed by ALT and AST respectively, to alpha-ketoglutarate to glutamate and pyruvate or oxaloacetate, respectively. Harmed liver cells will discharge their contents counting AST and ALT into the extracellular space. Ultimately the serum levels of ALT and AST will increase as a result of the released proteins in the circulation which will lead to high levels of these enzymes in other groups compared to control subjects. AST is localized in heart, brain, skeletal muscle and liver tissue. ALT is essentially localized to liver, skeletal muscle and heart tissue have lower enzymatic activities in it. (Ozer et al., 2008). Serum AST levels are increased in hepatic maladies (Hayes, 1994)

Moreover, the AST to ALT proportion is seen as a valuable parameter for supporting alcoholic hepatitis diagnosis (Finlayson, 1993; Harrison and Burt, 1993; Bacon et al., 1994). up to 83% of the hospitalized patients for alcoholic hepatitis has shown AST level more than twice the ALT level (Pinto et al., 1993). Moreover, a recent study recommended that a high AST/ALT proportion recommends progressed alcoholic liver illness (Nyblom et al., 2004). when cellular degeneration or destruction happens in liver an increase in the serum ALT is detected (Hassoun and Stohs 1995).

On the other hand, a statistically valuable reduction was shown in the AST level values of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH groups when we compared them to control and 20% EtOH group. According to our research this decrease in the AST enzyme levels maybe idicator of the hepatoprotective and antioxidant effect of *O. onites* essential oil and its 3 major components linalool, carvacrol and thymol, because in a study made by Uyanoglu, et al. carvacrol showed no

adverse effect on liver functions AST and ALT and concluded that the liver regeneration rate is improved by cavacrol (Uyanoglu et al., 2008). In spite of this, there was a statistically valuable increase in the ALT enzyme level values of *O. onites* + 20% EtOH groups when compare to the control and this may be because that some components in the *O. onites* infusion may have intracted with EtOH and lead to an adverse antagonistic effect of *O. onites* on the ALT enzyme level.

All of ethanol oxidation pathways within the liver result in generation of acetaldehyde, liver oxidize more than 90% of the acetaldehyde shaped from the oxidation of ethanol (Lindros, 1974); it is also, generally believed that the central location of acetaldehyde digestion system lies within the mitochondria. Constant ethanol utilization comes about in a noteworthy diminishment within the capacity of mitochondria in rodent liver to oxidize acetaldehyde (Cohen and Collins, 1970). The unaltered or indeed upgraded rates of ethanol oxidation (and so acetaldehyde era) coupled with the diminished capacity of mitochondria of alcohol-fed animals to oxidize acetaldehyd (Lieber and DeCarli, 1970; Lieber and DeCARLI, 1972.) may lead to an imbalance between generation and disposition of acetaldehyde. That is why after chronic ethanol utilization high levels of blood acetaldehyde is detected in rats (Koivula and Lindros, 1975) humans (Korsten et al., 1975) and baboons (Pikkarainen et al., 1979) and As with ethanol, protein synthesis is inhibited within the liver after the addition of acetaldehyde (Perin et al., 1974; Perin and Sessa, 1975; Sorrell et al., 1977). Conflicting results in protein synthesis was seen in vivo after the chronic administration of ethanol. After a few weeks of administration of a blend of fluid and solid diets containing ethanol a diminished incorporation of labeled amino acids into liver proteins was found in rats nourished the ethanol-containing diets described by (Porta et al., 1968; Mørland, 1974; Mørland and Sjetnan, 1976). Furthermore, Morland (Mørland, 1974) detected a decrease in the ability of alcohol fed rat liver to corporate amino acids into protein after stimulation with dexamethasone, and reduced activity of enzymes (tyrosine aminotransferase, tryptophan oxygenase) involved in protein metabolism.

All of these results and possibilities may clarify the valuable reduction in the total protein level of EtOH-fed group in coparison with the control group in our study. Also, in a study made by proctor *et.al.* a reduced protein synthesis rate was found in all

glands after acute ethanol administration (Proctor et al., 1993). Many studies on rats studies point out that utilization of alcohol on a long period result in a decrease in the amount of excreted saliva, an altered electrolyte concentration and protein synthesis reduction (Scott et al., 1989; Shori et al., 1994)

On the other hand, a valuable increase was shown in the total protein level of the *O. onites* infusion supplemented groups in comparison with EtOH group and it nearly elevated it to the control group level and this may be an indication of the antioxidant effect of *O. onites* in eliminating the free radicals formed from the ethanol intoxication and decreasing the severity of oxidative stress.

An alternation in lipid homeostasis is instigated by ethanol utilization. lipoproteins and intracellular lipid carrier proteins, are now established as the goals of short- and long-term ethanol utilization (Hannuksela et al., 1992; Wood et al., 2001). high-density lipoprotein (HDL) transport cholesterol from the peripheral tissues again to the liver by a procedure known as Reverse cholesterol transport (RCT). (Marmillot et al., 2007) found that that long-term ethanol consumption considerably impairs not only cholesterol efflux function of HDL through reducing its SM content but additionally cholesterol uptake through affecting presumably hepatocyte receptors for HDL.

One of the vital components that control cellular homeostasis and function are lipids. The liver takes a fundamental part in several stages of lipid synthesis, transportation and lipid digestion system. So, it is sensible that those who have extreme liver dysfunction to have an unusual lipid profile. patients with hepatic failure and serious hepatitis has a noticeable reduction in plasma triglyceride (TG) and cholesterol levels because of a decrease in lipoprotein biosynthesis. In chronic liver maladies commonly low levels of cholesterol and TG is observed as a result of diminished liver biosynthesis capacity (Halsted, 2004).

In our study a valuable reduction was shown in the total cholesterol level of EtOH group in contrast to the control this may have resulted from the changes in lipid homeostasis and liver degeneration induced by ethanol abuse. Hobson et al. have detailed that in elderly men (over 60) the normal serum cholesterol concentration in heavy consumers are lower than that of abstainers or direct consumers.

The effect of *O. onites* infusion on MDA content in the liver, kidney and erythrocyte tissues are presented in table 4.2. As it can be seen in the table, it was determined that the antioxidant properties of *O. onites* infusion against EtOH caused different effects in various tissues.

In the liver tissue a statistically valuable reduction was found in MDA content of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group in comparison with the control group. On the other side, there was a statistically valuable increase in MDA values of 20% EtOH in comparison to the control group. Also, there is a statistically valuable reduction in MDA valuess of 3% *O. onites* + 20% EtOH in comparison to the 20% EtOH group.

In the kidney tissue a statistically valuable increase in the MDA values of 20% ETOH, 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group was found in comparison with the control group. Also, there is a statistically valuable reduction in MDA valuess of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group in comparison with the 20% EtOH group.

In erythrocyte tissue a statistically valuable reduction was found in MDA values of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group in comparison with the control group. Although there was an increase in the MDA values of the 20% EtOH group in comparison with to the control group, it was not statistically significant. Also, when we compared the MDA valuess of 3% *O. onites* + 20% EtOH group to that of 20% EtOH group there was a valuable reduction. The comparison of other treatment groups were statistically insignificant.

Lipid peroxidation can be defiened as a process in which lipids containing carbon-carbon double bond(s), particularly (PUFAs) are attacked by oxidants such as nonradical species and free radical and they substract a hydrogen from the carbon with oxygen addition resulting in lipid peroxyl radicals and hydroperoxides (Ayala et al., 2014).

Among free radicals, the hydroxyl radicals shows an incredible capacity to react with numerous biological molecules, changing membrane structure and inducing lipoperoxides. Therefore LP may be of major importance in cell harm created by the free radical mechanism (Halliwell, 1987). Prove has gathered regarding the part of LP

and oxygen- derived free radicals within the development of alcoholic liver damage (Albano et al.,1993). It was suggessted by (Muller and Sies, 1987; Fridovich, 1989) that the acetaldehyde and ethanol metabolism can encourage lipid peroxidation by debilitating antioxidant substances or free radical generation resulting in oxidative stress in liver (Dianzani, 1985). In any case, recent information demonstrates that in organs such as brain and heart which has poor alcohol digestion systems ethanol can moreover initiate lipid peroxidation in extrahepatic tissues (Nordmann et al., 1990).

Lipid peroxidation shows up to be a major source of endogenous DNA harm in people, which may contribute essentially to cancer and other hereditary maladies connected to the way of life and dietary variables (Marnett, 2002).

High MDA content is a critical marker of LP and MDA is one of the major oxidation outcomes of peroxidized polyunsaturated fatty acids (Freeman and Crapo, 1981). Circulating MDA is one of the foremost commonly and broadly utilized oxidative stress biomarkers (Draper and Hadley, 1990; Giustarini et al., 2009). biological samples contain low amounts of MDA so highly sensitive methods are required for its detection, generally the whole MDA (free and bound) is assessed. In this way, thiobarbituric acid (TBA) and biological samples reacts together, after that MDA±TBA adducts are formed and then they are identified spectrophotometrically (Nair and Turner, 1984; Kosugi et al., 1987).

In our study, the reason for the significant increase in MDA content levels in the EtOH group in comparison to other groups is likely because alcohol administration essentially diminished the antioxidant activities of biological systems and resulted in the aggregation of free radicals which along these lines started lipid peroxidation (Wang et al., 2012) which in turn increased MDA levels. And the higher levels of hepatic lipid peroxidation may be connected to the fact that the larger part of ethanol metabolism happens within the liver (Molina et al., 2003). A vital marker of lipid peroxidation is increased MDA content (Freeman and Crapo 1981). Moreover, the bunch by Marnett detailed in 2003 that MDA is mutagenic in human cells (Niedernhofer et al., 2003).

On the other side, the valuable reduction seen in MDA content level in the *O. onites* infusion supplemented groups in comparison to the control and EtOH group in liver and erythrocyte tissue may be an indication of the antioxidant effect of *O. onites* in

eliminating the free radicals formed from the ethanol intoxication as in a study made by (Dogan and Celik, 2012; Bati et al., 2015)

But in the kidney tissue there was a valuable increase in MDA content level of *O. onites* infusion supplemented groups in comparison to the control group but showed a valuable reduction in the MDA content level of *O. onites* infusion supplemented groups in comparison with the EtOH group and this is an indication of the antioxidant effect of *O. onites* however, this antioxidant effect was not enough to lower the MDA level to the control group level.

The results of the effect of *O. onites* infusion on GSH enzyme activity in liver, kidney and erythrocyte tissues are presented in Table 4.3. As it can be seen in the table, it was determined that the antioxidant properties of *O. onites* infusion against EtOH caused different effects in various tissues.

A valuable increase was found in GSH values of liver tissue in 20% EtOH group insignificant control group. On the other hand, there was a statistically valuable reduction when we compared the 3% *O. onites* + 20% EtOH group to the control group. Although there was a decrease within the GSH value of 3% *O. Onites* infusion group compared with the control group, it was statistically insignificant. Also, there was a statistically valuable reduction within the GSH level values 3% *O. onites* + 20% EtOH group compared with the 20% EtOH group.

In kidney tissue, a statistically valuable reduction was shown in the GSH levels of 3% *O. onites* + 20% EtOH group when compared to the control group. Although there was an increase within the GSH values of 20% EtOH group when compared to the control group, it was statistically insignificant. On the other hand, the *O. onites* + 20% EtOH group showed a valuable decrease in comparison with the 20% EtOH group. The comparison of GSH values of other treatment groups was statistically insignificant.

GSH values of Erythrocyte tissue were statistically significantly higher in 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH groups in comparison with the control group. Also, there was a statistically valuable increase in the GSH level values of 3% *O. onites* + 20% EtOH group in comparison with the 20% EtOH group.

GSH is a water-soluble tripeptide composed of the amino acids glutamine, cysteine, and glycine. The thiol group may be a powerful reducing agent, rendering

GSH the foremost abundant intracellular small molecule thiol, coming to millimolar concentrations in some tissues. As a critical antioxidant, GSH plays a part within the detoxification of a variety of electrophilic compounds and peroxides through catalysis by glutathione S-transferases (GST) and glutathione peroxidases (GPx) (Anderson, 1998). moreover, GSH plays a basic part in securing cells from oxidative harm and the harmfulness of xenobiotic electrophiles and keeping up redox homeostasis (Forman et al., 2009.).

Intracellularly GSH can be found in either reduced (GSH) or oxidized (GSSG) state. For the cell to survive GSH: GSSG ratios must be kept at an ideal rate within the cell, so a tight regulation of the system is basic A lack of GSH puts the cell at chance for oxidative harm since GSH plays a major part in the evacuation of many reactive species. It isn't shocking that GSH imbalance is watched in a wide extend of diseases, for example, neurodegenerative disarranges, cancer, cystic fibrosis (CF), aging and HIV. (Townsend et al., 2003).

There is a harmony between the detoxification capacities of this organ and the High intracellular content of GSH in it. changes in liver GSH are either the cause or effect of a number of diseasess. For example when the mitochondrial GSH pools are drained in alcoholics, this will aggravate ROS harm creating cell death and eventually cirrhosis. A fractional inactivation of a particular mitochondrial membrane transport protein leads to decreased levels of mitochondrial GSH (Fernandez-Checa et al., 2002).

Long term alcohol exposure leads to physicochemical changes to the internal mitochondrial membrane so it will be unable to transport the already builtup cytosolic GSH to the mitochondria and when the amount of GSH is decreased in this patricular organelle hepatocytes become sensetized to the oxidative impacts of cytokines such as tumor necrosis factor (TNF) (Fernandez-Checa, 1998). Ethanol treatment results in the reductıon of GSH levels and diminishes the antioxidant activity (Das and Vasudevan, 2007)

However, in our study a valuable increase in the GSH level of EtOH group was seen in comparison with the control group this may be resulted as a defense mechanism from the tissues to overcome the large amount of ROS resulted from EtOH metabolism

and as a result of this the GSH level increased as in a study made by (Yayalacı et al., 2014).

On the other hand, the differences in the levels of antioxidant defense constituents in the tissues of rats fed with *O. onites* infusion and EtOH may be due to the fact that the cellular physiological response and adaptation differ from tissue to tissue or the different levels of exposure of these tissues to alcohol and *O. onites* bioactive active substances.

The results of the effect of *O. onites* infusion on SOD enzyme activity in liver, kidney and erythrocyte tissues are presented in Table 4.4. As it can be seen in the table, it was determined that the antioxidant properties of *O. onites* infusion against EtOH caused different effects in various tissues.

In the liver tissue, there was a statistically valuable reduction in SOD values of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group in comparison with the control group. Although there has been an increase in the SOD values of the 20% EtOH group in comparison with the control group, it was statistically insignificant. Also, there was a statistically valuable reduction in SOD values of 3% *O. onites* + 20% EtOH group in comparison with the 20% EtOH group.

In kidney tissue; a statistically valuable increase was shown in the SOD levels of 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group in comparison with the control group. Also, a statistically valuable increase was shown in the SOD levels of 3% *O. onites* + 20% EtOH group when we compaired it with the 20% EtOH group.

In erythrocyte tissue a statistically valuable reduction was shown in the SOD levels of 20% EtOH , 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH groups in comparison with the control group. The comparison of SOD values of other treatment groups was statistically insignificant.

One of the endogenous antioxidant defense system constituents is SOD and it is in charge of Superoxide radical dissemination and controlling the impacts of oxidative stress along with glutathione peroxidase and other constituents of antioxidant enzyme system. because any modifications in antioxidant action can lead to oxidative stress and these changes can be either suppression or induction of the enzyme. Our body uses

antioxidant enzymes such as SOD during oxidative stress to minimize the process of lipid peroxidation, hence, the action of this enzyme gets to be higher in early stages of stress but if this stress condition continues eventually this enzyme will deplet and will be unable to fight against free radicals, which implies that in progress stages of peroxidation the activity of SOD decreases. On the other hand, SOD action increments in case of short term Oxidative Stress or if it became very long-during.

SOD by the virtue of their capacity to scavenge $O_2^{-\bullet}$ play a noteworthy part in securing living cells against the mutagenicity and harmfulness of active O_2 (Scandalios, 1993). The noteworthiness and biological role of SOD are borne out in various studies with lower and higher eukaryotes, prokaryotes and higher plants as a defensive enzymes against O_2 toxicity (Fridovich, 1986; Hassan and Scandalios, 1990; Scandalios, 1990; Gralla and Kosman, 1992; Scandalios, 1992). Moreover, various studies with eukaryotes and prokaryotes shows that oxidative stress incite or increases the action of SOD (Scandalios, 1990; Bowler et al., 1992 ; Gralla and Kosman, 1992).

The impacts of chronic alcohol exposure on the cellular content or action of SODs are disputable, with reports of increments, no changes, or diminishing, depending on the model, diet, sum, and duration of alcohol feeding. (Cederbaum et al., 2009). In our study, the increase in the SOD activity of ethanol group may be due to the increase of superoxide radicals or the increment in SOD activity observed within the cell together was due to the size of the oxidative stresses and consequent adjustment to it (Koner et al., 1988). It is additionally conceivable that prime levels of oxidative stress could end in high SOD protein turnover, leading to the need for brand spanking new SOD catalyst synthesis to take care of SOD levels adequate for viable security (Scandalios, 1993).

It has been reported that the decrease in the antioxidant enzymes is resulted from the prolonged period of lipid peroxidation as a result of an increase in free radicals and so it overcame the antioxidant defense system (Meister and Anderson, 1983: Akkuş, 1995).

On the other hand, the differences in the levels of antioxidant defense systems in the tissues of rats fed with *O. onites* infusion and EtOH may be due to the fact that the cellular physiological response and adaptation differ from tissue to tissue or the

different levels of exposure of these tissues to alcohol and *O. onites* bioactive active substances.

The results of the effect of *O. onites* infusion on CAT enzyme activity in liver, kidney and erythrocyte tissues are presented in Table 4.5. As it can be seen in the table, it was determined that the antioxidant properties of *O. onites* infusion against EtOH caused different effects in various tissues.

In the liver tissue a statistically valuable reduction was shown in the CAT values of 3% *O. onites* + 20% EtOH group when compared to the control group. Although there has been an increase in the CAT values of the 20% EtOH group compared to the control group, it was statistically insignificant. Also, there has been a statistically valuable reduction in the CAT valuess of 3% *O. onites* + 20% EtOH group in comparison with the 20% EtOH group .

In the kidney tissue there is a statistically valuable reduction in the CAT values of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group in comparison with the control group. Although an increase was show in the CAT values of the 20% EtOH group compared to the control group, it was statistically insignificant. Also, there was a statistically valuable reduction in the CAT values of 3% *O. onites* + 20% EtOH group in comparison with the 20% EtOH group.

In the Erythrocyte tissue; a statistically valuable reduction was shown in the CAT values of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group in comparison with the control group. Although an increase was seen in the CAT values of the 20% EtOH group when compared to the control group, it was statistically insignificant. Also, a statistically valuable reduction was seen in the CAT valuess of 3% *O. onites* + 20% EtOH group in comparison with the 20% EtOH group. The comparison of CAT values of other treatment groups was statistically insignificant.

CAT is a prevalent constituent of antioxidant enzymes that is present in nearly all living tissues that utilize oxygen. The enzyme uses cofactors such as manganese or iron to catalyzes the reduction or degradation of H_2O_2 to atomic oxygen and water, eventually finishing the detoxification process stimulated by SOD (Marklund, 1984, Chelikani et al., 2004)

This enzyme is copious within the cell where it is constantly in search of hydrogen peroxide particles and CAT is exceedingly effective; in one second it can moulder millions of hydrogen peroxide particles. Basicaly this enzyme is found within the peroxisomes but mammalian cells lack this enzyme in their mitochondria (Radi et al.,1991). So the hydrogen peroxide in the mitochondria of mammalian cell is broken down to water and oxygen by another enzyme known as glutathione peroxidase. Catalase plays a vital part in eliminating higher intracellular H_2O_2 concentrations (Halliwell and Gutteridge, 2007).

In spite of the fact that low sums of Hydrogen peroxide tends to control a few physiological processes (Dröge, 2002), elevated concentrations of it are though to be exceptionally harmful to cells (Ercal, 2001). So, the capacity of CAT to successfully restrain H_2O_2 rate within the cells emphasize its value within the previously mentioned physiological operations also being the first line antioxidant defense enzyme. Different illness conditions and anomalies has been connected to the the lack or transformation of this enzyme (Góth et al., 2004).

Koller and Zámocky´ related oxidative DNA harm and the consequent chance of cancer vulnerability in people with hereditary polymorphisms and changed gene expression/ activity in catalase (Zámocky´ and Koller 1999).

According to Albano et al., (1998) and Cederbaum, (2001), modification in the levels of both nonenzymatic and enzymatic endogenous antioxidant systems and activation of free radical generation occur during the chronic alcohol utilization. This results in a cascade of impacts of oxidative stress , hence, influencing both the structural and functional integrity of cells and organelles membranes (De Leve et al., 1996).

The induction of this enzyme can be seen as an adaptive reaction to oxidative stress as it play a protective role against oxygen derived free radical-induced harm (Patlolla, et al., 2009). Comparable metabolic adjustments of catalase have been observed by other authors in different tissues of rats exposed to ethanol (Omodeo-Sale., 1997). Or an increment in CAT action in tissues would show upgraded ethanol tolerance by these specific tissues (Husain et al., 2001). moreover, there was an increment in catalase action in all examined patients enduring from oxidative stress (cardiovascular illnesses, diabetes, tumor, inflammation, dermatological maladies, anemia and Wilson's

disease) (Al-Abrash et al., 2000). The reason for the high levels of both SOD and CAT in our study may be related to high levels of SOD because it catalyzes the dismutation of superoxide anion to H_2O_2, which is, in turn, the substrate of CAT so to elemenat these high levels of H_2O_2 the catalase synthesis will increase .

On the other side, a valuable reduction was seen in the tissues of rats fed with *O. onites* infusion in cmparison with EtOH group and this is a sure sign of antioxidant effect of *O. onites* in protecting the cells from the toxic effect of ethanol metabolism and the oxidative stress formed from excess free radical production.

The results of the effect of *O. onites* infusion on GR enzyme activity in liver, kidney and erythrocyte tissues are presented in Table 4.6. As it can be seen in the table, it was determined that the antioxidant properties of *O. onites* infusion against EtOH caused different effects in various tissues.

In the liver tissue a statistically valuable reduction was seen in the GR values of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group in comparison with the control group. Although an increase was seen in the GR values of the 20% EtOH group when compared to the Control, it was statistically insignificant. Also, there was a statistically valuable reduction in the GR valuess of 3% *O. onites* + 20% EtOH group in comparison with the 20% EtOH group.

In the kidney tissue; a statistically valuable reduction was seen in the GR values of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group in comparison with the control group. Although an increase was seen in the GR values of the 20% EtOH group when compared to the control group, it was statistically insignificant. Also, there was a statistically valuable reduction in the GR valuess of 3% *O. onites* + 20% EtOH group in comparison with the 20% EtOH group.

In the erythrocyte tissue; a statistically valuable reduction was seen in the GR values of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group when we compaired them to the control group. Although an increase was seen in the GR values of the 20% EtOH group when compared to the control group, it was statistically insignificant. Also, there was a statistically valuable reduction in the GR valuess of 3% *O. onites* + 20% EtOH group in comparison with the 20% EtOH group. The comparison of GR values of other treatment groups was statistically insignificant.

One of the fundamental enzyme that recycles oxidized glutathione back to its reduced shape is Glutathione reductase (Couto et al., 2016). Pentose phosphate shunt predominantly keeps the the level of (NADPH) which acts as an reductant electron giver.

Both of GSH and GSSG take a main part in cellular redox homeostasis. By giving reducing counterparts to glutathione peroxidase, GSH helps to detoxify reactive oxygen species and also it helps to detoxify electrophilic xenobiotics along with glutathione S-transferase (Fagan and Palfey, 2010). The GSSG rate is kept up at ~1% of total glutathione concentration in normal physiological conditions, and any increments in GSSG rate are neutralized by its fast reduction by GR (Forman et al., 2002). However in serious oxidative stress condition GR activity is disabled resulting in high levels of GSSG within the cell which majorly can be effectively expelled through particular transporters (Gutierrez-Correa and Stoppani, 1997; Vander et al., 1997). Moreover, Glutathione reductase by increasing the level of reduced glutathione (GSSGR) within the process of aerobic glycolysis, plays a critical part in securing biological cell membranes, red cell enzymes and hemoglobin. Mild to moderately serious hemolytic anemia can be resulted from the insufficiency of this enzyme upon introduction to certain chemicals or drugs (Chang et al., 1978).

The high positive relationship between GR action and GSH level proposes that the increment in reductase action is the defense mechanism by which GSH accessibility is protected (Lukaszewicz-Hussain and Moniuszko-Jakoniuk, 2004). Or the high GR action may be due to the increment of peroxidative components in cells.

On the other hand, a valuable reduction was seen in the tissues of rats fed with *O. onites* infusion in cmparison with EtOH group and this is a sure sign of antioxidant effect of *O. onites* in protecting the cells from the toxic effect of ethanol metabolism and the oxidative stress formed from excess free radical production.

The results of the effect of *O. onites* infusion on GST enzyme activity in liver, kidney and erythrocyte tissues are presented in Table 4.7. As it can be seen in the table, it was determined that the antioxidant properties of *O. onites* infusion against EtOH caused different effects in various tissues.

In the liver tissue a statistically valuable reduction was seen in the GST values of 3% *O. onites* + 20% EtOH group in comparison with the control group. Although an increase was seen in the GST values of the 20% EtOH group when compared to the control, it was statistically insignificant. Also, there was a statistically valuable reduction in the GST valuess of 3% *O. onites* + 20% EtOH group when compared to the 20% EtOH group.

In the kidney tissue; a statistically valuable reduction was seen in the GST values of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group in comparison with the control group. Although an increase was seen in the GST values of the 20% EtOH group when compared to the control, it was statistically insignificant. Also, there was a statistically valuable reduction in the GST valuess of 3% *O. onites* + 20% EtOH group when compared to the 20% EtOH group.

In the erythrocyte tissue; a statistically valuable increase was seen in the GST values of 20% EtOH group when compared to the control group. On the other hand, a statistically valuable reduction was seen in the GST valuess of 3% *O. onites* + 20% EtOH group when compared to the 20% EtOH group. The comparison of GR values of other treatment groups was statistically insignificant.

GSTs possess different biological functions, they are included in the modification and synthesis of prostaglandins and leukotrienes, counting cell security against several poisonous particles and oxidative stress (Hayes et al., 2005). for instance, GSTs secure cellular DNA against oxidative harm that can induce DNA harm advancing carcinogenesis or lead to an increment of DNA transformations (Li et al., 2009)

GSTs are able to conjugate numerous therapeutic drugs, carcinogens and numerous oxidative metabolism products which are electrophilic and hydrophobic molecules to glutathione making them less harmful and predisposed to further alteration for release from the cell (Hayes et al., 2005). GSTs have a part in nomerous other catalytic functions in bonus its classical conjugation reactions,. GSTs catalyze the reduction of organic hydroperoxides to their matching alcohols by displaying glutathione peroxidase action. Fatty acids, phospholipids, and DNA hydroperoxides

created by oxidative harm to DNA and lipid peroxidation are among the compounds that this enzyme reduces (Hayes et al., 2005).

The increase in free radicals in the organism causes oxidative stress. So, to eliminate this stress the activity of antioxidant enzymes is increased. So, in our research we concluded that the increase in GST enzyme may be was due to the induction of an antioxidant adaptation system. An adaptive change against ethanol-induced lipid peroxide toxicity may be the reason for this increased GST activity (Sonde et al., 2000; Yayalaci et al., 2014; Turan and Celik, 2016). On the other hand, the *O. onites* supplemented groups showed a valuable reduction in contrast with the control and EtOH group which reflect the antioxidant effect of *O. onites* in lowering the toxic effects ethanol and protecting the cells from the oxidative stress.

The results of the effect of *O. onites* infusion on GSH-Px enzyme activity in liver, kidney and erythrocyte tissues are presented in Table 4.8. As it can be seen in the table, it was determined that the antioxidant properties of *O. onites* infusion against EtOH caused different effects in various tissues.

In the liver tissue a statistically valuable increase was seen in the GSH-Px values of 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH group in comparison with the control group. Although an increase in the GSH-Px values of the 20% EtOH group was seen when we compared it to the control group, it was statistically insignificant.

GSH-Px values of kidney tissue were statistically significantly higher in 20% EtOH and 3% *O. onites* + 20% EtOH groups in comparison to the control group. Although a reduction was seen in the GSH-Px value of 3% *O. onites* infusion group when compared to the control group, it was statistically insignificant. Also, a statistically valuable reduction was seen in the GSH-Px level values of 3% *O. onites* + 20% EtOH group in comparison with the 20% EtOH group.

In the erythrocyte tissue; a statistically valuable reduction was seen in the GSH-Px values of 3% *O. onites* infusion group when compared to the control group. Although an increase in the GSH-Px values of the 20% EtOH group was seen when compared to the control group, it was statistically insignificant.

GPx-1 uses GSH as an compel cosubstrate when it reduces hydrogen peroxide to water and generally the GPxs are homologous to the selenocysteine (Sec)- containing

mammalian GPx-1(Lubos et al., 2011). Accordingly, GPx-1 is a valuable antioxidant enzyme included in avoiding the destructive aggregation of H_2O_2 within the cell. This enzyme is found in all cells; found in mitochondria, cytosolic, and, in peroxisomal compartments of some cells and this enzyme is more viable than catalase at expelling intracellular peroxides beneath numerous physiological conditions (Cohen and Hochstein, 1963; Antunes et al., 2002).

One of the few cellular enzymes that may balance the general redox stress is GPx-1, vulnerability of cells to oxidative stress will advance when GPx-1 action is deminished because this will allow the aggregation of destructive oxidants, while overabundance GPx-1 may advance reductive stress which is identified by their need of fundamental ROS required for cellular signaling processes. Advance apoptotic pathways and diminished cell development can result from loss of basic ROS or abundance of oxidants. lipid hydroperoxides and hydrogen peroxide are inactivated by GPx-1 at the cost of GSH, which is oxidized to create GSSG. The enzyme glutathione reductase (GR) utilizes NADPH as a source of reducing reciprocals when it recycles GSSG back to GSH, and cellular stores of NADPH are keeps up by (G6PD) (Lubos et al., 2011).

It is conceivable that the rises watched in this enzyme represent a compensatory rise in antioxidant action in reaction to increased free radical generation (Mackenzie, 2002). Lipid peroxidation and increased action of free radicals over a certain period of time may increment the enzyme action. But it is detailed that antioxidant enzyme activities may diminish in case cellular antioxidant defense system is surpassed due to prolonged increment in free radical formation and lipid peroxidation (Meister and Anderson, 1983; Akkuş, 1995).

5.1. Conclusion

According to the biochemical data obtained in this study, it was summarized that EtOH caused an increase in the levels of serum enzymes and a decrease in TC and TP which are indicative of liver injury due to the damage caused in liver hepatocytes. Also, as a result of oxidative stress caused by EtOH, MDA the final product of lipid peroxidation and an indicator of free radical production, has increased in all tissues, whereas in many tissues it has caused fluctuations in the antioxidant defense system constituents. On the other hand, it was observed that the *O. onites* infusion supplemented groups attracted AST close to that of control group. In addition, *O. onites* infusion supplemented groups caused a decrease or attracted the increased MDA content in all tissues as a result of EtOH formed oxidative stress close to that of control groups.

Although these effects of *O. onites* cannot be stated, but it has been concluded that the contents of essential oil of oregano carvacrol and/or thymol as the major constituent(s) and other minor components such as p-cymene, c-terpinene, terpinen-4-ol, linalool, sabinene hydrate, and terpinen-4-ol (Kokkini, 1997; D'antuono et al., 2000). The major constituent of the Essential oil of *O. onites* is the phenolic compound carvacrol although linalool and thymol chemotypes are observed rarely in wild populations they may have hepatoprotective and antioxidant effect.

On the other hand, in rats fed with feeds containing *O. onites* infusion, the reason for its different levels of effects on lipid peroxidation and antioxidant defense systems levels in the tissues, may be due to the fact that the cellular physiological adaptation is different from tissue to tissue.

In this study, the protective efficacy of *O. onites* against necrosis was investigated. According to obtained results, lipid peroxidation and hence liver injury were prevented due to the antioxidant effect of *O. onites*.

The observations made at the end of our research led us to summarize that while administration of sub-chronic ethyl alcohol promotes MDA concentration fluctuations in the antioxidative systems and increases liver damage serum marker enzymes, the thyme infusion supplement provides protection against alcohol-induced liver injury and

oxidative stress. These observations, along with changes, might also suggest that such a test will also be of value in chemo preventive studies, and also be of interest to understand the molecular basis of the refractoriness of the protective role of *Origanum onites*. Also, we would like to study destructive effects of alcohol and the healing effects of *Origanum onites* against alcohol before coming to any conclusion. Nevertheless, the results suggest that regular intake of the functional food may be useful for the prevention of chronic degenerative liver diseases.

REFERENCES

Abdal Dayem, A., Hossain, M.K., Lee, S.B., Kim, K., Saha, S.K., Yang, G.M., Choi, H.Y., Cho, S.G., 2017. The role of reactive oxygen species (ROS) in the biological activities of metallic nanoparticles. ***International Journal of Molecular Sciences***, **18** (1): 120.

Aebi, H., 1974. ***Catalase, In Methods of Enzymatic Analysis*** (Bergemeyer, H U.,ed) Academic Press, New York-London. 673-684.

Akkus, I. 1995. ***Serbest Radikaller ve Fizyopatolojik Etkileri***. 1. Baski Konya: Mimoza Yayınları, **5**: 3-95.

Aksoy, Y., Balk, M., ÖĞÜŞ, H., Özer, N., 2005. The mechanism of inhibition of human erythrocyte catalase by azide. ***Turkish Journal of Biology***, **28** (2-4): 65-70.

Al-Abrash, A.S., Al-Quobaili, F.A., Al-Akhras, G.N., 2000. Catalase evaluation in different human diseases associated with oxidative stress. ***Saudi Medical Journal***, **21** (9): 826-830.

Albano, E., Clot, P., Tabone, M., Arico, S. and Ingelman-Sundberg, M., 1993. Oxidative damage and human alcoholic liver diseases. Experimental and clinical evidence. **In *Free Radicals: From Basic Science to Medicine***, Birkhäuser, Basel.

Albrich, J.M., McCarthy, C.A., Hurst, J.K., 1981. Biological reactivity of hypochlorous acid: implications for microbicidal mechanisms of leukocyte myeloperoxidase. ***Proceedings of the National Academy of Sciences***, **78** (1): 210-214.

Anderson, M.E., 1998. Glutathione: an overview of biosynthesis and modulation. ***Chemico-Biological Interactions***, **111**: 1-14.

Andoğan, B.C., Baydar, H., Kaya, S., Demirci, M., Özbaşar, D., Mumcu, E., 2002. Antimicrobial activity and chemical composition of some essential oils. ***Archives of Pharmacal Research***, **25** (6): 860-864.

Andrew, P.J., Mayer, B., 1999. Enzymatic function of nitric oxide synthases. ***Cardiovascular Research***, **43** (3): 521-531.

Anonim, 2010**.** ***http://www.ibreliler.com/tubives/bitki-04427-apiaceae-heracleum-persi-desf.html*****.** Erişim tarihi: 04.05.2016.

Antunes, F., Han, D., Cadenas, E., 2002. Relative contributions of heart mitochondria glutathione peroxidase and catalase to H_2O_2 detoxification in in vivo conditions. ***Free Radical Biology and Medicine***, **33** (9): 1260-1267.

Aslani, B.A., Ghobadi, S., 2016. Studies on oxidants and antioxidants with a brief glance at their relevance to the immune system**.** ***Life Sciences***, **146**: 163-173.

Ayala, A., Muñoz, M.F., Argüelles, S., 2014. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. ***Oxidative Medicine and Cellular Longevity***, 2014.

Aykaç G, Uysal M, Suha Yalcin A, 1985. The effect of chronic ethanol ingestion on hepatic lipid peroxide, glutathione, glutathione peroxidase and glutathione transferase in rats. ***Toxicology.***, **36** (1): 71–76.

Azcan, N., Kara, M., Demirci, B., Başer, K.H.C., 2004. Fatty acids of the seeds of *Origanum onites* L. and *O. vulgare* L. ***Lipids***, **39** (5): 487-489.

Bacon, B.R., Farahvash, M.J., Janney, C.G., Neuschwander-Tetri, B.A., 1994. Nonalcoholic steatohepatitis: an expanded clinical entity. ***Gastroenterology***, **107** (4): 1103-1109.

Badaloo, A. V., Marshall, K. G. (2014). Oxidative stress in childhood severe acute malnutrition. **In *Role of Oxidative Stress in Chronic Diseases***. Boca Raton, Florida: CRC Press.

Bánhegyi, G., Csala, M., Szarka, A., Varsányi, M., Benedetti, A., Mandl, J., 2003. Role of ascorbate in oxidative protein folding. ***Biofactors***, **17** (1-4): 37-46.

Baranauskaitė, J., Jakštas, V., Ivanauskas, L., Kopustinskienė, D.M., Drakšienė, G., Masteikova, R., Bernatonienė, J., 2016. Optimization of carvacrol, rosmarinic, oleanolic and ursolic acid extraction from oregano herbs (*Origanum onites* L., *Origanum vulgare* spp. hirtum and *Origanum vulgare* L.). ***Natural Product Research***, **30** (6): 672-674.

Barja, G., 2000. The flux of free radical attack through mitochondrial DNA is related to aging rate. ***Aging Clinical and Experimental Research***, **12** (5), 342-355.

Bashir, A., Perham, R.N., Scrutton, N.S., Berry, A., 1995. Altering kinetic mechanism and enzyme stability by mutagenesis of the dimer interface of glutathione reductase. ***Biochemical Journal***, **312** (2): 527-533.

Bati, B., Celik, I., Dogan, A., 2015. Determination of hepatoprotective and antioxidant role of walnuts against ethanol-induced oxidative stress in rats. ***Cell Biochemistry and Biophysics***, **71** (2): 1191-1198.

Baydar, H., Sağdiç, O., Özkan, G., Karadoğan, T., 2004. Antibacterial activity and composition of essential oils from Origanum, Thymbra and Satureja species with commercial importance in Turkey. ***Food Control***, **15** (3): 169-172.

Bayramoğlu, M., 2008. ***Artemisia taurica willd. ve Salvia kronenburgii Rech. Fil. Bitkilerinin Uçucu Yağlarının Antioksidan Özellikleri ve Ksantin Oksidaz Enzimine Etkileri*** (Master's thesis)**.** YYU, Institute of Science and Technology, Van

Beckman, J.S., Koppenol, W.H., 1996. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. ***American Journal of Physiology-Cell Physiology***, **271** (5): C1424-C1437.

Berg, J.M., Tymoczko, J.L., Gatto Jr, G.J., 2002. Stryer: ***Biochemistry***. WH Freeman and Company, **5**: 306-307.

Berg, J.M., Tymoczko, J.L., Stryer, L., 2002. ***Biochemistry***. 5th edition.WH Freeman, New York.

Berk, P., Korenblat, K., 2012. Approach to the patient with jaundice or abnormal liver tests. In ***Goldman's Cecil Medicine***. Twenty Fourth Edition. WB Saunders.

Beutler, E., Duron, O., Kelly, B.M., 1963. Improved method for the determination of blood glutathione. ***Journal of Laboratory and Clinical Medicine,*** **61** (5): 882-888.

Bielski, B.H., Cabelli, D.E., 1995. Superoxide and hydroxyl radical chemistry in aqueous solution. **In *Active Oxygen in Chemistry***. Springer, Dordrecht.

Bielski, B.H., Cabelli, D.E., Arudi, R.L., Ross, A.B., 1985. Reactivity of $HO_2/O^{-}2$ radicals in aqueous solution. ***Journal of Physical and Chemical Reference Data***, **14** (4): 1041-1100.

Bostancıoğlu, R.B., Kürkçüoğlu, M., Başer, K.H.C., Koparal, A.T., 2012. Assessment of anti-angiogenic and anti-tumoral potentials of *Origanum onites* L. essential

oil. ***Food and Chemical Toxicology***, **50** (6), 2002-2008.

Bowler, C., Montagu, M.V., Inze, D., 1992. Superoxide dismutase and stress tolerance. ***Annual Review of Plant Biology***, **43** (1): 83-116.

Carlberg, I., Mannervik, B., 1985. Glutathione reductase. **In *Methods in Enzymology,* 113**: 484-490.

Carney Almroth, B., 2008. ***Oxidative Damage in Fish Used as Biomarkers in Field and Laboratory Studies***. Department of Marine Ecology; Institutionen för marin ekologi.

Casareno, R.L.B., Waggoner, D., Gitlin, J.D., 1998. The copper chaperone CCS directly interacts with copper/zinc superoxide dismutase. ***Journal of Biological Chemistry***, **273** (37): 23625-23628.

Catalá, A., Díaz, M., 2016. Impact of lipid peroxidation on the physiology and pathophysiology of cell membranes. ***Frontiers in Physiology***, **7**: 423.

Cederbaum, A.I., Lu, Y., Wu, D., 2009. Role of oxidative stress in alcohol-induced liver injury. ***Archives of Toxicology***, **83** (6): 519-548.

Cerutti, P.A., 1985. Prooxidant states and tumor promotion. ***Science***, **227** (4685): 375-381.

Cetin, H., Erler, F., Yanikoglu, A., 2006. Toxicity of essential oils extracted from Origanum onites L. and Citrus aurentium L. against the pine processionary moth, Thaumetopoea wilkinsoni Tams. ***Folia Biologica***, **54** (3-4): 153-157.

Chae, H.Z., Kang, S.W., Rhee, S.G., 1999. Isoforms of mammalian peroxiredoxin that reduce peroxides in presence of thioredoxin. In ***Methods in Enzymology.*** Academic Press, **300**: 219-226.

Chang, J.C., van der Hoeven, L.H., Haddox, C.H., 1978. Glutathione reductase in the red blood cells. ***Annals of Clinical & Laboratory Science***, **8** (1): 23-29.

Cheeseman, KH., Slater, TF., 1993. An Introduction to radical biochemistry. ***Britsh Medical Bulletin,* 49**: 481–493.

Chelikani, P., Fita, I., Loewen, P.C., 2004. Diversity of structures and properties among catalases. ***Cellular and Molecular Life Sciences***, **61** (2): 192-208.

Chi-Tang Ho, ***Food Phytochemicals for Cancer Prevention***, vol. 11, 1st Edition, American Chemical Society, Washington DC, 1994, 428.

Chiueh, C.C., 1999. Neuroprotective properties of nitric oxide. ***Annals of the New York Academy of Sciences***, **890** (1): 301-311.

Cho, S.H., Lee, C.H., Ahn, Y., Kim, H., Kim, H., Ahn, C.Y., Yang, K.S., Lee, S.R., 2004. Redox regulation of PTEN and protein tyrosine phosphatases in H_2O_2-mediated cell signaling. ***Federation Of European Biochemical Societies***, **560** (1-3): 7-13.

Cohen, G., Collins, M., 1970. Alkaloids from catecholamines in adrenal tissue: possible role in alcoholism. ***Science***, **167** (3926): 1749-1751.

Cohen, G., Hochstein, P., 1963. Glutathione peroxidase: the primary agent for the elimination of hydrogen peroxide in erythrocytes. ***Biochemistry***, **2** (6): 1420-1428.

Cortez Pinto, H., Baptista, A., Camilo, M.E., Valente, A., Saragoca, A., Carneiro de mura, M., 1996. Nonalcoholic steatohepatitis: clinicopathological comparison with alcoholic hepatitis in ambulatory and hospitalized patients. ***Digestive Diseases and Sciences***, **41** (1): 172-179.

Coskun, S., Girisgin, O., Kürkcüoglu, M., Malyer, H., Girisgin, A.O., Kırımer, N., Baser, K.H., 2008. Acaricidal efficacy of Origanum onites L. essential oil against Rhipicephalus turanicus (Ixodidae). ***Parasitology Research***, **103** (2): 259-261.

Couto, N., Wood, J., Barber, J., 2016. The role of glutathione reductase and related enzymes on cellular redox homoeostasis network. ***Free Radical Biology and Medicine***, **95**: 27-42.

Czapski, G., Goldstein, S., 1995. The role of the reactions of· NO with superoxide and oxygen in biological systems: A kinetic approach. ***Free Radical Biology and Medicine***, **19** (6): 785-794.

d'Antuono, L.F., Galletti, G.C., Bocchini, P., 2000. Variability of essential oil content and composition of *Origanum vulgare* L. populations from a North Mediterranean Area (Liguria Region, Northern Italy). ***Annals of Botany***, **86** (3): 471-478.

Das, S.K., Vasudevan, D.M., 2007. Alcohol-induced oxidative stress. ***Life Science****s*, **81** (3): 177-187.

de Grey, A.D., 2002. HO2•: The forgotten radical. ***DNA and Cell Biology***, **21** (4): 251-257.

De Martino, L., De Feo, V., Formisano, C., Mignola, E., Senatore, F., 2009. Chemical composition and antimicrobial activity of the essential oils from three chemotypes of *Origanum vulgare* L. ssp. hirtum (Link) Ietswaart growing wild in Campania (Southern Italy). ***Molecules***, **14** (8): 2735-2746.

Demple, B., Levin, J., 1991 Repair systems for radical-damaged DNA. *In*: H. Sies (ed.): ***Oxidative Stress***, Academic Press, London, 119–154.

Devasagayam, T.P., Ippendorf, H., Werner, T., Martin, H.D., Siesa, H., 1992. Carotenoids, novel polyene polyketones and new capsorubin isomers as efficient quenchers of singlet molecular oxygen. In ***Lipid-Soluble Antioxidants: Biochemistry and Clinical Applications***, Birkhäuser, Basel.

Devlin, T.M., 2006. ***Textbook of Biochemistry with Clinical Correlations***. willy-liss, 6 th.

Diamond, I., Messing, R. O., 1994. Neurologic effects of alcoholism. ***Western Journal of Medicine,*** **161** (3): 279–287.

Rauch, A.E., Weininger, R., Pasquale, D., Burkart, P.T., Dunn, H.G., Weissman, C., Rydzak, E., 1994. Superwarfarin poisoning: a significant public health problem. ***Journal of Community Health***, **19** (1): 55-65.

Dianzani, M.U., 1985. Lipid peroxidation in ethanol poisoning: a critical reconsideration. ***Alcohol and Alcoholism***, **20** (2): 161-173.

Diler, O., Gormez, O., Diler, I., Metin, S., 2017. Effect of oregano (*Origanum onites* L.) essential oil on growth, lysozyme and antioxidant activity and resistance against Lactococcus garvieae in rainbow trout, Oncorhynchus mykiss (Walbaum). ***Aquaculture Nutrition***, **23** (4): 844-851.

Dizdaroglu, M., Jaruga, P., Birincioglu, M., Rodriguez, H., 2002. Free radical-induced damage to DNA: mechanisms and measurement. ***Free Radical Biology and Medicine***, **32** (11): 1102-1115.

Dogan, A., Celik, I., 2012. Hepatoprotective and antioxidant activities of grapeseeds against ethanol-induced oxidative stress in rats. ***British Journal of Nutrition***, **107** (1): 45-51.

Doğan, A., Çelik, İ., 2010, ***Etil Alkol ile Deneysel Oksidatif Stres Oluşturulan Sıçanlarda Üzüm (Vitis vinifera* L.*) Çekirdeğinin Karaciğer Koruyucu ve Antioksidan Rolünün Belirlenmesi*** (Master's thesis)**.** YYU Institute of Science and Technology, Van.

Dolphin, D., Poulson, R., Avramović, O., 1989. ***Glutathione: Chemical, Biochemical, and Medical Aspects***. John Wiley & Sons Inc.

Douki, T., Cadet, J., 1996. Peroxynitrite mediated oxidation of purine bases of nucleosides and isolated DNA. ***Free Radical Research***, **24** (5): 369-380.

Draper, H.H., Hadley, M., 1990. (43) Malondialdehyde determination as index of lipid Peroxidation. ***Methods in Enzymology,*** **186**: 421-431.

Droge, W., 2002. Free radicals in the physiological control of cell function. ***Physiological Reviews***, **82** (1): 47-95.

Ďuračková, Z., 1998. ***Free Radicals and Antioxidants in Medicine*** (I). *SAP, Bratislava*.

Economou, G., Panagopoulos, G., Tarantilis, P., Kalivas, D., Kotoulas, V., Travlos, I.S., Polysiou, M., Karamanos, A., 2011. Variability in essential oil content and composition of *Origanum hirtum* L., *Origanum onites* L., *Coridothymus capitatus* (L.) and *Satureja thymbra* L. populations from the Greek island Ikaria. ***Industrial Crops and Products***, **33** (1): 236-241

Ercal, N., Gurer-Orhan, H., Aykin-Burns, N., 2001. Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. ***Current Topics in Medicinal Chemistry***, **1** (6): 529-539.

Evan, A.P., Gardner, K.D., 1979. Nephron obstruction in nordihydroguaiaretic acid-induced renal cystic disease. ***Kidney International***, **15** (1): 7-19.

Evans, G.O., 2009. ***Animal Clinical Chemistry: a Practical Handbook for Toxicologists and Biomedical Researchers***. CRC Press.

Fagan RL., Palfey BA (2010) Flavin-dependent enzymes. In ***Comprehensive Natural Products II*** (Begley TP, ed.), 37–114. Elsevier, Amsterdam.

Fenton, H.J.H., 1894. LXXIII.—Oxidation of tartaric acid in presence of iron. ***Journal of the Chemical Society, Transactions***, **65**: 899-910.

Fernández-Checa, J.C., Colell, A., García-Ruiz, C., 2002. S-Adenosyl-L-methionine and mitochondrial reduced glutathione depletion in alcoholic liver disease. ***Alcohol***, **27** (3): 179-183.

Fernández-Checa, J.C., Kaplowitz, N., García-Ruiz, C., Colell, A., 1998. Mitochondrial glutathione: importance and transport. In ***Seminars in Liver Disease***. © 1998 by Thieme Medical Publishers, Inc. **18** (4): 389-401

Finlayson, N.D.C., 1993. Clinical features of alcoholic liver disease. ***Baillière's Clinical Gastroenterology***, **7** (3): 627-640.

Forman, H.J., Torres, M., Fukuto, J., 2002. Redox signaling. ***Molecular and Cellular Biochemistry***, **234** (1): 49-62.

Forman, H.J., Zhang, H., Rinna, A., 2009. Glutathione: overview of its protective roles, measurement, and biosynthesis. ***Molecular Aspects of Medicine***, **30** (1-2): 1-12.

Freeman, B.A., Crapo, J.D., 1981. Hyperoxia increases oxygen radical production in rat lungs and lung mitochondria. ***Journal of Biological Chemistry***, **256** (21): 10986-10992.

Fridovich, I., 1986. Superoxide dismutases. ***Advances in Enzymology - and Related Areas of Molecular Biology***, **58** (6), 61-97.

Fridovich, I., 1989. Oxygen radicals from acetaldehyde. ***Free Radical Biology & Medicine***, **7** (5): 557-558.

Galicia-Moreno, M., Gutiérrez-Reyes, G., 2014. The role of oxidative stress in the development of alcoholic liver disease. ***Revista de Gastroenterología de México*** (English Edition), **79** (2): 135-144.

Gamble, P.E., Burke, J.J., 1984. Effect of water stress on the chloroplast antioxidant system: I. Alterations in glutathione reductase activity. ***Plant Physiology***, **76** (3): 615-621.

Garrison, W.M., 1987. Reaction mechanisms in the radiolysis of peptides, polypeptides, and proteins. ***Chemical Reviews***, **87** (2): 381-398.

Genestra, M., 2007. Oxyl radicals, redox-sensitive signalling cascades and antioxidants. ***Cellular Signalling***, **19** (9): 1807-1819.

Giustarini, D., Dalle-Donne, I., Tsikas, D., Rossi, R., 2009. Oxidative stress and human diseases: origin, link, measurement, mechanisms, and biomarkers. ***Critical Reviews in Clinical Laboratory Sciences***, **46** (5-6): 241-281.

Góth, L., Rass, P., Páy, A., 2004. Catalase enzyme mutations and their association with diseases. ***Molecular Diagnosis***, **8** (3): 141-149.

Gralla, E.B., Kosman, D.J., 1992. Molecular genetics of superoxide dismutases in yeasts and related fungi. **In** ***Advances in Genetics***. Academic Press. **30**: 251-319

Grande, F., Hay, L.J., Heupel, H.W., Amatuzio, D.S., 1960. Effect of ethanol on serum cholesterol concentration in dog and man. ***Circulation Research***, **8** (4): 810-819.

Gutierrez-Correa, J., Stoppani, A.O.M., 1997. Inactivation of yeast glutathione reductase by Fenton systems: effect of metal chelators, catecholamines and thiol compounds. ***Free Radical Research***, **27** (6): 543-555.

Gutteridge, J.M., Halliwell, B., 2010. Antioxidants: molecules, medicines, and myths. ***Biochemical and Biophysical Research Communications***, **393** (4): 561-564.

Gutteridge, J.M., 1995. Lipid peroxidation and antioxidants as biomarkers of tissue damage. ***Clinical Chemistry***, **41** (12): 1819-1828.

Gutteridge, J.M., Paterson, S.K., Segal, A.W., Halliwell, B., 1981. Inhibition of lipid peroxidation by the iron-binding protein lactoferrin. ***Biochemical Journal***, **199** (1): 259-261.

Ha, T.J., Nihei, K.I., Kubo, I., 2004. Lipoxygenase inhibitory activity of octyl gallate. ***Journal of Agricultural and Food Chemistry***, **52** (10): 3177-3181.

Haber, F., Weiss, J., 1934. The catalytic decomposition of hydrogen peroxide by iron salts. ***Proceedings of the Royal Society***, **147** (861): 332-351.

Halliwell, B., Gutteridge, J.M., 1990. The antioxidants of human extracellular fluids. ***Archives of Biochemistry and Biophysics***, **280** (1): 1-8.

Halliwell, B., Gutteridge, J.M., 2015. ***Free Radicals in Biology and Medicine***. Oxford University Press, USA.

Halliwell, B., Gutteridge, J.M.C., 2007 ***Free Radicals in Biology and Medicine***, 4th edition, Clarendon Press, Oxford

Halliwell, B., Gutteridge, J.M.C., 1999. Free radicals, other reactive species and disease. ***Free Radicals in Biology and Medicine***, **3**: 617-783.

Halliwell, B., Whiteman, M., 2004. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean?. ***British Journal Of Pharmacology***, **142** (2): 231-255.

Halliwell, B., 1987. Oxidants and human disease: some new concepts. ***Federation of American Societies for Experimental Biology***, **1** (5): 358-364.

Halliwell, B., Clement, M.V., Long, L.H., 2000. Hydrogen peroxide in the human body. ***Federation of European Biochemical Societies***, **486** (1): 10-13.

Halsted, C.H., 2004, August. Nutrition and alcoholic liver disease. In ***Seminars in Liver Disease*** **24** (3): 289-304. Copyright© 2004 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA.

Hannuksela, M., Marcel, Y.L., Kesäniemi, Y.A., Savolainen, M.J., 1992. Reduction in the concentration and activity of plasma cholesteryl ester transfer protein by alcohol. ***Journal of Lipid Research***, **33** (5): 737-744.

Harrison, D.J., Burt, A.D., 1993. Pathology of alcoholic liver disease. ***Baillière's Clinical Gastroenterology***, **7** (3): 641-662.

Hassan H.M., Scandalios J.G., 1990 Superoxide dismutases in aerobic organisms. *In* R Alscher, J Cumming, eds, ***Stress Responses in Plants: Adaptation to Acclimation Mechanisms***. Wiley-Liss, New York, 175-179

Hassoun EA., Stohs SJ (1995) Comparative studies on oxidative stress as a mechanism for the fetotoxic of TCDD, endrin and lindane in C57BL/6J and DBA/2J mice. ***Teratology,*** **51**: 186–192

Hayes, A. W., 1994. ***Principles and Methods of Toxicology*** (3rd edition).Raven Press.

Hayes, J.D., Flanagan, J.U., Jowsey, I.R., 2005. Glutathione transferases. ***Annual Review of Pharmacology and Toxicology***, **45**: 51-88.

Helander, A., Tabakoff, B., CENTRES, W.I.S., 1997. Biochemical markers of alcohol use and abuse: experiences from the pilot study of the WHO/ISBRA collaborative project on state and trait markers of alcohol. ***Alcohol and Alcoholism***, **32** (2): 133-144.

Hermes-Lima, M., 2004. Oxygen in biology and biochemistry: role of free radicals. ***Functional Metabolism: Regulation and Adaptation***, **1**: 319-66.

Hernández, J.A., López-Sánchez, R.C., Rendón-Ramírez, A., 2016. Lipids and oxidative stress associated with ethanol-induced neurological damage. ***Oxidative Medicine and Cellular Longevity***, *2016*.

Hietala, J., Koivisto, H., Anttila, P., Niemelä, O., 2006. Comparison of the combined marker GGT–CDT and the conventional laboratory markers of alcohol abuse in heavy drinkers, moderate drinkers and abstainers. ***Alcohol and Alcoholism***, **41** (5): 528-533.

Holovská, K., Lenártová, V., Rosival, I., Kiˇcinková, M., Majerˇciaková, A., Legáth, J., 1998. Antioxidant and detoxifying enzymes in the liver and kidney of pheasants after intoxication by herbicides MCPA and ANITEN. ***Journal of Biochemical and Molecular Toxicology***, **12** (4): 235-244.

Huang, X.J., Choi, Y.K., Im, H.S., Yarimaga, O., Yoon, E., Kim, H.S., 2006. Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) detection techniques. ***Sensors***, **6** (7): 756-782.

Husain, K., Scott, B.R., Reddy, S.K., Somani, S.M., 2001. Chronic ethanol and nicotine interaction on rat tissue antioxidant defense system. ***Alcohol***, **25** (2): 89-97.

Ignarro, L.J., Buga, G.M., Wood, K.S., Byrns, R.E., Chaudhuri, G., 1987. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. ***Proceedings of the National Academy of Sciences***, **84** (24): 9265-9269.

Ipek, E., Zeytinoglu, H., Okay, S., Tuylu, B.A., Kurkcuoglu, M., Baser, K.H.C., 2005. Genotoxicity and antigenotoxicity of Origanum oil and carvacrol evaluated by Ames Salmonella/microsomal test. ***Food Chemistry***, **93** (3): 551-556.

Ischiropoulos, H., Al-Mehdi, A.B., 1995. Peroxynitrite-mediated oxidative protein modifications. ***Federation of European Biochemical Societies***, **364** (3): 279-282.

Ishii, H., Kurose, I., Kato, S., 1997. Pathogenesis of alcoholic liver disease with particular emphasis on oxidative stress. ***Journal of Gastroenterology and Hepatology,*** **12**: 272-282.

İkiz, F., Püskülcü, H., Eren, Ş., 1996. ***İstatitiğe Giriş*** (Bilgisayar Örnekleri ile Geliştirilmiş 4. Baskı). E Ü, İzmir, Bornova. 343-346.

Jain, S. K., McVie, R., Duett, J. 1989. Erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes. ***Diabetes,*** **38**: 1539–1543.

Jomova, K., Valko, M., 2011. Advances in metal-induced oxidative stress and human disease. ***Toxicology***, **283** (2-3): 65-87.

Kanofsky, J.R., 1984. Singlet oxygen production by lactoperoxidase: halide dependence and quantitation of yield. ***Journal of Photochemistry***, **25** (2-4): 105-113.

Kirimer, N., Başer, K.H.C., Tümen, G., 1995. Carvacrol-rich plants in Turkey. ***Chemistry of Natural Compounds***, **31** (1): 37-41.

Kohen, R., Nyska, A., 2002. Invited review: Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. ***Toxicologic Pathology***, **30** (6): 620-650.

Koivula, T., Lindros, K.O., 1975. Effects of long-term ethanol treatment on aldehyde and alcohol dehydrogenase activities in rat liver. ***Biochemical Pharmacology***, **24** (21): 1937-1942.

Kokkini, S., 1997, May. Taxonomy, diversity and distribution of Origanum species. In ***Proceedings of the IPGRI International Workshop on Oregano***). Valenzano, Bari: CIHEAM.

Kolankaya, D., Selmanoğlu, G., Sorkun, K., Salih, B., 2002. Protective effects of Turkish propolis on alcohol-induced serum lipid changes and liver injury in male rats. ***Food Chemistry.,*** **78**: 213-217.

Koner, B.C., Banerjee, B.D., Ray, A., 1988. Organochlorine pesticide induced oxidative stress and immune suppression in rats. ***Indian Journal of Experimental Biology,*** **36**: 395-398.

Kontos, H.A., Wei, E.P., Ellis, E.F., Jenkins, L.W., Povlishock, J.T., Rowe, G.T., Hess, M.L., 1985. Appearance of superoxide anion radical in cerebral extracellular space during increased prostaglandin synthesis in cats. ***Circulation Research***, **57** (1): 142-151.

Korsten, M.A., administration: Difference between alcoholic and nonalcoholic subjects. ***New England Journal of Medicine***, **292** (8): 386-389.

Korukluoglu, M., Gurbuz, O., Sahan, Y., Yigit, A., Kacar, O., Rouseff, R., 2009. Chemical characterization and antifungal activity of *Origanum onites* L. essential oils and extracts. ***Journal of Food Safety***, **29** (1): 144-161.

Kosugi, H., Kato, T., Kikugawa, K., 1987. Formation of yellow, orange, and red pigments in the reaction of alk-2-enals with 2-thiobarbituric acid. ***Analytical Biochemistry***, **165** (2): 456-464.

Kotan, R., Cakir, A., Ozer, H., Kordali, S., Cakmakci, R., Dadasoglu, F., Dikbas, N., Aydin, T., Kazaz, C., 2014. Antibacterial effects of *Origanum onites* against phytopathogenic bacteria: Possible use of the extracts from protection of disease caused by some phytopathogenic bacteria. ***Scientia Horticulturae***, **172**: 210-220.

Krinsky, N.I., 2001. Carotenoids as antioxidants. ***Nutrition***, **17** (10): 815-817.

Krishnamurthy, P., Wadhwani, A., 2012. Antioxidant enzymes and human health. Antioxidant enzyme. ***Rijeka: InTech***.

Kumar, S., 2011. Free radicals and antioxidants: human and food system. ***Advances in Applied Science Research***, **2** (1): 129-135.

Kuppusamy, P., Zweier, J.L., 1989. Characterization of free radical generation by xanthine oxidase. Evidence for hydroxyl radical generation. ***Journal of Biological Chemistry***, **264** (17): 9880-9884.

Lagouri, V., Boskou, D., 1995. Screening for antioxidant activity of essential oils obtained from spices. In ***Developments in Food Science***. Elsevier, **37**: 869-879

Lagouri, V., Blekas, G., Tsimidou, M., Kokkini, S., Boskou, D., 1993. Composition and antioxidant activity of essential oils from oregano plants grown wild in Greece. ***Zeitschrift für Lebensmittel-Untersuchung und Forschung***, **197** (1): 20-23.

Lahir, Y.K., 2015. Lipid oxidation in biological systems: Biochemical, Biological and Biophysical aspects. ***Global Journal of Bio-science and biotechnology***, **4**: 224-233.

Lieber, C.S., DeCARLI, L.M., 1972. The role of the hepatic microsomal ethanol oxidizing system (MEOS) for ethanol metabolism in vivo. ***Journal of Pharmacology and Experimental Therapeutics***, **181** (2): 279-287.

Lieber, C.S., DeCarli, L.M., 1970. Hepatic microsomal ethanol-oxidizing system in vitro characteristics and adaptive properties in vivo. ***Journal of Biological Chemistry***, **245** (10): 2505-2512.

Lieber, C.S., 1980. Alcohol, protein metabolism, and liver injury: Respective role of dietary protein and alcohol in the pathogenesis of liver injury. ***Gastroenterology***, **79** (2): 373-390.

Lin, Y.S., Hung, S.C., Wei, Y.H., Tarng, D.C., 2009. GST M1 polymorphism associates with DNA oxidative damage and mortality among hemodialysis patients. ***Journal of the American Society of Nephrology***, **20** (2): 405-415.

Lindros, K.O., 1974. Acetaldehyde oxidation and its role in the overall metabolic effects of ethanol in the liver in regulation of hepatic metabolism. In ***Proceedings of the Alfred Benson Symposium VI***. Munksgaard Copenhagen.

Lobo, V., Patil, A., Phatak, A., Chandra, N., 2010. Free radicals, antioxidants and functional foods: Impact on human health. ***Pharmacognosy Reviews***, **4** (8): 118.

Lubos, E., Loscalzo, J., Handy, D.E., 2011. Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. ***Antioxidants & Redox Signaling***, **15** (7): 1957-1997.

Lukaszewicz-Hussain, A., Moniuszko-Jakoniuk, J., 2004. Liver Catalase, Glutathione Peroxidase and Reductase Activity, Reduced Glutathione and Hydrogen Peroxide Levels in Acute Intoxication with Chlorfenvinphos, an Organophosphate Insecticide Introduction. ***Polish Journal of Environmental Studies***, **13** (3).

Lundquist, F., Tygstrup, N., Winkler, K., Mellemgaard, K., Munck-Petersen, S., 1962.

Ethanol metabolism and production of free acetate in the human liver. ***The Journal of Clinical Investigation***, **41** (5): 955-961.

Mackenzie, I.R., 2002. Pathogenesis of Neurodegenerative Disorders. 2001. Edited by Mark P. Mattson. Published by Humana Press. 294 pages. C $211.20 approx. ***Canadian Journal of Neurological Sciences***, **29** (2): 196-197.

Mandal, S., Yadav, S., Yadav, S., Nema, R.K., 2009. Antioxidants: a review. ***Journal of Chemical and Pharmaceutical Research***, **1** (1): 102-104.

Mannervik, B., Guthenberg, C., 1981. [28] Glutathione transferase (human placenta). In ***Methods in Enzymology***. Academic Press, **77**: 231-235

Markert, C.L., Shaklee, J.B., Whitt, G.S., 1975. Evolution of a gene. ***Science***, **189** (4197): 102-114.

Marklund, S.L., 1984. Extracellular superoxide dismutase and other superoxide dismutase isoenzymes in tissues from nine mammalian species. ***Biochemical Journal***, **222** (3): 649-655.

Marklund, S.L., 1990. Analysis of extracellular superoxide dismutase in tissue homogenates and extracellular fluids. ***Methods in Enzmology,*** **186**: 260-265.

Marmillot, P., Munoz, J., Patel, S., Garige, M., Rosse, R.B., Lakshman, M.R., 2007. Long-term ethanol consumption impairs reverse cholesterol transport function of high-density lipoproteins by depleting high-density lipoprotein sphingomyelin both in rats and in humans. ***Metabolism***, **56** (7): 947-953.

Marnett, L.J., 2000. Oxyradicals and DNA damage. ***Carcinogenesis***, **21** (3): 361-370.

Marnett, L.J., 2002. Oxy radicals, lipid peroxidation and DNA damage. ***Toxicology***, **181**: 219-222.

Massey, V., Williams, C.H., 1965. On the reaction mechanism of yeast glutathione reductase. ***Journal of Biological Chemistry***, **240** (11): 4470-4480.

Matés, J.M., Pérez-Gómez, C., De Castro, I.N., 1999. Antioxidant enzymes and human diseases. ***Clinical Biochemistry***, **32** (8): 595-603.

Maxwell, S.R., Lip, G.Y., 1997. Reperfusion injury: a review of the pathophysiology, clinical manifestations and therapeutic options. ***International Journal of Cardiology***, **58** (2): 95-117.

McCord, J.M., Fridovich, I., 1969. Superoxide dismutase, ***Journal of Biological Chemistry,*** **244** (22): 6049-6055.

McIntyre, M., Bohr, D.F., Dominiczak, A.F., 1999. Endothelial function in hypertension: the role of superoxide anion. ***Hypertension***, **34** (4): 539-545.

Mehta, S.K., Gowder, S.J.T., 2015. Members of antioxidant machinery and their functions. In ***Basic Principles and Clinical Significance of Oxidative Stress***. InTech.

Meister, A., Anderson, M.E., 1983. Glutathione. ***Annual Review of Biochemistry***, **52** (1): 711-760.

Michelson, A.M., McCord, J.M., Fridovich, I., 1977. Superoxide and superoxide dismutases. In ***EMBO Workshop on Superoxide and Superoxide Dismutases 1976: Banyuls, France)***. Academic Press.

Mitruka, B.M., Rawnsley, H.M., 1977. Clinical biochemical and hematological reference values in normal experimental animals. ***Clinical Biochemical and Hematological Reference Values in Normal Experimental Animals***.

Molina, M.F., Sanchez-Reus, I., Iglesias, I., Benedi, J., 2003. Quercetin, a flavonoid antioxidant, prevents and protects against ethanol-induced oxidative stress in mouse liver. ***Biological and Pharmaceutical Bulletin***, **26** (10): 1398-1402.

Morales-Gonzalez, J.A. ed., 2013. ***Oxidative Stress and Chronic Degenerative Diseases-a Role for Antioxidants***. InTech.

Mørland, J., Sjetnan, A.E., 1976. Effect of ethanol intake on the incorporation of labelled amino acids into liver protein. ***Biochemical Pharmacology***, **25** (19): 2125-2130.

Mørland, J., 1974. Effect of chronic ethanol treatment on tryptophan oxygenase, tyrosine aminotrans-ferase and general protein metabolism in the intact and perfused rat liver. ***Biochemical Pharmacology***, **23** (1): 21-35.

Mukherji SM, Singh SP. ***Reaction Mechanism in Organic Chemistry***. Madras: Macmillan India Press; 1986.

Murthy, K.N.C., 2001. ***Evaluation of Antioxidant Activity of Pomegranate (Punicagranatum) and Grapes (Vitis vinifera)***. a thesis, Rajiv Gandhi University of Health Science, Banglore, India..

Müller, A., Sies, H., 1987. Alcohol, aldehydes and lipid peroxidation: current notions. ***Alcohol and Alcoholism (Oxford, Oxfordshire). Supplement***, **1**: 67-74.

Nair, V., Turner, G.A., 1984. The thiobarbituric acid test for lipid peroxidation: structure of the adduct with malondialdehyde. ***Lipids***, **19** (10): 804-805.

Nathan, C., 2003. Specificity of a third kind: reactive oxygen and nitrogen intermediates in cell signaling. ***The Journal of Clinical Investigation***, **111** (6): 769-778.

Niedernhofer, L.J., Daniels, J.S., Rouzer, C.A., Greene, R.E., Marnett, L.J., 2003. Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells. ***Journal of Biological Chemistry***, **278** (33): 31426-31433.

Niemelä, O., 2002. Serum diagnosis of alcoholic liver disease and markers of ethanol intake. ***Ethanol and the Liver***, 411-449.

Nimse, S.B., Pal, D., 2015. Free radicals, natural antioxidants, and their reaction mechanisms. ***Rsc Advances***, **5** (35): 27986-28006.

Nordberg, J., Arner, E.S., 2001. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. ***Free Radical Biology and Medicine***, **31** (11): 1287-1312.

Nordmann, R., Ribière, C., Rouach, H., 1990. Ethanol-induced lipid peroxidation and oxidative stress in extrahepatic tissues. ***Alcohol and Alcoholism***, **25** (2-3): 231-237.

Nordmann, R., Ribière, C., Rouach, H., 1992. Implication of free radical mechanisms in ethanol induced cellular injury. ***Free Radical Biology and Medicine,*** **12**: 219-240.

Nyblom, H., Berggren, U., Balldin, J., Olsson, R., 2004. High AST/ALT ratio may indicate advanced alcoholic liver disease rather than heavy drinking. ***Alcohol and Alcoholism***, **39** (4): 336-339.

Omodeo-Sale, F., Gramigna, D., Campaniello, R., 1997. Lipid peroxidation and antioxidant systems in rat brain: effect of chronic alcohol consumption. ***Neurochemical Research***, **22** (5): 577-582.

Otero Regino, W., Velasco, H., Sandoval, H., 2009. The protective role of bilirubin in human beings. ***Revista Colombiana de Gastroenterologia***, **24** (3): 293-301

Ott, C., Jacobs, K., Haucke, E., Santos, A.N., Grune, T., Simm, A., 2014. Role of advanced glycation end products in cellular signaling. ***Redox Biology***, **2**: 411-429.

Ozer, J., Ratner, M., Shaw, M., Bailey, W., Schomaker, S., 2008. The current state of serum biomarkers of hepatotoxicity. ***Toxicology***, **245** (3): 194-205.

Özdemir, B., Ekbul, A., Topal, N.B., Sarandöl, E., Sağ, S., Başer, K.H.C., Cordan, J., Güllülü, S., Tuncel, E., Baran, I., Aydinlar, A., 2008. Effects of *Origanum onites* on endothelial function and serum biochemical markers in hyperlipidaemic patients. ***Journal of International Medical Research***, **36** (6): 1326-1334.

Özkan, A., Erdoğan, A., 2011. A comparative evaluation of antioxidant and anticancer activity of essential oil from *Origanum onites* (Lamiaceae) and its two major phenolic components. ***Turkish Journal of Biology***, **35** (6): 735-742.

Paglia, D. E., Valentine, W. N., 1967. Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase. ***Journal of Laboratory and Clinical Medicine,*** **70**: 158.

Pari, L., Suresh, A., 2008. Effect of grape (*Vitis vinifera* L.) leaf extract on alcohol induced oxidative stress in rats. ***Food and Chemical Toxicology***, **46** (5): 1627-1634.

Patlolla, A.K., Barnes, C., Yedjou, C., Velma, V.R., Tchounwou, P.B., 2009. Oxidative stress, DNA damage, and antioxidant enzyme activity induced by hexavalent chromium in Sprague-Dawley rats. ***Environmental Toxicology: An International Journal***, **24** (1): 66-73.

Perin, A., Sessa, A., 1975. In vitro effects of ethanol and acetaldehyde on tissue protein synthesis. ***The role of acetaldehyde in the action of ethanol. 6th Int Congr Pharmacol. Helsinki: The Finish Foundation for Alcohol Studies***, 105-22.

Perin, A., Scalabrino, G., Sessa, A., Arnaboldi, A., 1974. In vitro inhibition of protein synthesis in rat liver as a consequence of ethanol metabolism. ***Biochimica et Biophysica Acta (BBA)-Nucleic Acids and Protein Synthesis***, **366** (1): 101-108.

Petrucci, R.H., Harwood, W.S., Herring (2002) ***General Chemistry***, (8th Ed), Prentice Hall, New York.

Phaniendra, A., Jestadi, D.B., Periyasamy, L., 2015. Free radicals: properties, sources, targets, and their implication in various diseases. ***Indian Journal of Clinical Biochemistry***, **30** (1): 11-26.

Pikkarainen, P.H., Salaspuro, M.P., Lieber, C.S., 1979. A method for the determination of "free" acetaldehyde in plasma. ***Alcoholism: Clinical and Experimental Research***, **3** (3): 259-261.

Pizzale, L., Bortolomeazzi, R., Vichi, S., Überegger, E., Conte, L.S., 2002. Antioxidant activity of sage (*Salvia officinalis* and *S. fruticosa*) and oregano (*Origanum onites* and *O. indercedens*) extracts related to their phenolic compound content. ***Journal of the Science of Food and Agriculture***, **82** (14): 1645-1651.

Polidori, M.C., Stahl, W., Eichler, O., Niestroj, I., Sies, H., 2001. Profiles of antioxidants in human plasma. ***Free Radical Biology and Medicine***, **30** (5): 456-462.

Pomposiello, P.J., Demple, B., 2002. ***Global Adjustment of Microbial Physiology During Free Radical Stress.***

Porta, E.A., Koch, O.R., Gomez-Dumm, C.L., Hartroft, W.S., 1968. Effects of dietary protein on the liver of rats in experimental chronic alcoholism. ***The Journal of***

Nutrition, **94** (4): 437-446.

Proctor, G.B., Shori, D.K., Preedy, V.R., 1993. Protein synthesis in the major salivary glands of the rat and the effects of re-feeding and acute ethanol injection. ***Archives of Oral Biology***, **38** (11): 971-978.

Prütz, W.A., 1996. Hypochlorous acid interactions with thiols, nucleotides, DNA, and other biological substrates. ***Archives of Biochemistry and Biophysics***, **332** (1): 110-120.

Pryor, W.A., Jin, X., Squadrito, G.L., 1994. One-and two-electron oxidations of methionine by peroxynitrite. ***Proceedings of the National Academy of Sciences***, **91** (23): 11173-11177.

Radi, R., Turrens, J.F., Chang, L.Y., Bush, K.M., Crapo, J.D., Freeman, B.A., 1991. Detection of catalase in rat heart mitochondria. ***Journal of Biological Chemistry***, **266** (32): 22028-22034.

Rains, J.L., Jain, S.K., 2011. Oxidative stress, insulin signaling, and diabetes. ***Free Radical Biology and Medicine***, **50** (5): 567-575.

Randox Lab. Ltd., 1996. ***Ransod Süperoxide Dismutase Enzim Kiti***, Ransel glutathione peroxidase enzim kiti**. Randox Lab. Ltd.,55 Diamond Road, UK.**

Repetto, M., Semprine, J., Boveris, A. 2012. Lipid peroxidation: chemical mechanism, biological implications and analytical determination. In Lipid peroxidation. InTechOpen.

Rhee, S.G., Kang, S.W., Jeong, W., Chang, T.S., Yang, K.S., Woo, H.A., 2005. Intracellular messenger function of hydrogen peroxide and its regulation by peroxiredoxins. ***Current Opinion in Cell Biology***, **17** (2): 183-189.

Rizzi, R., Caroli, A., Bolla, P., Acciaioli, A., Pagnacco, G., 1988. Variability of reduced glutathione levels in massese ewes and its effect on daily milk production. ***Journal of Dairy Research,*** **55**: 345-353.

Roche, M., Rondeau, P., Singh, N.R., Tarnus, E., Bourdon, E., 2008. The antioxidant properties of serum albumin. ***Federation of European Biochemical Societies***, **582** (13): 1783-1787.

Rosman, A.S., Lieber, C.S., 1994. Diagnostic utility of laboratory tests in alcoholic liver disease. ***Clinical Chemistry***, **40** (8): 1641-1651.

Sağdıç, O., 2003. Sensitivity of four pathogenic bacteria to Turkish thyme and oregano hydrosols. ***LWT-Food Science and Technology***, **36** (5): 467-473

Sandquist, A.R., Briviba, K., Sies, H., 1994. (37) singlet oxygen quenching by carotenoids. In ***Methods in Enzymology***. Academic Press, **234**: 384-388

Santo, A., Zhu, H., Li, Y.R., 2016. Free radicals: from health to disease. ***Reactive Oxygen Species***, **2** (4): 245-63.

Scandalios, J.G., 1990. Response of plant antioxidant defense genes to environmental stress. In ***Advances in Genetics***. Academic Press, **28**: 1-41

Scandalios, J.G., 1992. ***Molecular Biology of Free Radical Scavenging Systems***. Cold Spring Harbor Laboratory Press.

Scandalios, J.G., 1993. Oxygen stress and superoxide dismutases. ***Plant Physiology***, **101** (1): 7.

Scott, J., Berry, M.R., Woods, K., 1989. Effects of acute ethanol administration on stimulated parotid secretion in the rat. ***Alcoholism: Clinical and Experimental Research***, **13** (4): 560-563.

Shahidi, F., Zhong, Y., 2010. Novel antioxidants in food quality preservation and health promotion. ***European Journal of Lipid Science and Technology***, **112** (9): 930-940.

Shanlin, F.U., Stocker, R., Davıes, M.J., 1997. Biochemistry and pathology of radical-mediated protein oxidation. ***Biochemical Journal***, **324** (1): 1-18.

Shori, D.K., Proctor, G.B., Teare, J. and Preedy, V.R., 1994. Indices of protein synthesis and RNA translating activities in the major salivary glands of rat and comparison to synthetic rates in liver. ***Biochemical Society transactions***, ***22*** (2): 182S.

Sies, H., 1991. ***Oxidative Stress: Oxidants and Antioxidants***. Academic Pr.

Sivas, H., Tomsuk, Ö., 2011. Antiproliferative and apoptotic effects of the essential oil of *Origanum onites* and carvacrol on Hep-G2 cells. ***Anadolu University Journal Of Science And Technology–C Life Sciences and Biotechnology***, **1** (2): 171.

Sonde, V., D'souza, A., Tarapore, R., Pereira, L., Khare, M.P., Sinkar, P., Krishnan, S., Rao, C.V., 2000. Simultaneous administration of diethylphthalate and ethyl alcohol and its toxicity in male Sprague–Dawley rats. ***Toxicology***, **147** (1): 23-31.

Sorrell, M.F., Tuma, D.J., Schafer, E.C., Barak, A.J., 1977. Role of acetaldehyde in the ethanol-induced impairment of glycoprotein metabolism in rat liver slices. ***Gastroenterology***, **73** (1): 137-144.

Stamler, J.S., 1994. Redox signaling: nitrosylation and related target interactions of nitric oxide. ***Cell***, **78** (6): 931-936.

Standman, E.R., Levine, R.L., 2000. Protein oxidation. ***Annals of the New York Academy of Sciences***, **899**: 191-208.

Suslow, T.V., 2004. Oxidation-reduction potential (ORP) for water disinfection monitoring, control, and documentation.

Temple, M.D., Perrone, G.G., Dawes, I.W., 2005. Complex cellular responses to reactive oxygen species. ***Trends in Cell Biology***, **15** (6): 319-326.

Tonk, F.A., Yüce, S., Bayram, E., Giachino, R.R.A., Sönmez, Ç., Telci, I., Furan, M.A., 2010. Chemical and genetic variability of selected Turkish oregano (*Origanum onites* L.) clones. ***Plant Systematics and Evolution***, **288** (3-4): 157-165.

Townsend, D.M., Tew, K.D., Tapiero, H., 2003. The importance of glutathione in human disease. ***Biomedicine & Pharmacotherapy***, **57** (3-4): 145-155.

Turan, A., Celik, I., 2016. Antioxidant and hepatoprotective properties of dried fig against oxidative stress and hepatotoxicity in rats. ***International Journal of Biological Macromolecules***, **91**: 554-559.

Uyanoglu, M., Canbek, M., Aral, E., Baser, K.H.C., 2008. Effects of carvacrol upon the liver of rats undergoing partial hepatectomy. ***Phytomedicine***, **15** (3): 226-229.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M., Telser, J., 2007. Free radicals and antioxidants in normal physiological functions and human disease. ***The İnternational Journal of Biochemistry & Cell Biology***, **39** (1): 44-84.

Vallyathan, V., Shi, X., 1997. The role of oxygen free radicals in occupational and environmental lung diseases. ***Environmental Health Perspectives***, **105** (suppl 1): 165-177.

Van Zutphen, L.F.M., Baumans, V., Beynen, A.C., 2003. ***Laboratuvar Hayvanları Biliminin Temel İlkeleri.*** Medipres yayıncılık, Malatya.

Vander Jagt, D.L., Hunsaker, L.A., Vander Jagt, T.J., Gomez, M.S., Gonzales, D.M., Deck, L.M., Royer, R.E., 1997. Inactivation of glutathione reductase by 4-hydroxynonenal and other endogenous aldehydes.***Biochemical Pharmacology***, **53** (8): 1133-1140.

Vašková, J., Vaško, L., Kron, I., 2012. Oxidative processes and antioxidative metaloenzymes. In ***Antioxidant Enzyme***. InTech.

Von Sonntag, C., 2006. ***Free-Radical-Induced DNA Damage and its Repair*** Berlin: Springer.

Wang, M., Zhu, P., Jiang, C., Ma, L., Zhang, Z., Zeng, X., 2012. Preliminary characterization, antioxidant activity in vitro and hepatoprotective effect on acute alcohol-induced liver injury in mice of polysaccharides from the peduncles of Hovenia dulcis. ***Food and Chemical Toxicology***, **50** (9): 2964-2970.

Wink, D.A., Mitchell, J.B., 1998. Chemical biology of nitric oxide: insights into regulatory, cytotoxic, and cytoprotective mechanisms of nitric oxide. ***Free Radical Biology and Medicine***, **25** (4-5): 434-456.

Winterbourn, C.C., Kettle, A.J., 2000. Biomarkers of myeloperoxidase-derived hypochlorous acid. ***Free Radical Biology and Medicine***, **29** (5): 403-409.

Winterbourn, C.C., 1985. Comparative reactivities of various biological compounds with myeloperoxidase-hydrogen peroxide-chloride, and similarity of oxidant to hypochlorite. ***Biochimica et Biophysica Acta (BBA)-General Subjects***, **840** (2): 204-210.

Wood, W.G., Avdulov, N.A., Chochina, S.V., Igbavboa, U., 2001. Lipid carrier proteins and ethanol. ***Journal of Biomedical Science***, **8** (1): 114-118.

Xia, E., Rao, G., Remmen, H.V., Heydari A.R., Richardson, A., 1994. Activities of antioxidant enzymes in various tissues of male fischer 344 rats are altered by food restriction. ***Journal of Nutrition,*** **125**: 195-201.

Yayalacı, Y., Celik, I., Batı, B., 2014. Hepatoprotective and antioxidant activity of linden (*Tilia platyphyllos* L.) infusion against ethanol-induced oxidative stress in rats. ***The Journal of Membrane Biology***, **247** (2): 181-188

Yurt, B., Çelik, İ., 2010. ***Etil Alkol ile Oksidatif Stres Oluşturulan Sıçanlarda Kayısı ve Kayısı Çekirdeğinin Karaciğer Koruyucu ve Antioksidan Etkilerinin Belirlenmesi.*** Doktora tezi, YYÜ Fen Bil. Ens. Van.

Zámocký, M., Koller, F., 1999. Understanding the structure and function of catalases: clues from molecular evolution and in vitro mutagenesis. ***Progress in Biophysics and Molecular Biology***, **72** (1): 19-66.

DeLeve, L.D., Wang, X., Kuhlenkamp, J.F., Kaplowitz, N., 1996. Toxicity of azathioprine and monocrotaline in murine sinusoidal endothelial cells and hepatocytes: the role of glutathione and relevance to hepatic venoocclusive disease. ***Hepatology***, **23** (3): 589-599.

Zeytinoglu, H., Incesu, Z., Baser, K.H.C., 2003. Inhibition of DNA synthesis by carvacrol in mouse myoblast cells bearing a human N-RAS oncogene. ***Phytomedicine***, **10** (4): 292-299.

APPENDIX 1

DENEYSEL OLARAK ETIL ALKOL ILE OKSIDATIF STRES OLUŞTURULAN SIÇANLARDA KEKIK (*Origanum onites L.*) INFIZYONUNUN KARACIĞER KORUYUCU VE ANTIOKSIDAN ROLÜNÜN BELIRLENMESI

Hazırlayan: Shreen Abdulkareem MUSA **Danışman:** Prof. Dr. İsmail ÇELİK

EXTENDED TURKISH SUMMARY
GENİŞLETİLMİŞ TÜRKÇE ÖZET

1. GİRİŞ

Geçtiğimiz 40 yıl boyunca, serbest radikallerin hem toksik hem de faydalı türler olarak ikili rolü üzerine çok sayıda araştırmalar yapılmıştır. Serbest radikaller, normal hücresel metabolizmanın yan ürünleri olarak üretilir veya dış çevremizdeki kimyasallar tarafından üretilir (örneğin, sigara dumanı, hava ve su kirliliği, güneş ışığına maruz kalma, gama ışıması ve bazı kemoterapötik ilaçlar). Düşük ila orta dereceli konsantrasyonlarda, serbest radikaller etkilerini hücre sinyalleşme kaskadlarının düzenlenmesi yoluyla gösterirler. Ancak, yüksek konsantrasyonlarda, tüm makromoleküllere zarar verir, DNA hasarına, lipid peroksidasyonuna, protein modifikasyonuna ve sonunda hücre ölümüne neden olurlar. Serbest radikaller yaşlanma, ateroskleroz, iskemik kalp hastalığı, kanser ve Alzheimer hastalığı gibi bir takım koşulların patogenezinde rol oynamıştır (Santo ve ark., 2016).

İnsan vücudunun doğal antioksidan savunma sistemi ile birlikte doğa karmaşık bir antioksidan savunma sistemi bahşetmiştir. Bunlar glutatyon peroksidaz ve glutatyon redüktaz katalaz ve süperoksit dismutaz gibi enzimatik antioksidanların yanı sıra, tiyol antioksidanlar, melatonin, koenzim Q ve metal şelatlama proteinleri gibi enzimatik olmayan antioksidanlar olup bunlar aşırı serbest radikallere karşı savaşmak göçlü moleküllerdrir. Ayrıca, C vitamini, E vitamini, karotenoidler, polifenoller ve iz elementler gibi besin antioksidanlarının, reaktif türlerin zararlı etkilerini en aza indirmeye yardımcı oldukarı için yüksek antioksidan potansiyeline sahip doğadan temin

edilen moleküllerir. Ayrıca bağışıklık sistemi; kontrolsüz serbest radikal üretimi ve savunma mekanizmasını olumsuz etkileyebileceği için oksidan ve antioksidan dengeye karşı aşırı derecede hassastır (Aslani ve Ghobadi, 2016). Vücut sıvıları ve dokularındaki serbest radikallerin kimyasal biyobelirteçlerini tahmin edebilme yeteneği, hastalık süreçlerine katkıda bulunan mekanizmaları anlamada önemli bir adımdır (Santo ve ark., 2016).

Anahtar kelimeler: Antioksidan, Oksidatif stres, Serbest radikaler

2. KAYNAK BİLDİRİŞLERİ

Sağdıç, O., 2003, İki kekik (*Thymus vulgaris* L. ve *Thymus serpyllum* L.) ve üç oregano (*Origanum vulgare* L., *Origanum onites* L. ve *Origanum majorana* L.) hidrosollerini kullanarak dört patojenik bakteriye karşı potansiyel bir doğal antimikrobiyal olarak inhibitör etkilerini (*Escherichia coli* ATCC 25922, *E. coli* O157: H7 ATCC 33150, *Staphylococcus aureus* ATCC 2392 ve *Yersinia enterocolitica* ATCC 1501) üzerinde test ettiler. Yaapılan bu çalışmanın sonuçlarına göre ik kekik hidrozollerini gıdaların korunmasında ve içeceklerde kullanma olasılığını rapor etmişlerdir.

Özdemir ve ark., 2008, ilaç tedavisi gerektirmeyen hafif hiperlipidemili 48 hastada *Origanum onites L*. endotelyal fonksiyon ve antioksidatif durum üzerine olan etkilerini araştırdılar. Elde edilen sonuçlara göre, hafif hiperlipidemisi olan hastalarda *Origanum onites* distilatının lipid profilleri, antioksidan durumu ve brakiyal arterin akış ve nitrogliserin aracılı dilatasyonu üzerinde yararlı etkileri olduğu sonucuna varmışlar.

Kotan ve ark., 2014, Bitki kaynaklı patojen bakterilere karşı, *Origanum onites* L.'nin ekstraktlarını, uçucu yağın ve saf metabolitlerinin antibakteriyel aktivitesini belirlemek için bir deney yapmış olup, *Origanum onites*'lerinin heksan, kloroform ve aseton ekstreleri uygulamaları hastalık semptomlarını önemli ölçüde azaltmıştır ve ekstraktların bu antibakteriyel etkilerini fenolik içeriği olan karvakrolüne atfedilebildiğni rapor ettiler.

Evrim ve ark., 2005, *Origanum onites* L. ve karvakrol uçucu yağının genotoksik ve antijenotoksik etkilerini Ames ise Salmonella/mikrozomal testiyle değerlendirmişler. Her ikisinin sırasıyla S9 olan veya olmayan her iki suşta 4-nitro-o-fenilendiamin ve 2-aminofluorenin neden olduğu mutagenisiteyi kuvvetle inhibe ettiği gösterilmiştir ve bu sonuçlara dayanarak, uçucu yağın ve karvakrolün in vitro olarak anlamlı antimutagenisitesine işaret ettiler ve bu da kanserin önlenmesi için farmakolojik önemini gösterdiğini rapor etmişlerdir.

Uyanoğlu ve ark., 2008, yaptıkları araştırmada; sıçanlarda parsiyel hepatektomiyi takiben karaciğerin rejeneratif özelliği üzerine kekik yağından elde edilen karvakrolün olası etkilerini araştırdılar ve elde ettikleri sonuçlara dayanarak karvakrolün karaciğer rejenerasyon oranını arttırdığı sonucuna varmışlardır.

3. MATERYAL VE YÖNTEM

Çalışmamızda, VAN Yüzüncü Yıl Üniversitesi deneysel tıp uygulama ve araştırma merkezı tarafından sağlanan, 3-4 aylık ve 150-250 g ağırlığındaki 28 adet *Wistar albino* cinsi dişi sıçan kullanıldı. Kekik (*Origanum onites L.*), Van Yüzüncü Yıl Üniversitesi Ziraat Fakültesi Tarla Bitkileri Bölümü tıbbi ve aromatik bahçesinden alınmıştır. Bu çalışmada kullanılan 28 rat, her grupta rastgele seçilen 7 rat 4 gruba ayrıldı. Gruplar çalışmanın amacına uygun olarak aşağıdaki şekilde tasarlanmıştır:

i. Grup: Kontrol (K) Grubu (n = 7): Bu gruba hiçbir şey uygulanmadı, sadece standart sıçan yemi ve suyla beslendi.
ii. Grup: %20 Etil alkol (EtOH)) Grup (n = 7): Bu gruba standart sıçan yemi ve su yerine %20 EtOH verildi.
iii. Grup: %3 *Origanum onites* infüzyonu (*O. onites*) Grup (n = 7): Bu gruba standart sıçan yemi ve su yerine taze demlenen ve soğutulan %3 *Origanum onites* infüzyonu verildi.
iv. Grup: %3 *Origanum onites* + %20 Etil alkol %20 EtOH (%3 *O. onites* + %20 EtOH) Grup (n = 7): Bu gruba standart yem ve su yerine 3% *Origanum onite*s ve %20 etil alkol karışımı verildi.

Deney 30 gün sürdü. Deney periyodunun sonunda (30 gün), tüm farelere% 10 ketamin ile anestezi uygulandı ve sakrifiye edildi. Enjektörlerin yardımıyla kan (intrakardiyak) örnekleri alındı. Sıçanların böbrek, eritrosit ve karaciğer dokuları da

alındı ve daha sonra Eritrosit Paketi ve Doku homojenatları hazırlandı ve daha sonra deneyler gerçekleştirilmiştir.

3.1. Deney Protokolü

3.1.1. Serum parametrelerin okunması

AST ve ALT enzim seviyeleri ile TC ve TP (COBAS 8000 ISE, Seri No: 1296-08) otoanlizör cihazında cobas 8000 Roche biyokimya kitleri ile bakıldı.

3.1.2. Redükte glutatyon (GSH) tayini

Elde edilen eritrosit paketleri ve doku supernatantlarında sülfidril gruplarının DTNB 5,5'-ditiyobis (2-nitrobenzoik asit) ile reaksiyonu sonucu sarı rengin oluşumun spektrofotometrik ölçülmesi ile belirlenmiştir (Beutler 1963).

3.1.3. Lipid peroksidasyon (MDA) tayini

Yağ asitlerinin, serbest radikallerle reaksiyonu sonucu oluşan peroksidasyon ürünlerinden MDA, tiyobarbiturik asit (TBA) ile renkli forma girmesi ile ölçülmüştür (Jain ve ark., 1989).

3.1.4. Glutatyon peroksidaz (GSH-Px) enzim tayini

GSH-Px, kümen hidroperoksitin GSH varlığında indirgenmesini katalizlemektedir. Kümen hidroperoksidin indirgenmesiyle oluşan glutatyonun formu GSSG, GR ve NADPH varlığında NADPH'ın $NADP^{+}$'ye yükseltgenmesiyle indirgenir ve enzim aktivitesi, 340 nm'de absorbanstaki değişime göre belirlenmiştir (Paglia ve Valentine, 1967).

3.1.5. Katalaz (CAT) enzim tayini

CAT enziminin aktivite tayini, 37 ^{o}C 240 nm'de H_2O_2'in tüketilme esasına dayanan spektrofotometrik metoda göre tespit edilmiştir (Aebi, 1974).

3.1.6. Glutatyon *S*-transferaz (GST) enzim tayini

GST, 1-kloro-2,4-dinitrobenzen (CDNB) ile glutatyonun -SH grubu arasındaki tepkimeyi katalizler. Enzim aktivitesi 340 nm'de 37º C'de CDNB ile glutatyon konjugasyon şiddetini ölçerek tespit edilmiştir (Mannervik ve Guthenberg, 1981).

3.1.7. Glutatyon redüktaz (GR) enzim tayini

GR, GSSG'nin NADPH tarafından GSH'a indirgenmesini katalize eder. GR aktivitesi (EU), 37 ºC'de 340 nm dalga boyunda dakika başına harcanan NADPH miktarından hesaplanmıştır (Carlberg ve Mannervik, 1985).

3.2. İstatistiksel analiz

Ortalama ve standart sapma (X±SD), Minitab hazır program kullanılarak standart metotlara göre; grup ortalamaları arasındaki fark ise Tek Yönlü Varyans Analizi (One Way ANOVA-Turkey) testi kullanılarak hesaplandı. $p \leq 0.05$ olan değerler istatistiksel olarak anlamlı kabul edildi (İkiz ve ark.,1996).

4. TARTIŞMA VE SONUÇLAR

Biyomedikal literatürler, serbest radikallerin ve diğer reaktif türlerin (ROS) insan hastalıklarına karıştığı iddialarıyla doludur. Romatoid artrit, Rett sendromu ve hemorajik şok, kardiyomiyopati ve kistik fibrozis ile intestinal iskemi, AIDS ve hatta erkek tipi kellik arasında değişen 200'den fazla bozuklukta rol oynarlar (Halliwell ve Gutteridge, 2015). Kekik infüzyonu ve etil alkolün subkronik uygulamalarına maruz

kalan Sıçanların serum ve çeşitli dokularından alınan örneklerde, kekik infüzyonunun karaciğer koruyucu ve antioksidan rolü için; karaciğer harabiyeti için serum AST, ALT enzimleri ile TP ve TC seviyeleri ve karaciğer, böbrek ve eritrosit dokularında antioksidan savunma unsurlarından CAT, SOD, GSH-Px, GST, GR enzim aktiviteleri ile GSH seviyeleri ve MDA içerikleri tespit edilerek antioksidan rolü ortaya konulmuştur. Hem ALT hem de AST serum enzim seviyelerinde; %20 EtOH grubu, kontrol grubuna kıyaslandığında istatistiksel olarak anlamlı bir artış gösterdi. Çalışmamızda bu enzimlerin seviyelerindeki artış, etil alkolün toksik etkilerinin bir sonucu olarak karaciğerin metabolizmasındaki ve detoksifikasyon aktivitesindeki değişikliklerin bir sonucu olabilir. Çünkü bazı araştırmacılar, serum kökenli karaciğer kaynaklı enzimler, AST ve ALT konsantrasyonlarının, alkolik hastalarda sık sık arttığını bildirilmiştir (Niemelä, 2002, Rosman ve Lieber, 1994). Ayrıca, EtOH ile beslenen grubun total protein seviyesinde kontrol grubuyla karşılaştırıldığında önemli bir düşüş vardı, bunun nedeni etiketli amino asitlerin karaciğer proteinlerine dahil edilmesinin azalmasından kaynaklanıyor olabilir. Öte yandan, kekik infüzyon destekli gruplarda, EtOH grubuyla karşılaştırıldığında toplam protein seviyesinde anlamlı bir artış vardı ve bu durum neredeyse kontrol grubu seviyesinde kadar yükseldi ve bu etki kekik infüzyonunun antioksidan etkisinin bir göstergesi olarak etanol zehirlenmesinden oluşan protein sentez inhibisyon şiddetini azaltmak suretiyle olmuş olabilir. Çalışmamızda, EtOH grubunun total kolesterol seviyesindeki kontrol grubuna kıyasla anlamlı bir düşüş vardı, bunun nedeni lipid homeostazı ve etanolün aşırı kullanımı ile indüklenen karaciğer dejenerasyonundaki değişikliklerden kaynaklanıyor olabilir. Çalışmamızda, EtOH grubundaki MDA düzeylerinin diğer gruplara göre anlamlı derecede arttığı dikkat çekmektedir. Çünkü alkol uygulamasının biyolojik sistemlerin antioksidan aktivitelerini önemli ölçüde azalttığı ve sonradan MDA seviyelerini arttıran lipid peroksidasyonunu başlatan serbest radikallerin birikmesiyle sonuçlanması muhtemeldir (Wang ve ark, 2012). Dolayısıyla, hepatik lipid peroksidasyonunun daha yüksek seviyeleri, etanol metabolizmasının çoğunluğunun karaciğerde meydana gelmesi ile bağlantılı olabilir (Molina ve diğ., 2003).

Bununla birlikte, çalışmamızda GSH düzeyinde EtOH grubunun kontrol grubuyla karşılaştırıldığında anlamlı bir artış olduğu, bunun etil alkol

metabolizmasından kaynaklanan büyük miktarda ROS'un üretiminin üstesinden gelmek için dokulardan bir savunma mekanizması olduğu ve bunun sonucunda GSH düzeyi, yapılan bir çalışmada olduğu gibi artmış olabilir (Yaylacı ve ark., 2014). Çalışmamızda, etanol grubunun SOD aktivitesindeki artış, süperoksit radikallerinin artışı nedeniyle olabilir. Hücrede oksidatif streslerin büyüklüğü ve buna bağlı adaptasyon ile birlikte SOD aktivitesinde bir artış gözlendiği (Koner ve ark. 1988) ortaya konulmuştur. Ayrıca, yüksek seviyelerde oksidatif stresin yüksek SOD üretimine yol açabileceği ve bunun da etkili koruma için yeterli SOD enzim sentezi gereksinimi doğurabileceği düşünülebilir (Scandalios, 1993). CAT enziminde önemli bir artış görüldü ve bu enzim oksijen serbest radikal kaynaklı hasara karşı koruyucu bir role sahip olduğundan, onun indüksiyonu oksidatif strese karşı adaptif bir cevap olarak anlaşılabilir (Patlolla ve ark. 2009). Katalazın benzer metabolik adaptasyonları, diğer araştırmacılar tarafından etanola maruz kalan çeşitli sıçan dokularında gözlemlenmiştir (Omodeo-Sale., 1997). Keza, dokulardaki CAT aktivitesindeki bu artış, belirli dokular tarafından arttırılmış olması etanol toleransı olduğunu gösterir (Husain ve ark., 2001). GR aktivitesi ile GSH düzeyi arasındaki yüksek pozitif korelasyon, redüktaz aktivitesindeki artışın GSH mevcudiyetinin korunduğu savunma mekanizması olduğunu göstermektedir (Lukaszewicz-Hussain ve Moniuszko-Jakoniuk, 2004). Ayrıca, yüksek GR aktivitesi hücrelerdeki peroksidatif bileşenlerin artmasından kaynaklanıyor olabilir. Organizmadaki serbest radikallerin artması oksidatif strese neden olur. Dolayısıyla, bu stresi ortadan kaldırmak için antioksidan enzimlerin aktivitesi artar. Bu nedenle araştırmamızda GST enzimindeki artışın bir antioksidan adaptasyon sisteminin indüklenmesi nedeniyle olabileceği sonucuna vardık. Artan GST aktivitesi, etanolün neden olduğu lipit peroksit toksisitesine karşı adaptif bir değişimi yansıtabilir (Turan ve Çelik, 2016, Sonde ve diğerleri, 2000, Yaylacı ve diğerleri, 2014). Ayrıca, GSH-Px enzim seviyesinde bir artış gözlendi ve bu enzimde gözlenen yükselmelerin, serbest radikal oluşumuna cevaben antioksidan aktivitesinde telafi edici bir artışı temsil etmesi mümkündür (Mackenzie, 2002). Ayrıca, lipid peroksidasyonu ve belirli bir zaman diliminde serbest radikallerin artan aktivitesi, enzim aktivitesini artırabilir kanaatindeyiz.

CURRICULUM VITAE

Shreen Abdulkareem MUSA was born on August 2, 1992 in Duhok province, Iraq. She completed primary, secondary and high school in Duhok Province. She graduated from Biology Department in Duhok University in 2015. She speak Kurdish, Arabic, English and Turkish. She started her MSc. at Van Yuzuncu Yil University in Van -Turkey on February 2017.

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