ISBN 978-2-38236-236-5

LIVRE DE LYON

OXIDATIVE STRESS AND ANTIOXIDANT DEFENSE SYSTEM

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Editor
Assoc. Prof. Dr. Aysel GÜVEN

Health Sciences

LIVRE DE LYON
2021

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LIVRE DE LYON
Lyon 2021

Oxidative Stress and Antioxidant Defense System

Editor •. Assoc. Prof. Dr. Aysel GÜVEN • Orcid: *0000-0001-7511-7105*
Cover Design • Clarica Consulting
Book Layout • Mirajul Kayal
First Published • December 2021, Lyon

ISBN: 978-2-38236-236-5

Publisher • Livre de Lyon
Address • 37 rue marietton, 69009, Lyon France
website • http://www.livredelyon.com
e-mail • livredelyon@gmail.com

PREFACE

This book presents all the studies on oxidative stress and antioxidant defense systems of Health Sciences, Oral and Dental Health, Pharmacy, Nursing and Health Services Schools, together with new information and technologies, and all studies on scientific developments. While the book is a good resource in every period of academic life, it is aimed to benefit everyone who is interested in the subject.

In the book, the sources of free radicals that we encounter in every period of life, the damage they cause and the endogenous and exogenous antioxidants that try to prevent these damages will be presented in the light of the updated literature and will be an important source for the studies to be done.

"Knowledge grows as it is shared," I say respectfully.

Editor
Associate Professor Aysel GÜVEN

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CHAPTER 1

THE EFFECTS OF PESTICIDES AT SUBLETHAL DOSES ON THE LEVELS OF OXIDATIVE STRESS AND BIOCHEMICAL PARAMETERS IN SOME ECONOMICALLY IMPORTANT FISHES

İnan KAYA [1] & Haci Ahmet DEVECİ[2] & Müge MAVİOĞLU KAYA[3] & Ayla DEVECİ[4] & Mehmet Ali KIRPIK[5] & Muhitdin YILMAZ[6]

[1] (Assoc. Prof. Dr.) Kafkas University, e-mail: inankaya_@hotmail.com
Orcid: 0000-0003-2900-4067
[2] (Assoc. Prof. Dr.) Gaziantep University, h.ahmet_deveci@hotmail.com
Orcid: 0000-0002-3862-1991
[3](Assist. Prof. Dr.) Kafkas University, m.mavioglu@hotmail.com
Orcid: 0000-0003-1276-3745
[4] (Lect.) Kilis 7 Aralık University, ayladeveci92@gmail.com
Orcid: 0000-0003-2574-0251
[5] (Prof. Dr.) Kafkas University, mhmtalikrpk@gmail.com
Orcid: 0000-0003-0156-8127
[6] (Prof. Dr.) Sinop University, muhittinylmaz@gmail.com
Orcid: 0000-0002-1733-9719

1. Introduction

In the world where global warming is felt more and more every hour, environmental disasters occur due to the change in the ecological qualities of the aquatic environment. In the natural environment, pollutants pass into the body of living organisms through nutrients and environmental factors. Because of the nutrients and water used by aquatic organisms living in polluted waters for their vital functions, pollutants pass into the fish's body through the digestive and respiratory systems and are generally kept in the adipose tissue (Güvenç and Aksoy, 2007; Kilercioğlu et al., 2015).

According to the Stockholm Convention which was in force in 2004, countries are required to eliminate pollutants that cannot be destroyed in nature

until 2025 (Secretariat of the Stockholm Convention 2005; Kilercioğlu et al., 2015). The meticulous implementation of this convention will also positively affect the existence of aquatic organisms and contribute to the preservation of the living standards of societies that are economically dependent on them. Sea saliva (mucilage) emerging in the Marmara Sea increasingly in recent years is one of the most recent examples of disasters caused by environmental pollutants. Due to the trade center and being under the influence of dense population, the lack of dissolved oxygen in the water caused by pollution in the Marmara Sea and other factors create mucilage by disrupting the ecological balance, and it can be said that serious economic and health problems are inevitable for people at the top of the food chain due to this disaster (Keleş et al., 2020).

In order to ensure the sustainability of fish production and life in harmony with the environment, biochemical parameters in tissues such as blood, gill, liver, kidney or brain are frequently included in recent studies. More than one biochemical indicator is used in different ways in different studies to evaluate the response to toxic conditions. In the sum of all these parameters, it is tried to evaluate the interaction between the environment and fish in a sustainable ecosystem (Li et al., 2010; Ferrari et al., 2007; Arslan et al., 2016; Fırat and Aytekin, 2018; Karataş et al., 2019; Ghelichpour and Mirghaed, 2019).

Seafood contains many nutrients that are beneficial for humans and are easily absorbed from the digestive system. This is why it makes it one of the best sources of nutritional components (Bourre and Paquotte, 2008; Aci et al., 2020). In this respect, fish are also economically important. Some economically important fish species from fish families are carp (*Cyprinus carpio*) and transcaucasian barb (*Capoeta capoeta*) from Cyprinidae, trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss)* from Salmonidae, catfish (*Clarias gariepinus*) from Clariidae, Nile tilapia (*Oreochromis niloticus*) from Cichlidae, perch (*Dicentrarchus labrax*) from Moronidae and there are many studies conducted in the world on these fish species (Selcuk et al., 2010; Kaya et al., 2014; Nur et al., 2017; Yılmaz et al., 2012; Aci et al., 2020; Yalcin et al., 2001; Arslan et al., 2007; Şahan et al., 2016; Özden and Erkan 2008).

In this chapter, it is aimed to contribute to the understanding of relationship between environmental pollution, aquaculture, nutrition and health by trying to reveal the effects on oxidative stress and biochemical parameters of pesticides that can be mixed into the aquatic environment due to domestic, agricultural and industrial activities on some economically important fish species through a literature review.

1.1. Oxidative Stress and Biochemical Parameters

Reactive oxygen species (ROS) called free radicals are formed as a result of normal metabolic reactions in living organisms. These are also called oxidants. In addition, there are defense mechanisms called antioxidants that sweep away these harmful species in the organism. There is a harmony between these oxidants and the antioxidant system. Whenever oxidant production increases and there is an imbalance between them, then a situation called oxidative stress occurs. Oxidative stress damages the cellular structures in the organism. As a result, various pathological conditions occur which can progress even to death (Dorval and Hontela, 2003; Kaya and Karapehlivan, 2017; Başer et. al., 2020; Gelen et al., 2021; Kükürt et al., 2021, Nur et al., 2021).

In recent studies, many parameters such as reduced glutathione (GSH), lipid peroxidation (LPO) in terms of metabolic intermediates, superoxide dismutase (SOD), glutathione peroxidase (GSHPx), catalase (CAT) from enzymes or total antioxidant/oxidant capacity (TAS/TOS) as indicators of oxidative stress have been used (Kıral et al., 2008; Alkan et al., 2014; Deveci et al., 2017; Berkoz et al., 2019; Uçar et al., 2020; Kaya et al., 2021, Dogan et al., 2021a). Since oxidative stress is induced by many toxic agent including pesticides used in agriculture or industry, the effect of pro-oxidant factors in fish organism can be used to assess pollution (Güven et al., 2008; Slaninova et al., 2009; Kaya et al., 2012).

The levels of many biochemical parameters such as lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), alkaline phosphatase (ALP), acetylcholine esterase (AChE), total protein (TP), glucose and urea are evaluated in the diagnosis and follow-up of metabolic and clinical conditions in organisms (Zaki et al., 2009; Sekin et al., 1996; Cenesiz et al., 2005; Uçar et al., 2020, Dogan et al., 2021b). The together explanation of findings about oxidative stress and biochemical parameters in fish has great importance for health of human and animal with environmental and aquatic toxicology (Table 1 and 2).

2. Fishes, Pesticides, Oxidative Stress and Biochemical Parameters

2.1. Carp (Cyprinus carpio L., 1758)

Carp is widely distributed in almost all fresh waters of Turkey and constitutes approximately 1/3 of the total freshwater fish catch (Balık et al., 2006). Therefore, when evaluated in terms of fisheries, it has an important economic share in Turkey.

In carps (*C. carpio*) exposured to low (0.04125 ppm), medium (0.0825 ppm) and high (0.165 ppm) levels of propargite as an organosulfide pesticide was reported increased of levels of calcium, magnesium, phosphorus and chlorine from serum electrolytes and decreased in the iron levels (Tulgar and Çelik, 2019). It was noted that a similar situation was observed in the calcium level of *C. carpio* fish exposed to Sencor 70 WG (metribuzin 70% W/V) at a dose of 250.2 mg/L (Velisek et al., 2009b). In another study conducted in young carp, it was reported that the sublethal toxicity of propoxur as a carbamate pesticide at a dose of 5 mg/L caused a significantly increase in plasma phosphorus, sodium and chlorine levels (Gül et al., 2012). It was noted a decrease in chlorine levels, an increase in sodium and phosphorus levels for sublethal cyfluthrin (10 µg/L) toxicity in *C. carpio*. (Sepici-Dinçel et al., 2009).

It has been noted that sublethal doses of Propargite cause an increase in glucose levels of carp (Tulgar and Çelik, 2019). In another study, endosulfan (0.26, 0.52 and 0.78 ppb) toxicity was reported to cause increased glucose levels (Chandrasekar and Jayabalan, 1993). Gül et al., (2012) reported that sublethal propoxur toxicity increased plasma glucose levels in young carp. One study reported increased glucose levels in carp fish exposed to sublethal toxicity of lufenuron (0.11 mg/L) and flonicamid (4.3 mg/L) as pesticides (Ghelichpour and Mirghaed, 2019).

It has been noted that propargite (0.04125 ppm, 0.0825 ppm and 0.165 ppm) toxicity in carp significantly increased albumin concentrations and decreased globulin concentrations (Tulgar and Çelik, 2019). In another study conducted in carp, it was noted that the sublethal toxicity of propoxur caused a statistically important increase in plasma total protein levels (Gül et al., 2012). An increase in plasma cortisol and C-reactive protein (CRP) levels was detected in carp for sublethal 10 µg/L cyfluthrin toxicity (Sepici-Dinçel et al., 2009). In a study, it was reported that cortisol levels decreased in carp exposure to sublethal toxicity of 0.11 mg/L dose of lufenuron and 4.3 mg/L dose of flonicamide pesticides (Ghelichpour and Mirghaed, 2019).

It has been reported that sublethal propargite causes a decrease in triglyceride (TG) levels and an increase in cholesterol (CHOL) and low-density lipoprotein (LDL) levels in carp fish (Tulgar and Çelik, 2019). Sublethal toxicity of propoxur in carp resulted in increased plasma CHOL and TG levels (Gül et al., 2012).

As a result of propargite toxicity in carp, the activity increases in of ALT, ALP, AST, CK and LDH enzymes has been reported (Tulgar and Çelik, 2019). Similarly, in AST and ALT values of *C. carpio* exposed to diazinon at

0.5, 1.0 and 2.0 mg/L doses (Ahmad, 2011), LDH level of *C. carpio* exposed to Roundup® (3.5, 7 and 14 ppm) (Gholami-Seyedkolaei et al., 2013) and CK level of *C. carpio* exposed to Talstar EC 10 (57.5 µg/L, active ingredient 100 g/L bifenthrin) (Velisek et al., 2009a) has been recorded increases. It was noted that the sublethal toxicity of propoxur caused an increase in plasma AST activity of carp (Gül et al., 2012). In a study, it was reported that chlorpyrifos (0.52, 0.26 and 0.13 mg/L) administration caused an increase in protein carbonyl and 8-Hydroxy-2'-deoxyguanosine (8-OHdG) levels and a decrease in acetylcholinesterase (AChE) levels (Berkoz et al., 2019). In addition, it was reported that AST and ALT activities increased in carp exposed to Roundup® at 3.5, 7 and 14 ppm doses (Gholami-Seyedkolaei et al., 2013).

Sublethal 10 µg/L cyfluthrin toxicity was stated to lead to a important increase in the malondialdehyde (MDA) levels as a lipid peroxidation production in brain tissue in addition to a decrease in total antioxidant levels in *C. carpio* (Sepici-Dinçel et al., 2009). Berkoz et al. (2019), 0.52, 0.26 and 0.13 mg/L doses of chlorpyrifos application caused an important rising in MDA levels in *C. carpio* brain tissue, increased levels of glutathione (GSH), but GSHPx, CAT and SOD activities were found to decrease. In the same study, it was found that chlorpyrifos administration caused an increase in protein carbonyl and 8-OHdG levels and a decrease in AChE levels (Berkoz et al., 2019). In an study, exposure to sublethal chlorpyrifos (0.0112 mg/L and 0.0224 mg/L) was found to cause AChE inhibition in *C. carpio* (Ramesh and David, 2009).

Nitric oxide (NO) as a lipophilic molecule with unpaired electrons is produced by nitric oxide synthase during the conversion of arginine to citrulline. Although it is not a reactive radical, it is important because it can form reactive intermediates (Moncada et al. 1989; Kükürt et al., 2021). In a study, it was noted that 0.04 mg/L and 0.08 mg/L chlorpyrifos toxicity significantly increased NO and MDA levels in *C. carpio* (L. 1758) (Deveci et al., 2016).

Sialic acid (SA) derived from neuraminic acid by N-acetylation is found in the structure of biological membranes, macromolecules and receptors (Schauer, 1982; Varki, 1992). It has been reported in many studies that various factors that cause adverse effects in living organisms significantly increase total sialic acid levels (Deveci et al., 2016; Deveci et al., 2017; Kaya et al., 2019). In a study conducted in *C. carpio*, it was noted that chlorpyrifos toxicity (0.04 mg/L and 0.08 mg/L) significantly increased total sialic acid (TSA) levels (Deveci et al., 2016).

Paraoxonase enzyme (PON1) is a glycoprotein calcium dependent for hydrolyze organophosphates and is found in in soft tissues such as body fluid

and tissues. This enzyme which binds to HDL in serum is known as a important antioxidant defense compound in reducing lipid peroxidation (Aviram et al., 1998; Deveci et al., 2015; Kaya et al., 2015; Kaya et al., 2016). In a study investigating the effects of non-lethal doses of 0.025 mg/L, 0.5 mg/L and 1 mg/L malathion on PON1 and ARE enzyme activities in *C. carpio*, it was found that exposure to malathion toxication had a decreasing effect on serum and liver PON1 and ARE enzyme activities of fish (Kılıç and Yonar, 2017).

2.2. Transcaucasian barb (Capoeta capoeta [Guldenstaedt, 1773])

It has been reported that the level of MDA, which is a marker that increases during lipid peroxidation and oxidative stress, changes significantly in *Capoeta capoeta* administered sublethal doses of glyphosate (0.01 and 0.02 mg/L) (Kaya et al., 2012). It has been reported that TSA levels, which generally increase during tissue damage and during oxidative stress, show an increasing feature in sublethal glyphosate concentrations (Kaya et al., 2012; Yılmaz et al., 2017). In addition, in a study on Kars Creek transcaucasian barb (*Capoeta capoeta* [Guldenstaedt, 1773]), serum TAS and TOS levels directly related to oxidative stress were analyzed following exposure to a dose of sublethal glyphosate as an herbicide. significantly increased. It was also reported that the HDL levels of fish treated with glyphosate were also significantly reduced compared to the control group which was not administered glyphosate (Deveci et al., 2017).

2.3. Trout (Salmo trutta L., 1758)

Brown trout (Salmo trutta L., 1758) is a naturally common fish species in the freshwaters of Europe, including Turkey. This fish is used wherever it commons as a sustainable resource and is found an internationally important share for commercial and aquaculture (Arslan et al. 2007).

In a study examining effects for brown trout of propiconazole (1-(2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl)-1H-1,2,4-triazole) 0, 23, 102 and 296 µg/L for 25 g fish and 0, 8.3, 23, 93, 313 and 606 µg/L for 50 g fish as a fungicide has been reported that in fish whose glutathione S-transferase (GST) activities were measured, a significant increase was observed at 93 and 313 µg/L doses in 50 g fish and suppressed at 606 µg/L doses. Also, increase of GST was observed at doses of 23 and 102 µg/L in the 25 g fish group (Egaas et al. 1999).

In a study evaluated ALP, AST, ALT, LDH, CK, glucose (GLU) and total protein (TP) levels in the sera of Caspian brown trout (*Salmo trutta caspius* Kessler, 1877) exposed to sublethal 0.0026 mg/L chlorpyrifos toxicity for 10, 20 and 30 days was reported that exposure to chlorpyrifos caused to increase

in LDH, CK, ALP, ALT, GLU and TP levels. In addition, it was reported that cortisol concentration increased after exposure for 10 and 20 days and decreased after exposure for 30 days (Adel et al., 2017).

Deltamethrin (1.0 and 2.0 µg/L) exposure in brown trout (*Salmo trutta fario*) was recorded to cause a statistically rise in MDA, ALT and AST levels and also a decline in CAT and AChE activities with blood total protein and albumin content (Karataş et al., 2019). After 21 days of exposure of *Salmo trutta fario* to different concentrations of chlorpyrifos (CPF) (0.25, 0.5 and 1 ug/L) was reported that SOD, GPx, glutathione reductase (GR), GST and acetylcholinesterase (AChE) activities decreased but glucose-6-phosphate dehydrogenase (G6PD), CAT activity, GSH and MDA levels increased (Uçar et al., 2020). It was observed that exposure of *Salmo trutta fario* to different doses of chlorpyrifos (0.25, 0.5 and 1 ug/L) for 21 days resulted in a decrease in GPx, SOD, GST, GR and AChE activities and increase GSH and MDA concentrations with glucose-6-phosphate dehydrogenase (G6PD) and CAT activity (Uçar et al., 2020).

2.4. Rainbow Trout (Oncorhynchus mykiss L., 1758)

Trout which known as cold water fish is farmed in pools and dam ponds. It is stated that it can reach 200-250 g and up to 5 kg in the marine environment. Rainbow trout *(O. mykiss)* is one of the most preferred fish species in aquaculture in Turkey due to its easy harvesting and feeding, good adaptability and resistance to diseases. It has been reported that rainbow trout which is common in nature, can be an important biomarker that reveals the effects of environmental pollutants in aquatic environments (Çelikkale, 1991; Hartavi, 1998; Arslan et al., 2016).

Dorval and Hontela (2003) investigated the effects of different levels of application doses of endosulfan as an organochlorine pesticide on *O. mykiss* enzyme activities and LPO and reported that endosulfan increased MDA level with CAT, GPx and GST activities. Li et al., (2020) investigated the oxidative stress parameters and CAT, SOD, GR and GPx enzyme activities in rainbow trout, which they applied propiconazole at different doses (0.2, 50, 500 µg/L) and days (7, 20, 30 days). According to this study, at the end of the application for 7 days, the antioxidant defense system responded with adaptation against the pesticide's effect, but there was a significant loss of antioxidant enzyme activities in the applications for 20 and 30 days. Accordingly, it has been reported that long-term pesticide applications can cause serious oxidative damage (Li et al., 2010).

It was reported that carboxylesterase activity was inhibited in all groups in *O. mykiss* exposure carbaryl and azinphosmethyl for 24, 48, 96 hours and GSH levels in liver and kidney tissues decreased in both pesticides. In the same study,

they found that while the CAT activity increased in carbaryl application for 24 hours, it decreased in applications for 48 and 96 hours. On the other hand, they reported that azinphosmethyl increased CAT enzyme activity in all applications (Ferrari et al.,2007).

In a study which added *Origanum vulgare* extract at different rates (2, 6, 10, 14 g/kg) to the feed of rainbow trout exposed to diazinon pesticide was found that GPx, SOD and CAT activity levels for liver of groups received 2, 6 and 10 g/kg extract after expose to diazinon increased. However, it was noted that GPx, SOD and CAT activities decreased in the groups received 14 g/kg *Origanum vulgare* and this situation could be related to toxic effects of high-dose origanum extract on antioxidant enzymes and hepatocytes (Rafieepour et al., 2020).

Nur and Deveci (2018) investigated the oxidative stress parameters and the histological changes caused by glyphosate in gill of rainbow trout (*O. mykiss*) in which they applied glyphosate at 1.25 mg/L and 2.5 mg/L doses for 14 days. According to this study, it was determined that the total oxidant level (TOS) increased, while total antioxidant level (TAS) and paraoxonase (PON) activity decreased depending on the increasing dose in glyphosate applied fish. In the examination of the gill tissue, it was determined that there were significant histological changes in both glyphosate groups compared to the control group.

Uçar et al., (2021), in their study examining hematological and biochemical impacts of fipronil insecticide in rainbow trout reported that there were significant changes in hematological parameters with the effect of fipronil. In the same study, it was noted that there was a dose connected limitation in the CAT, SOD, PON, ARE and GP_X enzyme activity levels, while there was a statistically important increase in MDA level and MPO activity as biochemical parameters. Alak et al., (2019) investigated the protection of N-acetylcysteine (NAC) to support the antioxidant defense system in rainbow trout applied cypermethrin pesticide for 14 days. In this study, they reported that AChE, CAT, SOD, PON, ARE and GP_X enzyme activity levels decreased, MDA level and MPO activity increased as a result of oxidative damage composed by cypermethrin in fish brain tissue and NAC applications prevented these changes.

2.5. Catfish (Clarias gariepinus Burchell, 1822)

Clarias gariepinus (*C. gariepinus)* is reported as a common freshwater fish species, found in Turkey, the Middle East, and throughout Central and Southern

Africa. This species lives in all environments such as lakes, ponds and rivers with both deep and shallow water (Yalçin et al., 2001).

In copper, sodium, cortisol, urea, and ALT and AST levels were reported significant increases for *C. gariepinus* exposed to malathion at a concentration of 4.5 mg/L for 98 hours (Zaki et al., 2009). In *C. gariepinus* exposed to 0.05, 0.10, 0.20, 0.30 and 0.40 ppm paraquat dichloride doses for 4 days has been noted to decrease of plasma AST activity and total protein concentration with increase of urea concentration (Ogamba et al., 2011). In this fish exposed to malathion at levels of 0.5, 1.0 and 2.0 mg/L for 4 weeks was reported that plasma glucose level increased and plasma protein level decreased. It has also been reported that toxication causes a decrease in liver and muscle glycogen and increases AST and ALT activities. In addition, it was stated that calcium and magnesium ion concentrations were also affected, whereas these effects were not statistically significant (Ahmad, 2012).

It was reported that exposure to carbofuran at *C. gariepinus* 0.16 and 0.49 mg/L concentrations for 35 days resulted in a statistically decrease in G6PDH, LDH, CAT, SOD, GST and GSH concentrations, and an important increase in cortisol and lipid peroxidation. It was also noted that testosterone levels in males and 17β-estradiol levels in females are decreased (Ibrahim and Harabawy, 2014). In another study of the researchers, an important increase in ALT and AST levels and a decline for ALP activity was recorded in *C. gariepinus* fish exposed to toxication at the same doses. In addition, it was stated that there was significantly increase in plasma GLU, total lipid, creatinine and urea levels and decrease in globulin, albumin and total protein concentrations (Harabawy and Ibrahim, 2014). It was reported that exposure to Thiobencarb (CITRON®) at a dose of 0.72 ppm for 3, 9 and 15 days in African catfish (*C. gariepinus*) could be caused a statistically decrease in serum AST and ALT activities as well as an important reduction in glucose and total protein concentrations (Elias et al., 2020).

African catfish (*C. gariepinus*) by Ayanda et al. (2014) were exposed to different doses of paraquat (0.0035, 0.007 and 0.014 mg/L) and glyphosate (0.0265, 0.053 and 0.106 mg/L) for 8 weeks. It was noted that there was a significant rising for the SOD, CAT, LPO and GPx enzyme activity levels of fish.

2.6. Nile tilapia (Oreochromis niloticus)

Nile tilapia is one of the most cultivated fish in the world among freshwater fish and is fed as omnivores. They are fish that are easy to breed and resistant to environmental conditions. It has a strong immune system, are highly resistant to environmental pollutants. They are frequently used in aquatoxicological

researches because they can live in water contaminated by environmental pollutants and are the primary food source for humans (Fırat and Aytekin, 2018). Figueiredo-Fernandes et al., (2006) investigated the antioxidant enzyme activities at different temperatures (17 and 27ºC) in *Oreochromis niloticus*, which they applied a single dose (0.5 L-1) paraquat. According to this study, it was reported that paraquat caused a important rising in GR, GST and SOD activities. Durmaz et al., (2005) determined that CAT, GPx and SOD activities from antioxidant enzymes increased in *O. niloticus* that they applied diazinon in different periods (1, 7, 15 and 30 days). In the same study, it was reported that there was a similar increase in the levels of MDA as lipid peroxidation product.

Fırat and Aytekin (2018) applied thiamethoxam pesticide at different concentrations (60 and 120 mg/L) and days (4 and 14 days) in *O. niloticus* and reported that oxidative stress parameters were adversely affected in fish tissues. In the same study, it was determined that the GSH level and CAT activity of thiamethoxam at different concentrations (60 and 120 mg/L) were increased in the gill and intestinal tissues in 4-day applications compared to the control group, and CAT activity and GSH level decreased in 14-day application. Also, in this study, it was reported that thiamethoxam increased MDA level and SOD activity in gill and intestinal tissues compared to the control group in 4 and 14-day applications. Meng et al., (2018) SOD at concentrations above 0.2 µg/L in *O. niloticus* to which methomyl was applied at different concentrations (0, 0.2, 2, 20 or 200 µg/L) and days (0, 6, 12, 18, 24 and 30) and reported significant increases in CAT activities. Peixoto et al., (2006) reported that administration of oxyfluorfen at different times (7, 14 and 21 days) and concentrations (0.3 and 0.6 mg/L) to Nile tilapia increased CAT, SOD, GR and GST activities.

2.7. Perch (Dicentrarchus labrax)

Dicentrarchus labrax is a commercial fish with a significant function in the pelagic food webs of European estuaries and coastal regions. This fish is widely used in various researches for the toxic effects of different pollutants because it has many convenient features such as care and transportation (Gravato and Santos, 2003; Varo et al., 2003; Lemaire et al., 1996; Lemaire-Gony et al., 1995). Almeida et al., (2010) in their study with the organophosphate pesticide phenitrothion, reported that the administration of different doses of phenitrothion to *D. labrax* fish for 96 hours caused dose-dependent changes in GR, CAT and GPx activities and a significant increase in SOD activity.

Banni et al., (2011) showed that the GST activity of *D. Labrax* which they evaluated nickel and chlorpyrifos toxication was higher in the chlorpyrifos group than nickel group, but the highest activity was in the nickel plus chlorpyrifos

group. Hernández-Moreno et al., (2011) determined that brain AChE and muscle cholinesterase (ChE) activities decreased significantly in *D. labrax* which they applied pesticide carbofuran. They also reported that there were significant changes in the activities of the antioxidant enzymes GST, CAT, SOD, and GPx. Varo et al., (2003) determined that ChE activity decreased significantly in *D. labrax* which they applied the organophosphate pesticide dichlorvos.

Table 1. Effects of Pesticides in Sublethal Doses on the Levels of Oxidative Stress Parameters in Some Economically Important Fishes

	General Statue	Reference Study or Studies
GPx	↕	Dorval and Hontela 2003↑; Durmaz et al., 2005↑; Ayanda et al., 2014↑; Uçar et al., 2020↓
GR	↑	Uçar et al., 2020↓; Figueiredo-Fernandes et al., 2006↑; Peixoto et al., 2006↑
SOD	↕	Uçar et al., 2020↓ ; Alak et al., 2019↓; Ibrahim and Harabawy, 2014↓; Ayanda et al., 2014↑; Figueiredo-Fernandes et al., 2006↑; Durmaz et al., 2005↑; Peixoto et al., 2006↑; Almeida et al., 2010↑
GST	↕	Uçar et al., 2020↓; Dorval and Hontela 2003↑; Ibrahim and Harabawy, 2014↓; Figueiredo-Fernandes et al., 2006↑; Peixoto et al., 2006↑
CAT	↕	Durmaz et al., 2005↑; Peixoto et al., 2006↑; Uçar et al., 2020↑ ; Karataş et al., 2019↓; Dorval and Hontela 2003↑; Ferrari et al.,2007↑; Alak et al., 2019↓; Ibrahim and Harabawy, 2014↓; Ayanda et al., 2014↑
MPO	↑	Uçar et al., 2021
PON1	↓	Deveci et al., 2017; Nur and Deveci 2018; Alak et al., 2019
ARE	↓	Alak et al., 2019
MDA	↑	Kaya et al., 2012; Uçar et al., 2020; Karataş et al., 2019; Uçar et al., 2021; Deveci et al., 2016
GSH	↕	Uçar et al., 2020↑; Ibrahim and Harabawy, 2014↓
TOS	↑	Nur and Deveci 2018; Deveci et al., 2017
TAS	↓	Nur and Deveci 2018; Deveci et al., 2017
TSA	↑	Kaya et al., 2012
NO	↑	Deveci et al., 2016

↓, ↑ or ↕: The levels of parameters parameter decrease, increase or uncertain. GPx: Glutathion peroxidase, GR: Glutathion reductase, SOD: Superoxide dismutase, GST: Glutathion S-transferase, CAT: Catalase, MPO: Myeloperoxidase, PON1:Paraoxonase 1, ARE: Arylesterase, MDA:

Malondialdehyde, GSH: Reducted glutathion, TOS: Total oxidant statue, TAS: Total antioxidant statue, TSA: Total sialic acid, NO: Nitric oxide

Table 2. Effects of Pesticides in Sublethal Doses on the Levels of Biochemical Parameters in Some Economically Important Fishes

	General Statue	Reference Study or Studies
G6PDH	↕	Uçar et al., 2020; Ibrahim and Harabawy, 2014
LDH	↑	Adel et al., 2017; Tulgar and Çelik, 2019
ALT	↑	Karataş et al., 2019; Zaki et al., 2009; Ahmad, 2011
ALP	↑	Tulgar and Çelik, 2019; Adel et al., 2017
AST	↑	Karataş et al., 2019; Zaki et al., 2009; Ahmad, 2011
CbE	↓	Ferrari et al.,2007
AChE	↓	Karataş et al., 2019; Hernández-Moreno et al., 2011; Berkoz et al., 2019; Uçar et al., 2020
ChE	↓	Hernández-Moreno et al., 2011
CK	↑	Tulgar and Çelik, 2019; Adel et al., 2017
CRP	↑	Sepici-Dinçel et al., 2009
HDL	↓	Deveci et al., 2017
Alb	↓	Karataş et al., 2019; Ibrahim and Harabawy, 2014
TP	↓	Karataş et al., 2019; Ibrahim and Harabawy, 2014
Chol	↑	Tulgar and Çelik, 2019↑ Gül et al., 2012
Globulin	↓	Ibrahim and Harabawy, 2014
Cortisol	↑	Sepici-Dinçel et al., 2009
Total lipid	↑	Ibrahim and Harabawy, 2014
Glucose	↑	Ibrahim and Harabawy, 2014; Tulgar and Çelik, 2019
Creatinin	↑	Ibrahim and Harabawy, 2014
Urea	↑	Zaki et al., 2009; Ibrahim and Harabawy, 2014

↓, ↑ or ↕: The levels of parameters parameter decrease, increase or uncertain. G6PDH: Glutathion-6-phosphate dehydrogenase, LDH: Lactate dehydrogenase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, CbE: Carboxyl esterase, AchE: Acetyl cholinesterase, AST: Aspartate aminotransferase, ChE: Cholinesterase, CK: Creatin kinase, HDL: High density lipoprotein, Alb: Albumin, Chol: Kolesterol, CRP: C-reactive protein, TP: Total protein

3. Conclusion

Pesticides which have an important share among environmental pollutants compose many changes in the biochemical structure of economically important

fish. Looking at previous studies, it was found that pesticide applications was more intensive on *Cyprinus carpio, Capoeta capoeta, Salmo trutta, Oncorhynchus mykiss, Clarias gariepinus, Oreochromis niloticus* and *Dicentrarchus labrax* between economically important fish species. It is understood that pesticide type, fish species, dose and time cause significant and different effects on pollution and toxicity. These changes can be considered as risky for health in general. In addition, it was concluded that more comprehensive studies should be carried out for the economically important fish species scanned and examined in the literature, and urgent measures should be taken in terms of environment and health due to pesticides.

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CHAPTER 2

THE ROLE OF OXIDATIVE STRESS IN ALZHEIMER'S DISEASE

Mehmet BERKÖZ

(PhD., Assistant Professor Doctor), Van Yuzuncu Yil University,
Faculty of Pharmacy,
Department of Biochemistry, Van, TURKEY,
e-mail: mehmet_berkoz@yahoo.com
Orcid: 0000-0003-4219-8054

1. Alzheimer's Disease and its Characteristics

Aging is a process and a rule that works for every living being on earth. Individuals are born and the moment they are born, the biological clock begins to advance. As a concept, old age covers a certain period of this process. The World Health Organization (WHO) accepts the old age period when individuals are 65 years or older. The number of elderly people over the age of 65 in the world is 703 million according to 2019 data, and this number is expected to exceed one and a half billion in 2050 (1). As life expectancy rises, so do age-related illnesses, such as Alzheimer's disease. Alzheimer's disease is a progressive neurodegenerative disease that presents with loss of memory, poor judgment, impaired cognitive abilities, confusion, and changes in behavior and personality (2). A number of noncognitive changes also occur in 90% of Alzheimer's disease patients. These include apathy, depression, anxiety, agitation, aggression, aberrant motor movements, and psychosis (3). The earliest symptom is typically the inability to retrieve newly acquired information, but this will eventually progress into the inability of the individual to do simple daily tasks (4).

There are two classifications of Alzheimer's disease – familial and sporadic (5). Early onset familial Alzheimer's disease (EOFAD) has an onset age of less than 65 years old, with a mean age of 45 years. It occurs in individuals with a family history of dementia and only accounts for approximately 0.5% of Alzheimer's disease cases (6). There are currently 227 genetic mutations that have been linked with EOFAD and predominately occur in amyloid

precursor protein (*APP*), presenilin 1 (*PSEN1*), or presenilin 2 (*PSEN2*) (7). These mutations alter the processing of APP, resulting in increased production of amyloid-beta (Aβ), increased aggregation, or decreased clearance (8). The vast majority of Alzheimer's disease cases are categorized as sporadic, with an age of onset over 65 years with multiple genetic and environmental factors contributing to pathogenesis (9). While age of onset, presentation, and life expectancy can vary depending on type, all forms are comparable in pathology, disease progression, and biomarkers (5). Mutations discovered in EOFAD have allowed for further research into the etiology and pathogenesis of Alzheimer's disease through the creation of transgenic mouse models (10).

The pathophysiological process begins decades prior to clinically detectable symptoms in both EOFAD and sporadic Alzheimer's disease (11). The duration for the disease is 8-10 years after symptoms begin to manifest (5). The progression of Alzheimer's disease can be categorized into three stages: preclinical Alzheimer's disease, mild cognitive impairment (MCI) due to Alzheimer's disease, and dementia due to Alzheimer's disease. The stages of Alzheimer's disease are diagnosed using a multitude of tests, including neuropsychological examinations, neural imaging, and biomarker measurements (12).

As the population of baby boomers enter their elder years, the prevalence of Alzheimer's disease will reach epidemic proportions with an estimated 6.2 million Americans with the disease in 2021. By 2060, the number of people aged 65 and older with Alzheimer's disease may nearly triple to a projected 13.8 million unless an effective intervening treatment is discovered. Between 2000 and 2019, deaths attributed to Alzheimer's disease increased by 145 percent, while those attributed to the number one cause of death, heart disease, decreased by 14 percent (13). Data for 2014 found continuing decreases in mortality for heart disease but a rise of 8.1 percent for Alzheimer's disease (14). One can only imagine what these statistics will look like mid-century if these trends continue with no interventional therapies to alter the progression.

2. Cellular Changes and Pathology in Alzheimer's Disease

The classic neurochemical and pathological hallmarks of Alzheimer's disease includes selective neuronal death, synapse loss and damage, decreases in specific neurotransmitters, mitochondrial dysfunction, inflammation, increases in reactive oxygen species (ROS), and depositions of extracellular senile plaques and intracellular neurofibrillary tangles (NFTs) (15,16). The senile

plaques are comprised of aggregated amyloid-beta (Aβ) proteins, and the NFTs are composed of hyperphosphorylated tau proteins, which can either aggregate into paired helical filaments (PHFs) or remain as straight filaments (17,18).

2.1. Tau Pathology and Cytotoxicity Mechanisms

Tau proteins are microtubule-associated proteins that are predominantly found in the axons of neurons. The primary function of tau is to bind to and stabilize microtubules and promote their polymerization (19). When tau becomes phosphorylated, it negatively affects its ability to bind to microtubules, thus impairing its function (20) . While the mechanisms underlying PHF formation are still unknown, it is thought that phosphorylation of tau and the dissociation from microtubules causes an increase in the tau pool which may be more resistant to degradation by proteases or more prone to aggregation (21). Tau pathology is believed to spread transcellularly, with the hyperphosphorylated tau released into the extracellular space by affected neurons serving as the seed for further propagation (22).

There are six tau isomers in humans, with the most common being the 3R and 4R variants (23). Diseases with the appearance of aberrant aggregations of tau within specific neuronal populations are referred to as tauopathies (24). The composition of tau differs among the tauopathy (17). For example, Pick's disease is characterized by 3R, whereas both progressive supranuclear palsy and corticobasal degeneration have a higher prevalence of 4R (25). Alzheimer's disease has a mixture of 3R and 4R, with the ratio of 3R to 4R being highly variable. Tau filament deposition and NFTs closely correlate with both cognitive decline and cell death, while Aβ dysregulation and deposition far precedes cognitive deficits and plateaus upon reaching the earliest clinical stage of Alzheimer's disease, MCI (26).

2.2. Amyloid-beta Pathology and Cytotoxicity Mechanisms

The genetic mutations that lead to EOFAD are predominantly found either in the *APP*, *PS1*, or *PS2* genes in a dominant manner (5,7). Additionally, Aβ deposition is found in cognitively normal individuals prior to the onset of symptoms (27,28). For these reasons, the Amyloid Cascade Hypothesis of Alzheimer's disease remains the primary theory in the field to explain onset and progression of this disease, as well as the main therapeutic focus to treat it. This hypothesis suggests that the accumulation of toxic Aβ proteins, either due to increased production or decreased degradation, is the cause for all downstream

effects that occur in Alzheimer's disease including NFTs, neurodegeneration, synaptic dysfunction, mitochondrial dysfunction, increased oxidative stress, cell death, as well as the disease itself (29).

Aβ is a 39-42 amino acid peptide that results from the cleavage of one or more transcripts of APP (30). This cleavage is performed by three different secretase enzymes: α (ADAM), β (BACE), and γ (31). The full length APP has been found to carry out several roles. Some of these include cell adhesion, acting as a cell surface receptor, regulating synaptic morphology and plasticity, and cell division (32). However, it is difficult to pinpoint a main function, as both overexpression and knock-out of the gene also affects the fragments that result from its proteolytic cleavage.

The cleavage is generally divided into two competing pathways. One creates non- amyloidogenic products through ADAM cleavage, while the other creates amyloidogenic products through BACE cleavage (33). ADAM10 is the dominant α-secretase and BACE1 is the dominant β-secretase in neurons (34,35). Regardless of the pathway taken, γ- secretase cleavage follows with differing results. Cleavage by ADAM10 results in soluble APPα (sAPPα) and a C-terminal fragment (CTF), CTF-83, which is subsequently cleaved by γ-secretase to yield p3 and APP intracellular domain (AICD) fragments (36). Cleavage by BACE results in soluble APPβ (sAPPβ) and CTFs (most commonly CTF-99), which are subsequently cleaved by γ- secretase to yield Aβ and AICD fragments (37).

Both sAPPα and sAPPβ have been shown to activate microglia, stimulate neurite outgrowth, and play a role in the differentiation of neural progenitors. Data regarding the toxicity of soluble forms and fragments of amyloid-beta is conflicting. However, sAPPα has been shown to have a generally neuroprotective effect being approximately 100-fold more potent than sAPPβ against excitotoxicity, Aβ-related toxicity, and glucose deprivation (38).

Furthermore, sAPPα has been found to block tau phosphorylation and activate neuronal insulin receptors, the importance of which will be discussed later. On the other hand, sAPPβ is primarily viewed as the more toxic soluble species (38). For example, it has the ability to be further cleaved and bind to death receptor DR6, activating neuronal death and degeneration. Thus, sAPPα and its fragment, CTF-83, are generally viewed as protective in disease pathogenesis while sAPPβ and its fragment, CTF-99, are viewed as pathogenic (39).

The γ-secretase consists of four subunits, including PS1 and PS2, which comprise the catalytically active center of the complex. The main products resulting from γ-secretase cleavage on βCTF fragments are Aβ1-40 (Aβ40) and

Aβ1-42 (Aβ42) (37). Aβ40 is the predominant product under basal conditions, with a yield of around 90% compared to less than 10% of Aβ42 (38). However, levels of Aβ42 and the ratio of Aβ42 to Aβ40 are increased in Alzheimer's disease brains. Aβ40 is generally considered to be the less toxic form and has demonstrated the ability to inhibit the oligomerization of Aβ42 (40). Aβ42 promotes the deposition of plaques, while Aβ40 has been found to inhibit that deposition in vivo in a transgenic mouse model (41). Aβ can undergo a variety of post-transcriptional modifications, including phosphorylation, nitration, and pyroglutamate conversion, which contribute to aggregation. While the exact function of Aβ is not fully known, studies have shown that Aβ has a duality whereby it can exhibit both cytotoxic and protective effects (42).

The initial Amyloid Cascade Hypothesis was formulated based upon studies of EOFAD, which accounts for less than 1% of Alzheimer's disease cases (6,10). However, it does not address the etiology of the more prominent sporadic form (42). First, approximately 25-30% of patients that have a considerable amount of senile plaques present with no or very little cognitive impairment (43). This is found in multiple lines of transgenic mice, as well. The deposition of plaques occur months prior to cognitive impairment, with synaptic dysfunction occurring prior to plaque deposition (44). Furthermore, unless the mouse is also expressing a mutant tau transgene, no NFTs are detected, as would be indicated by the hypothesis (45).

Based on these conflicts, the original hypothesis has since been revised naming soluble non-fibrillar oligomeric APP fragments (ADDLs) as the most toxic species (38). ADDL stands for Aβ-derived diffusible ligands and are also referred to as Aβ oligomers (46). The basis for an alternate, ADDL-based view of the Amyloid Cascade Hypothesis is supposed to account for the above mentioned disconnect. In depth study of oligomeric structures, which are estimated to contain 3–24 peptide monomers, find them capable of inducing cell death and rapidly disrupting long-term potentiation (LTP) in organotypic hippocampal slices. LTP is a form of synaptic plasticity that is believed to be a cellular model of memory (47,48). Increasing evidence suggests that LTP impairment is one of the earliest events that occurs following increases in levels of Aβ (47).

The overall revision of the hypothesis is that the immediate consequence of elevated Aβ42 is increased ADDL formation, not increased Aβ deposition. In turn, the increased production of ADDLs leads to functional deficits as a result of ADDL-triggered aberrant synaptic signaling, rather than from neuronal death instigated by plaques and fibrils. Together, this hypothesis is meant to better

account for the earliest subtle deficits (i.e. synaptic changes) to more severe late-stage deficits and structural damage (i.e. tau hyperphosphorylation and NFTs) caused by persistent signaling disruptions that occur after the chronic presence of ADDLs (49). Despite the attempt of the ADDL-based hypothesis to better address Alzheimer's disease etiology, several aspects remain unaddressed. For example, it is known that plaque and NFT load are inversely correlated with oxidative stress, indicating that this pathology may be a cellular mechanism to relieve an increased challenge of oxidative stress on the aging brain (50,51).

Aβ has an interesting duality whereby it can act as both an antioxidant and a pro-oxidant, depending on monomeric or oligomeric form (52). Numerous studies have shown that Aβ exhibits neurotrophic, neuroprotective, metal chelating, and antioxidant abilities at physiological concentrations (53). Aβ in monomeric form protects neurons from metal-induced ROS and death, however this neuroprotective activity is lost when Aβ becomes oligomerized and aggregated. While Aβ has shown toxicity, it is mainly done in vitro using high concentrations of aged Aβ, or in vivo using aged animals that face a higher amount of oxidative stress (54).

The Aβ chelation properties, specifically of Cu^{2+}, Zn^{2+}, Fe^{2+}, and Fe^{3+} have been proposed to turn on antioxidant activities (55). As Aβ42 has a higher binding affinity for these metals than Aβ40, it follows that amyloid plaques constituent mostly of Aβ42 along with the inclusion of these metals (56). Furthermore, Aβ has been found to be neurotoxic when dissolved in Fe (III) containing media but not in media free of Fe^{3+} (57). This may indicate that the toxicity of Aβ is potentiated by metal ions, such as iron, copper, and zinc (58). Furthermore, large injections of fibrillary human Aβ42 caused less neuronal death, while human Aβ40 killed a similar amount of neurons when compared to an equivalent saline dose (59). Additionally, the injection of Aβ containing 1.0 mM iron is significantly less toxic than injections of 1.0 mM iron alone (60). These findings give added credence to the idea that Aβ acts as a metal chelator to provide neuroprotection and is not toxic without the addition of metals.

Increased production of Aβ is accompanied by a decrease in the production of ROS, particularly the highly reactive hydroxyl radical (·OH) (61). Moreover, when Aβ is bound to a specific amount of Cu^{2+} and Zn^{2+} it has been found to catalyze the dismutation of the highly reactive superoxide radical ($O_2 \cdot^-$) into the less reactive H_2O_2, indicating antioxidant activity (62). As mice with PS1 mutations show a significantly decreased activity of the antioxidant enzymes Cu-Zn superoxide dismutase and glutathione reductase prior to plaque deposition, it could be postulated that increased Aβ production is a compensatory

response to diminished levels of antioxidant enzymes and thus increased levels of oxidative stress (63).

The trapping of these metals to reduce more harmful oxidative effects is purported to cause the aggregation of Aβ, which can then exhibit toxic effects. These findings, along with the fact that pro-inflammatory processes, oxidative stress, mitochondrial dysfunction, and neuronal death are seen prior to detectable Aβ deposition has led to alternative hypotheses. These hypotheses ascribe the initiation of Alzheimer's disease pathogenesis to fundamental cellular processes, namely, mitochondrial, intracellular calcium, and oxidative balance dysregulation rather than pathology (64,65). These processes may trigger the induction of classic pathology in order to repair, replace, and restore function to the impacted cells as a consequence of aging and increased oxidative stress (66).

3. Brain Aging and Neuronal Oxidative Stress

Aging is associated with progressive neurological decline, and age is the largest risk factor for neurodegenerative disorders like Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis (67). Features associated with aging such as energy imbalance, protein aggregation, genomic instability, mitochondrial dysfunction, and transition metal dyshomeostasis are thought to promote neurodegenerative disease. However, emerging evidence suggests that oxidative stress may be a central effector of all these features that drives pathogenesis of neurodegenerative diseases. This is supported by data showing that indices of oxidative stress appear in early stages of pathogenesis and are elevated in neuronal populations most afflicted during pathogenesis. Interestingly, different neuronal populations seem to exhibit different vulnerabilities to oxidative stress based on phenotypic characteristics (68). Compelling evidence suggests that this phenomenon, called selective neuronal vulnerability (SNV), may dictate the regional specificity of neuropathology in different neurodegenerative disorders (69,70).

The notion that neurons respond differently to oxidative stress is not surprising given the brain contains billions of neurons with diverse functions, distributions, and morphologies. Neurons vary morphologically by axon length, synaptic distance, dendrite complexity, and myelin content and differ functionally according to neurotransmitter type such as glutamate, acetylcholine, GABA, dopamine or others. Inherent in such diversity is an assortment of unique expression profiles and chemical reactions that govern neuronal specificity and sensitivity to oxidative stress. It thus stands to reason that SNV may explain heterogeneity in affected neuronal populations of common neurodegenerative disorders. For example, neurons of the frontal cortex, entorhinal cortex, and

CA1 region of the hippocampus exhibit SNV to oxidative stress and are the most afflicted in Alzheimer's disease (71). On the other hand, Parkinson's disease is characterized by neurodegeneration of dopaminergic neurons of the substantia nigra, while Amyotrophic Lateral Sclerosis is restricted primarily to spinal motor neurons and motor tracts of the cortex (72). SNV to oxidative stress is likely predicated on several cellular factors. One is the basal level of ROS generation due to a higher ATP demand. This is exemplified by CA1 hippocampal neurons that have a high metabolic rate and rely on endogenous ROS for long term potentiation and memory formation (73). Support for this can be found in numerous studies demonstrating sensitivity to ROS toxicity in CA1 neurons which induced mitochondrial dysfunction and ROS production (73,74). While transcriptional profiling of CA1 neurons does indicate acute induction of compensatory antioxidant genes, maintaining redox homeostasis is an energy intensive process that declines with age due to mitochondrial dysfunction and elevated mitochondrial ROS production, both of which skew to a more oxidizing environment. Redox-active metabolites can also sensitize select neurons to oxidative stress. In dopaminergic neurons of the substantia nigra pars compacta, iron accumulates with age at relatively high levels which increases ROS burden through Fenton reactions (75,76). Increased ROS in these neurons can oxidize dopamine itself yielding the highly neurotoxic compound 6-hydrodopamine (77). Progressive age-related loss of calcium balancing capacity can also sensitize select neurons to oxidative stress, particularly in glutaminergic neurons. Disrupted calcium efflux, increased calcium influx, and decreased calcium buffering simultaneously occur with age leading to elevated intracellular calcium $[Ca^{2+}]_i$ (78). Elevated $[Ca^{2+}]_i$ can lead to increased ROS through stimulation of mitochondrial metabolism and activation of nitric oxide synthases (79). In glutaminergic neurons, elevated ROS seems to drive increased $[Ca_{2+}]_i$ leading to the accumulation of $[Ca^{2+}]_i$ which can induce further Ca^{2+} release from ER stores and initiate caspase-dependent apoptosis. Increased $[Ca^{2+}]_i$ can also hyperactivate glutaminergic synapses leading to excess glutamate release at the risk of glutamate excitotoxicity (80). As expected, glutaminergic neurons are the most sensitive to Ca^{2+} dysregulation during oxidative stress which may in part explain why the majority of synapses lost in age-associated neurodegenerative disorders are glutaminergic (80,81). Another interesting attribute that contributes to SNV is neuron size and axonal length. Neurons with longer projections have a larger surface area that is more exposed to damaging insults like protein aggregates. Maintaining membrane integrity for long distance neurotransmission is also energy intensive and requires large quantities of ATP.

Indeed, pyramidal neurons of the hippocampus, dopaminergic neurons of the substantia nigra, and upper and lower motor neurons are in fact relatively larger neurons and undergo significant neurodegeneration in Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis respectively (82).

4. Lipid Peroxidation in Alzheimer's Disease Pathogenesis

The etiology of Alzheimer's disease for sporadic and late onset cases is unknown, although oxidative stress is thought to be an important contributor to disease progression and/or initiation. This is corroborated by a plethora of evidence from human and mouse tissues showing the presence of ROS mediated damage markers strongly associated with Aβ formation. Oxidative stress is an early event that has been shown to precede Aβ plaque formation, NFT aggregation, and cognitive decline in various mouse models. In fact, oxidative stress can directly exacerbate Aβ deposition and tau phosphorylation, both of which promote further oxidative stress, leading to a vicious cycle of progressive oxidative stress burden (83,84).

The most damaging effects from oxidative stress in neurons is lipid peroxidation (LPO) damage to membranes (described in detail in section A of this chapter). LPO damages neuronal membranes and alters the function of membrane proteins that can lead to disruption of synaptic function. LPO is prominent in Alzheimer's disease due to the ability of Aβ peptides to directly initiate LPO events in neuronal membranes. This prooxidant effect seems to be the product of Aβ42 oxidation at Met35 which generates a sulfoxide modification that allows the peptide to oligomerize with phospholipid membranes and propagate LPO (85). Convincing evidence of this was generated by replacing Met35 on Aβ42 with Cys which dramatically reduced Aβ42 derived LPO indicating that the oxidation of Met35 is likely important for the overt toxicity of Aβ42. Indeed, the majority of Aβ in plaques isolated from Alzheimer's disease patients contain the sulfoxide modification on Met35, further supporting the idea of a feed-forward relationship between Aβ and LPO in neurons (60).

Reactive aldehydes like Malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE) have become established surrogates for LPO damage in blood and tissues of Alzheimer's disease patients (86). These "toxic second messengers" covalently modify an array of macromolecules and disrupt cellular function. While the link between reactive aldehyde formation and Alzheimer's disease has long been observed, the functional effects of reactive aldehydes in Alzheimer's disease pathogenesis are becoming more appreciated through the use of redox proteomics. These tools have the ability to detect and identify

oxidatively modified proteins as a result of LPO at various stages of Alzheimer's disease pathogenesis (87).

HNE is the most abundant and toxic reactive aldehyde product of LPO and as such, is one of the most examined modifications using redox proteomics (88). In a series of studies, the Butterfield group has characterized HNE-modified proteins from human brain tissue representing different stages of neurological decline from MCI to late Alzheimer's disease. A variety of HNE-modified proteins were consistently found in all stages and included proteins related to energy metabolism (α-enolase, pyruvate kinase, and lactate dehydrogenase), antioxidant defense (peroxiredoxin, glutamate synthase), structural support (α-tubulin, actin), proteasomal function (UCH-L1), as well as proteins involved in tau phosphorylation and APP processing (89). Interestingly, HNE-modified proteins involved in energy metabolism were particularly enriched in MCI brains. This included hits for pyruvate kinase, phosphoglucomutase-1, α-enolase, ATP synthase, and glyceraldehyde 3-phosphate dehydrogenase (90). Because all these proteins are in some way involved in ATP synthesis, it was surmised that HNE-modification and inactivation of one or more of these enzymes might contribute to the decreased glucose metabolism that occurs in prodromal stage of Alzheimer's disease. Coincidently, other groups have reported diminished activity for all these enzymes in Alzheimer's disease brain (91). Taken together, these studies provide compelling evidence for a mechanistic link between LPO and energy imbalance in MCI and early Alzheimer's disease.

In addition to Alzheimer's disease, redox proteomic tools have identified a consortium of HNE modified proteins in in other neurodegenerative diseases including Parkinson's disease, Amyotrophic Lateral Sclerosis, and Huntington's disease (92). These include proteins involved in energy metabolism, antioxidant response, cell cycle regulation, and protein homeostasis (93). Furthermore, HNE has also been shown to be present in pathogenic protein aggregates associated with diseases such as α-synuclein (Parkinson's disease), SOD1 (Amyotrophic Lateral Sclerosis), HTT proteins (Huntington's disease), and Aβ (Alzheimer's disease) (94). Overall, studies using redox proteomic techniques have yielded data that support the importance of lipid peroxidation in pathogenesis of neurodegenerative diseases and those data provide new insights into mechanisms driving neurodegenerative disorders (95).

5. Mitochondrial Dysfunction

Mitochondria are organelles that are essential for normal energy metabolism of ATP, distribution, trafficking, calcium modulation, cellular redox state

regulation, ionic regulation, apoptosis, and are integral for cellular signaling, communication, and survival. Mitochondrial dysfunction translates in decreased cellular respiration, increase in ROS, calcium overload, hypometabolism, and cell death (96). The brain, which accounts for 2% of body's weight and consumes 20-25% of the oxygen, has no way to store energy, which makes the mitochondria crucial for cerebral function. Synaptic mitochondria, which are generated in the neuronal soma and further transported to synapses, undergo continuous activation to sustain the high energy demand at synaptic terminals and maintain synaptic function and transmission, normally through glucose oxidation. As a result, any defect or alteration in mitochondria severely compromises synaptic function. Synaptic mitochondria, which are more vulnerable than non-synaptic counterparts, undergo increased oxidation during aging, and are more susceptible to calcium insult and Aβ accumulation that interferes with organelle function and distribution/trafficking along axons (97).

Maintaining membrane potential is of utmost importance in order for mitochondria to function properly, and its loss leads to interference in the electron transport chain, mitochondrial swelling, mPTP pore formation, cyt c release, and apoptosis (98). Alterations in mitochondria function affect ATP production through oxidative phosphorylation, resulting in disruption of bioenergetic processes and eventually cell death. In Alzheimer's disease, a calcium imbalance may be due to mitochondrial dysfunction. Normally, mitochondrial Ca^{2+} uniporter accumulates excess Ca^{2+} in mitochondria, while Na^{+} /Ca^{2+}- and H^{+}/Ca^{2+}-antiporters release Ca^{2+} in the cytoplasm to maintain homeostasis (99). Aβ is thought to increase calcium levels by promoting its release from the endoplasmic reticulum, upon which mitochondria take up the cytosolic calcium in an attempt to regulate its levels and avoid calcium overload which can lead to ROS generation, alterations in cell signaling, neuronal excitotoxicity, and cell death (100).

Aβ has been demonstrated to interfere with the mitochondria's fusion and fission processes, which are normal functions that alter the organelles morphology to adjust to different conditions and cellular demands (96). In this sense, it was shown that Aβ activates Drp1 (dynamin related protein 1), a protein essential for fission, through S-nitrosylation to promote excessive mitochondrial fragmentation, thus impairing mitochondrial transport and dynamics and leading to synaptic degeneration in Alzheimer's disease. Aβ has been demonstrated to be toxic to mitochondria. Aβ, either directly or through other stressors, can cause loss of mitochondrial membrane potential. Once Aβ enters the mitochondria, it can interfere with the electron transport chain by disrupting the bioenergetic

profiles, such as decreasing the activity of cytochrome C oxidase by preventing its binding to cyt C. Bioenergetic defects have been shown in Alzheimer's disease patients, especially in cytochrome c oxidase (also known as complex IV of the electron transport chain) implicating mitochondrial dysfunction in the disease pathophysiology (101). Decreased levels of essential mitochondrial enzymes such as α-ketoglutarate dehydrogenase and pyruvate dehydrogenase have also been reported in Alzheimer's disease patients. In vitro studies have shown that Aβ can also prompt the release of cyt c from the mitochondria into the cytoplasm triggering a cascade of caspase activation and eventually leading to apoptosis (102).

Notably, Aβ is not the only culprit in the mitochondria alterations associated with Alzheimer's disease. Tau has also been shown to affect mitochondrial function, as seen in transgenic mice as well as in neuronal cells challenged with tau. Both cases translated into impaired mitochondrial function with defects in the respiratory chain and reduced ATP levels (103). All this mounting evidence indicate that mitochondrial dysfunction is a major contributor to the pathobiology of age-associated neurodegenerative disorders and is tightly linked with loss of synapses and Alzheimer's disease pathogenesis.

6. Targeting Oxidative Stress as a Treatment for Alzheimer's Disease

Given the strong association of oxidative stress with Alzheimer's disease, numerous clinical studies using antioxidant therapies have been carried out. While antioxidant interventions in animal studies have showed promising results, their use in human Alzheimer's disease patients has been largely unsuccessful in modifying the disease (104). The most widely tested antioxidants for treatment of Alzheimer's disease include vitamin E, vitamin C, N-acetyl cysteine, coenzyme Q_{10}, and alpha-lipoic acid (105). Several reasons have been proposed to account for the failure of antioxidant therapies in Alzheimer's disease patients. Firstly, improper dosage or duration of treatment may have decreased the bioavailability of the antioxidant (106). This may be due to variance in basal ROS level among patients where some individuals may need much higher and longer doses. Secondly, ROS stress targets may change at different stages of the disease which would require a combinatorial and stage specific treatment regimen (107). Finally, timing of antioxidant therapy may have been too late to prove effective. Most clinical studies to date involved patients with late stage Alzheimer's disease, a time when other pathophysiological factors such as inflammation and protein aggregation are contributing to neurological damage.

Epidemiological studies have in fact shown that vitamin E can mitigate age associated MCI providing some support for a prophylactic effect of antioxidant supplementation in preclinical stages of Alzheimer's disease (108).

Hemeoxygenase-1 (HO-1) is one of the major antioxidant responses by the cell against oxidative stress induced in Alzheimer's disease. HO-1 regulates the cellular oxidative stress by oxidizing the heme to biliverdin, free ferrous iron and carbon monoxide. The biliverdin is further converted into bilirubin that scavenges the reactive oxygen species and prevents lipid peroxidation. HO-1 is expressed in small populations of scattered neurons and glia (109). The nuclear factor erythroid related factor 2 (Nrf2) is identified as the activator for HO-1 in the brain (110). On an oxidative challenge, the Nrf2 transcription factor moves into the nucleus and binds to the Antioxidant Response Elements (ARE) sequences to activate the antioxidant and anti-inflammatory target genes, specifically HO-1 (111). The upregulation of HO-1 in the brain has been associated with the neurodegeneration and ageing (110). This adaptive protective mechanism of the cell in the neurodegenerative subject is often reported to be a result of Nrf2-dependent activation (109). Also, the Nrf2-dependent upregulation of HO-1 on treatment with flavonoids has been reported to provide protection against the induced neurodegeneration in mice(112).The microglia HO-1 and astrocytic HO-1 levels have been reported to be higher in the Alzheimer's disease patient brain compared to their control subjects (109). Besides the protective effects of HO-1 as antioxidant, there have been reports providing evidence for the detrimental effects of HO-1 (113). The breakdown products of HO-1, the ferrous iron and carbon monoxide aggravate intracellular oxidative stress and promotes free radical generation resulting in mitochondrial and cellular damage (114). The excessive HO-1 upregulation in the glial cells have been reported to promote dysfunctional energy metabolism by affecting iron metabolism and mitochondrial functioning (113). Target inhibition of HO-1 in the glia has displayed therapeutic effects in cell cultures and transgenic mouse model of Alzheimer's disease (110).

7. Conclusion

In conclusion, studies have shown that oxidative stress is associated with some of the main pathological processes in Alzheimer's disease, including Aβ-induced neurotoxicity, tau pathology, and mitochondria dysfunction. Presence of at least one of the factors such as excessive ROS generation, mitochondrial dysfunction, abnormal Aβ accumulation or tau pathologies can result in

oxidative stress and cause abnormal accumulation of abnormal transition metals. Abnormal accumulation of Aβ and tau proteins can induce oxidative stress, leading to neurotoxicity, increase Aβ production and aggregation, facilitate phosphorylation and polymerization of tau protein, and ultimately increase the formation of intracellular ROS, dramatically advancing the progression of Alzheimer's disease. Oxidative stress is an important factor contributing to the development of Alzheimer's disease. Elimination or reduction of intracellular ROS generation, inhibition of neuronal toxicity, regulation of mitochondria function and metal homeostasis, suppression of Aβ production and aggregation, reduction of phosphorylation and polymerization of tau protein can delay the onset or slow the progression of Alzheimer's disease. Therefore, treatment with natural or synthetic antioxidant molecules may be a new approach targeting the prevention of Alzheimer's disease by suppressing the molecular pathways involved in the pathogenesis of Alzheimer's disease.

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CHAPTER 3

OXIDATIVE STRESS AND LIVER

Reşit Uğran[1] & Abdulkadir Levent[2]
[1]*(Dr.) Batman University, e-mail:* resitugran@gmail.com
Orcid: 0000-0003-3431-8351
[2]*(Prof.Dr.) Batman University*, leventkadir@hotmail.com
Orcid: 0000-0001-5792-419X

1. Introduction

The production of free radicals in the human body is an unavoidable ongoing process. Among the main reasons for this are genetic factors such as aging, metabolism and stress, as well as frying, grilling, additives, alcohol, cigarettes and coffee; drugs and the immune system (constantly produced oxy-radicals and reactive oxygen species) can also be counted (1). Free radical and antioxidant imbalance cause oxidative damage to proteins and nucleic acids. The harmful oxidative reaction involving strong oxidizing compounds damages cells and tissues, leading to aging and chronic diseases. A free radical (pro-oxidant) is any species capable of independent existence containing one or more unpaired electrons. Free radicals such as superoxide anion radical, hydroxyl radicals, free lipid radicals, nitrogen dioxide and free nitric oxide radicals together with hydrogen peroxide, oxygen and ozone form the main forms of reactive oxygen species (ROS) in vivo (2). Radicals are necessary as they are involved in signal transduction to sustain life and defend against bacteria, parasites and toxins. At the same time, Leukocytes also use exogenous free radicals to kill microbes. This, in turn, causes DNA mutation and damage to the cell membrane, causing cancer as well as some heart, liver, lung, skin and similar diseases (3,4).

Radical changes in environmental factors, taken toxins and drugs cause liver to be affected the most, as they are metabolized in the liver. Meanwhile, liver cells work to keep the production of oxygen species in balance. Imbalance and oxidative damage following the redox reaction often lead to a range of diseases without jaundice, such as subclinical hepatitis, inflammatory necrotic hepatitis, liver cirrhosis, and cancer (5).

Studies show that oxidative stress may be responsible to varying degrees in the onset and/or progression of various diseases. Oxidative stress, neurodegenerative diseases (Alzheimer's diseases [AD], Parkinson's disease [PD], amyotrophic lateral sclerosis [ALS], heart diseases (coronary heart diseases [CHD], atherosclerosis, stroke/ischemia), obesity, kidney diseases (urolithiasis and diabetic) nephropathy), lung diseases (asthma, pulmonary fibrosis and lung cancer), eye disease (cataract, age-related macular degeneration [AMD], diabetic retinopathy [DR], autoimmune uveitis [AIU], retinitis pigmentosa [RP]), skin diseases, reproductive system diseases, blood diseases (beta thalassemia, acute lymphoblastic leukemia [ALL], joint disorder (rheumatoid arthritis, temporomandibular [TMB] joint disorders, systemic lupus erythematosus [SLE]), liver and pancreas diseases, diabetes, Wilson diseases and brain diseases associated with many diseases (6,7).

2. Oxidative Stress

Oxidative stress has been defined as a disorder in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses that may lead to tissue damage (8). Commonly identified ROS are superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and singlet oxygen ($_1O^2$); They are produced by biological systems as metabolic byproducts (9,10). Processes such as protein phosphorylation, activation of various transcriptional factors, apoptosis, immunity, and differentiation all depend on an appropriate production of ROS and its availability in cells, which must be kept at a low level. (11). ROS is mainly produced by mitochondria during both physiological and pathological conditions, i.e. $O_2^{\bullet-}$ can be generated by lipoxygenases (LOX) and cyclooxygenases (COX) and endothelial and inflammatory cells during arachidonic acid metabolism (12). Although these organelles have an intrinsic ROS scavenging capacity, this is not sufficient to meet the cellular need to scavenge the amount of ROS produced by mitochondria (13). Cells use an antioxidant defense system primarily based on enzymatic components such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) to protect themselves from cellular damage caused by ROS (14,15).

From a general perspective, it has long been recognized that ROS are important in a number of essential cellular functions, including cell proliferation, inflammation, apoptosis, and gene expression. However, ROS cause cellular damage by indeterminately reacting with biomolecules such as free radicals,

lipid membranes, protein and DNA in particular. It also impairs intracellular and intercellular vital functions. Therefore, oxidative stress affects all organs in the body (16).

2.1. Sources of Free Radicals

ROS production is mainly based on enzymatic and non-enzymatic reactions. Enzymatic reactions that can generate ROS are those related to the respiratory chain, prostaglandin synthesis, phagocytosis, and the cytochrome P450 system (8).

$O_2^{\bullet-}$ is produced by NADPH oxidase, xanthine oxidase and peroxidases. Once formed, H_2O_2, OH•, peroxynitrite (ONOO−), hypochlorous acid (HOCl), etc. participates in various reactions that produce. H2O2 (non-radical) is produced by multiple oxidase enzymes, namely amino acid oxidase and xanthine oxidase. OH•, which is the most reactive of all free radical species in vivo, is produced by the reaction of $O_2^{\bullet-}$ with H_2O_2, Fe^{2+} or Fe^{2+} in the presence of a Fenton catalyst. Nitric oxide radical (NO•), which plays some important physiological roles, is synthesized as a result of arginine-citrulline oxidation by nitric oxide synthase (NOS) (17). When oxygen reacts with organic compounds or when cells are exposed to ionizing radiation, non-enzymatic reactions may be responsible for free radical production. Non-enzymatic free radical generation can also occur during mitochondrial respiration (18-20).

Free radicals are produced from both endogenous and exogenous sources. Exogenous free radical production can occur as a result of exposure to environmental pollutants, heavy metals (Cd, Hg, Pb, Fe and As), some drugs (cyclosporine, tacrolimus, gentamicin and bleomycin), chemical solvents, cooking (smoked meat). When these exogenous compounds enter the body, they are broken down or metabolized and free radicals are produced as by-products. Free radicals can be beneficial to the organism when they are kept at low or medium concentrations. Free radicals play an important role in detoxification, apoptosis and phagocytosis. It helps maintain cellular homeostasis in the human body by taking part in various signaling pathways. Free radicals are also thought to be involved in different metabolic and cellular processes such as gene expression, transcription, signal transduction, wound healing, and oxygen sensing. Free radicals may also be involved in the production of prostaglandins and the hydroxylation of various amino acids such as proline and lysine. In summary, free radicals are very important for human health when they are kept at low or moderate levels (21,22).

Endogenous free radicals target vital cellular components such as lipids, proteins and DNA. Lipid peroxidation is a common free radical reaction phenomenon that can interfere with fluidity, and some toxic metabolites are formed as a result of membrane permeability. These toxic metabolites are called 2nd messenger, which can exert their harmful effects away from the production site. Protein, which is an important cellular component that is directly affected by free radicals and initiates the oxidation reaction, therefore changes its protein structure and enzymatic activities. Protein peroxidases, an unstable oxidized product, interact with transition metal ions and accumulate slowly in the body, causing neurodegenerative diseases such as Alzheimer's disease. Again, the free radical attacks the C4-C5 double bond of both pyrimidines and purines, and the poly (ADP-ribose) synthetize enzyme is activated, resulting in the cleavage of NAD+ to aid in DNA fragmentation and repair. Finally, when degradation is widespread, NAD+ levels can drop drastically and cell death can occur (23,24).

Because increased levels of various biomarkers of oxidative damage are found in many chronic diseases and are also associated with risk factors for the development of these diseases, there are several pathological conditions involving excessive oxidative stress. Increased oxidative stress is found in chronic high alcohol intake, obesity, smoking, and chronic exposure to air pollutants. The finding of higher oxidative stress levels during diabetes or stress hyperglycemia, chronic obstructive pulmonary disease, chronic and acute inflammation, cancer and ischemia-reperfusion further supports the pathogenetic role hypothesis of oxidative stress. Similarly, epidemiological findings have shown a link between the increasing prevalence of various disorders and depletion of antioxidant stores, including cancer, heart disease, accelerated aging, and neurodegenerative diseases (25,26).

3. Oxidative Stress and Liver

The liver, the largest organ in the body after the skin and also the largest compound gland, is located in the abdominal cavity. It has various vital functions such as bile production, storage of fat, glycogen and some vitamins (Vitamins A and B), detoxification, synthesis (fibrinogen, protombin, globulin etc.), phagocytosis and blood production in the embryonal period, and disposal of metabolic wastes. The liver is an exocrine gland because it discharges its secretions into the duodenum through the bile ducts, and an endocrine gland by giving the synthesized substances directly to the blood (27).

The liver is one of the important organs affected by ROS. Oxidative stress has recently been recognized as one of the main factors in the pathological

changes observed in various liver diseases. During the metabolism process in hepatic cells, a large number of free radicals are produced which are balanced by the endogenous antioxidant defense system. These free radicals are known to induce oxidative stress to generate ROS, reactive nitrogen species (RNS) and reactive sulfur species (RSS). Free radicals are produced due to the inequality between prooxidants and antioxidants in the body (28). As the rate of free radical formation increases, oxidative stress increases. This leads to liver dysregulation and altered homeostasis, resulting in hepatic trauma. The liver is also an important center for free radicals; during metabolism, excessive free radicals are produced and cause liver damage. Liver induces the oxidation and degradation of many liver enzymes, including diamine oxidase, aldehyde dehydrogenase, tryptophan binary oxidase, dehydrogenase, and the cytochrome P450 enzyme system. Many free radicals found in the liver are products of endogenous or exogenous antioxidants (drugs such as acetaminophen) (30). Alcohol consumption, high-calorie diet, drug overdose, environmental pollutants, heavy metals and the like have been associated with the occurrence of liver damage through ROS production. The hepatocyte mitochondria and endoplasmic reticulum are central to ROS production in various forms of liver diseases (31). It is known that oxidative stress plays an important role in many diseases that occur in the liver. It has been reported that the main toxic mediator that triggers cell death in acute liver injury and hepatic inflammation is reactive oxygen species produced in neutrophils and Kupffer cells (32). There are many cells in the liver such as hepatocytes, hepatic stellate cells, Kupffer cells, and liver sinusoidal endothelial cells. Parenchymal cells are the primary cells in the liver that undergo oxidative stress-induced damage. Mitochondria, microsomes, and peroxisomes in parenchymal cells can generate ROS that regulate PPARα, which is mainly involved in liver fatty acid oxidation gene expression. In addition, Kupffer cells, hepatic stellate cells, and endothelial cells are potentially more exposed to oxidative stress-related molecules, making them more susceptible to oxidative stress. Various cytokines such as TNF-α can be produced in Kupffer cells induced by oxidative stress, which can increase inflammation and apoptosis. Hepatic stellate cells, on the other hand, are triggered by lipid peroxidation caused by oxidative stress and increase cell proliferation and collagen synthesis (33,34).

Living things have developed complex antioxidant systems to counteract reactive species and reduce their damage. These antioxidant systems include enzymes such as catalase, superoxide dismutase and glutathione peroxidase; It contains macromolecules such as albumin, ceruroplasmin and ferritin, and small molecules such as ascorbic acid, α-tocopherol, β-carotene, ubiquinol-10,

reduced glutathione (GSH), methionine, uric acid and bilirubin (35,36). Mammals have an advanced antioxidant system to maintain redox homeostasis in the liver. When ROS is at an excessive level, homeostasis will be disrupted and oxidative stress will occur, which plays a critical role in liver diseases as well as other chronic and degenerative disorders (37). Oxidative stress not only triggers hepatic damage by inducing irreversible changes in lipids, proteins, and DNA contents, and more importantly, by modulating pathways that control normal biological functions. Since these pathways regulate the transcription of genes, protein expression, cell apoptosis and hepatic stellate cell activation; oxidative stress is accepted as one of the pathological mechanisms resulting in the initiation and progression of various liver diseases such as chronic viral hepatitis, alcoholic liver diseases and non-alcoholic steatohepatiti (38,39). It has also been suggested that there are complex relationships between pathological factors, inflammation, free radicals and immune responses (39,40).

Recent studies have shown that ROS and RNS cause loss of liver function, production of harmful substances and a decrease in bile. In the body, toxins and drugs are metabolized in the liver, while liver cells work to keep the production of oxygen species in balance. After the redox reaction, imbalance and oxidative stress, especially alcoholic liver disease (ALD), non-alcoholic steatohepatitis (NASH) and hepatitis C, play an important role in the pathophysiological changes that progress to liver cirrhosis, hepatocellular carcinoma (HCC), and lead to many diseases such as cancer (5, 40,41).

3.1. Alcoholic Liver Disease (ALD)

Alcoholic liver disease (ALD) is a common disease especially seen in societies with high alcohol consumption and may cause cirrhosis if necessary interventions are not made in this process. This disease is caused by excessive consumption or long-term use of alcohol. Alcohol is known to cause oxidative stress in living cells of the body. In particular, liver metabolism initiates a series of pathological processes including toxic protein aldehyde compounds, endotoxins, immunological activity and proinflammatory cytokine release. In this disease, varying degrees of fibrosis and histopathologically steatosis and steatohepatitis are seen (44,45). The liver is the organ primarily responsible for the metabolic breakdown of alcohol. As alcohol intake increases, ethanol is eliminated pathophysiologically by cytochrome P450 and acetaldehyde produces toxic effects in the liver. A number of chemical processes take place in the breakdown of alcohol. These processes are primarily oxidation to acetaldehyde by the alcohol dehydrogenase enzyme. Then, acetaldehyde is

converted to acetic acid, and acetaldehyde dehydrogenase (ALDH) takes part in this conversion. All these transformations cause oxidation and free oxygen radicals are accumulated in subsequent processes. Increasing free oxygen radicals cause liver disorders that are very difficult to recover after a while (44-47). ROS and RNS production is stimulated in hepatic parenchymal cells through cytokine-induced oxidative stress signals and stimulation of Kupffer cells and inflammatory cells. Shifts in the balance of cytokines in hepatocytes, including tumor necrotic factor (TNF)-α, interleukin (IL)-1β, and IL-6, also contribute to liver injury in alcoholic hepatitis. Chronic alcohol consumption or fatty liver disease associated with obesity/type 2 diabetes are associated with mitochondrial defects (48). Changes in the mitochondrial genome and proteome result in loss of mitochondrial respiration, inability to maintain adequate ATP concentrations, and the formation of more ROS and RNS, which ultimately results in oxidative stress (49).

3.2. Nonalcoholic Steatohepatitis (NASH)

Nonalcoholic steatohepatitis (NASH) is strongly associated with overweight or obesity and the metabolic syndrome; It is defined by chronic liver damage and inflammation (hepatitis) caused by excessive lipid accumulation (steatosis) in the liver. By definition, it is not etiologically related to excessive alcohol consumption. NASH is defined histologically and diagnosed by liver biopsy findings (steatosis, hepatocyte damage, and liver inflammation). Cytokines such as tumor necrosis factor α (TNF-α), transforming growth factor β, interleukin 8 (IL-8) and Fas ligand, but not ROS/RNS (hydrogen peroxide, superoxide anion radical, peroxynitrite and hydroxyl radical) and lipid peroxidation by-products responsible for their rise. The sum of these events results in the development of NAFLD (50). The oxidative hepatic environment in obesity also supports signaling and activation of transcription programs (STAT-1 and STAT-3) that promote T cell recruitment and liver injury with disease progression up to malignant transformation (51). Simple hepatic steatosis also occurs with NASH with fibrosis and nonalcoholic fatty liver disease (NAFLD), a clinically defined disease that broadly encompasses NASH-associated cirrhosis (52.53). The severity of liver injury is generally related to the degree of hepatic metabolic stress, but there is substantial interindividual variability in NASH outcomes. While liver damage remains relatively stable in most individuals, it regresses in some and progresses in others (54). The intensity of hepatic fibrosis usually correlates with the degree of liver damage and inflammation (severity of NASH), but again, not all patients with NASH develop advanced liver fibrosis (55).

3.3. Cirrhosis

Cirrhosis is defined as the histological development of regenerative nodules surrounded by fibrous bands in response to chronic liver injury leading to portal hypertension and end-stage liver disease. The most common causes include alcoholic liver disease and hepatitis C, as well as factors such as alcohol consumption, age, obesity, insulin resistance or type 2 diabetes, hypertension, and hyperlipidemia. Fibrosis describes the encapsulation or replacement of injured tissue with a collagenous scar. Liver fibrosis results from the continuation of the normal wound healing response, which causes abnormal continuation of fibrogenesis (connective tissue production and deposition). Fibrosis progresses at variable rates depending on the cause of liver disease, environmental factors, and host factors (56-59). Cirrhosis is an advanced stage of liver fibrosis accompanied by deterioration of the hepatic vessels. The resulting vascular distortion leads to continuity of portal and arterial blood supply directly to the hepatic outflow (central veins) and compromises the exchange between hepatic sinusoids and adjacent liver parenchyma (hepatocytes). The hepatic sinusoids are lined with windowed endothelium, which rests on a permeable connective tissue layer in the cavity of Disse, which also contains hepatic stellate cells and some mononuclear cells. The other side of the Disse cavity is lined with hepatocytes. In liver cirrhosis, the space of Disse is filled with scar tissue and endothelial fenestrations are lost, a process known as sinusoidal capillarization (60). Histologically, cirrhosis is characterized by vascularized fibrotic septa that connect the portal tracts to each other and to the central vessels, resulting in the formation of islands of hepatocytes surrounded by fibrotic septa and lacking a central vessel. The main clinical consequences of cirrhosis are impaired hepatocyte (liver) function, increased intrahepatic resistance (portal hypertension), and the development of hepatocellular carcinoma. General circulatory abnormalities in cirrhosis (splanchnic vasodilation, vasoconstriction and hypoperfusion of the kidneys, water and salt retention, increased cardiac output) are closely associated with hepatic vascular changes and the resulting portal hypertension. Cirrhosis and its associated vascular distortion are traditionally considered irreversible, but recent studies suggest that regression or even reversal of cirrhosis may be possible (61.62).

3.4. Hepatocellular Carcinoma (HCC)

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver and is responsible for 1 million deaths per year worldwide. It is

the most common form of internal malignancy in some parts of the world and the most common cause of death from cancer. It is known that causes of HCC include chronic liver disease and cirrhosis, viral hepatitis (hepatitis c and hepatitis b) and excessive alcohol intake, aflatoxin exposure, smoking, obesity and diabetes (63, 64). There are also rare diseases that increase the risk of HCC, such as alpha1-antitrypsin deficiency, Wilson's disease, and tyrosinemia (65). The liver constantly adapts to changes in environmental conditions related to dietary xenobiotic, viral infections, and changes in the microbiota. The etiology of HCC, which is closely related to environmental factors, also indicates that epigenetic abnormalities may contribute to the initiation and progression of HCC. These environmental stresses lead to changes in DNA methylation, acetylation, chromatin modifications, long non-coding RNAs (lncRNAs) and miRNA, resulting in changes in the hepatic epigenome. The accumulation of these epigenetic modifications and alterations results in dysregulated expression of tumor suppressor genes and oncogenes, which ultimately manifests in the progression and metastasis of carcinogenesis of HCC. Thus, the treatment of the disease becomes difficult (66, 67).

3.5. Some Other Liver Diseases

Hepatocytes, liver cells, play a central role in alcohol or drug metabolism and increase the production of ROS in metabolic processes such as alcohol use or increased drug intake. In obesity, large amounts of free fatty acids (FFA) from visceral adipose tissue, as well as glucose and fat from the meal, come directly to the liver.

The excess amount of free fatty acids in hepatocytes exceeds the β-oxidation capacity of mitochondria and other organelles. It causes the hepatocyte to exceed the excretion capacity of very low-density lipoproteins (VLDL), which can lead to the development of diseases such as fatty liver or NASH. In these processes, mitochondria, peroxisomes, and endoplasmic reticulum metabolize high amounts of fatty acids in hepatocytes. It results in ROS production and oxidative stress. Extremely high levels of iron are stored in the hepatocytes of patients with NASH, alcoholic hepatitis, or hepatitis type C. Such excessive iron accumulation also causes oxidative stress in hepatocytes. The reason why hepatocytes have the highest antioxidant function compared to cells of other organs is that oxidative stress is readily induced in the hepatocytes, possibly as a result of lifestyle-related factors such as alcohol use or obesity (68). In addition, increased oxidative stress in hepatocytes is one of the important mechanisms of liver dysfunction (69). Oxidative stress is also thought to play

a role in the formation of hepatic encephalopathy found in patients with liver failure (2). Prolonged oxidative stress to hepatic cells can irreversibly destroy lipids, proteins and DNA involved in numerous pathways to control protein expression, gene transcription, hepatic stellate cell (HSC) activation and cell apoptosis (70).

There are studies reporting that oxidative stress causes many histological and histopathological changes in the liver tissue. Ibtissem et al. (71) reported that oxidative stress caused by methylthiophanate causes necrosis, infiltration of inflammatory leukocyte cells and hepatocyte vacuolation in the rat liver. It has been reported that oxidative stress induced by N-diethylnitrosamine causes various histological changes in the mouse liver, including areas of necrosis, apoptotic cells, and the presence of mitosis (72). Arsenic has been reported to cause DNA damage and apoptosis in rat hepatocytes (73). Sadik et al. (74) found the appearance of hepatocellular carcinoma with enlarged hyperchromatic nuclei and diffuse mitosis in diethylnitrosamine rat livers. In addition to nuclear pycnosis in mice, methotrexate has been reported to accumulate inflammatory and leukocytes in hepatocytes (75). Histopathological findings such as fatty degeneration, hydropic degeneration, fibrosis, mononuclear cell infiltration and regenerative nodules were detected in rat livers of cadmium due to toxicity (76).It has been determined that cyclophosphamide (CP) causes hydropic degeneration in the cytoplasm of hepatocytes in the livers of rats, deterioration in cytoplasm homogeneity, as well as dark staining and shrinkage as a result of chromatin condensation in the nuclei of some hepatocytes, irregularity in the nuclear borders and an increase in eosinophilia in the cytoplasm increased congestion and accumulation in erythrocytes have been reported in the vessels (77). It has been mentioned that cyclophosphamide causes sinusoidal dilatation, mononuclear cell infiltration, and vascular congestion in the liver (78). Koubaa et al. (79), reported that vanadium caused leukocyte infiltration, inflammatory disorders and liver lesions localized around the vena centralis in rats, as well as a vascular occlusion indicating the onset of a necrosis step. Yousef et al. (80), rat liver sections of paracetamol, large areas of hepatocellular necrosis, prominent cytoplasmic vacuolation in most of the centrilobular hepatocytes and swelling in the pycnotic nuclei, and hepatic sinusoids were almost invisible. Diabetes mellitus, commonly known as diabetes, is one of the most important health problems with a very high prevalence, morbidity and mortality. A group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both (81).

Increasing evidence, both in experimental and clinical studies, shows that oxidative stress plays a role in the pathogenesis of diabetes mellitus, and the resulting free radicals lead to a decrease in the antioxidant defense mechanism, which in turn leads to the risk and development of diabetes. It also shows that oxidative stress plays a very important role in the etiology of diabetic complications (36, 82). Alloxan, which is a diabetogenic agent, and STZ (streptozotocin) are generally used to create an experimental model of diabetes (83). The cytotoxic effect of STZ (streptozotocin) is mediated by free radicals and affects various organs of the body such as the pancreas, liver and kidneys (84, 85). It has been reported that STZ administration causes liver tissue damage along the cell cords around the vena centralis (86). It has been reported that in the liver samples of the diabetic group, there is dilatation in the sinusoids and vacuolization in the hepatocytes, especially around the vena centralis (87).

In experimental diabetes, deterioration of the radial arrangement of the cells around the vena centralis, widening and thickening of the vessel walls, diffuse degeneration in the cells, lipid accumulation and vacuolization, hydropic changes, degeneration and reduction in the organelles, development of fibrosis and dilatation in the sinusoids were observed in the liver tissue in general (88, 89). It was also determined that there were fibrosis and inflammatory cell infiltration in the portal areas (90). Deprem et al. (91), diabetes mellitus causes a decrease in GPx1 activity, known as one of the antioxidant enzymes, and the outer surfaces of some cell nuclei become irregular; it has been reported that there are lesions such as swelling and enlargement (megalocytosis) in the nuclei as well as cytoplasmic invaginations with eosinophilic character.

Dilatation of the sinusoids, hepatocellular macrovesicular vacuolization and spotty necrosis have been observed due to diabetes (92). Taslidere et al. (93) observed histological changes such as inflammatory cell infiltration around the portal triad in rat livers of STZ, and also reported that glycogen storage was less in hepatocytes compared to other groups. In diabetic animals, hepatocytes with intensely stained nuclei and vacuoles were detected in the liver, and local fibrous areas were observed between these cells; in severely affected hepatocytes, the nuclei degenerate and appear very small, while in the nuclei, the chromatin appears clumped and dark; It was observed that there was a significant increase in the amount of connective tissue and collagen around the vessels in the periportal areas (94.95). Abdultawab and Ayuob (96) reported that alloxan in rat liver, as well as small, dark hepatocyte nuclei lacking nuclear membrane, membranous cell organelles such as rER and Golgi vesicles were fragmented and many mitochondrial membranes and crystals were disrupted.

4. Conclusion

Oxidative stress and free radicals are known to be harmful to human health. Numerous studies show that free radicals actually contribute to oxidative stress, causing the initiation and progression of various pathologies. Since oxidative stress has been determined to play an important role in the pathogenesis of many clinical conditions and aging, several studies have been conducted to investigate the therapeutic effects of antioxidant therapy. In light of the vital role of oxidative stress in liver diseases, antioxidants are understandably considered a good therapeutic strategy for the treatment of liver disorders. To date, study results are inconclusive and controversial; however, the therapeutic efficacy of certain antioxidants has been proven. Several studies have shown that curcuminoids protect DNA against ROS and support hepatocytes during injury and cirrhosis (97). Ekin et al. (98) reported that Hypericum perforatum L. reduced the oxidative stress level caused by 7,12 Dimethylbenz[a]Anthracene in rats. Research in the field of chronic HCV has revealed improvement after antiviral therapy supplemented with silymarin (milk thistle extract), which preserves GSH in hepatocytes. Ascorbic acid, lipoic acid, quercetin (a flavonoid antioxidant), and mitoquinone (a mitochondria-targeted antioxidant) have also shown beneficial effects in patients with chronic HCV infection. The antioxidant properties of resveratrol reduce hepatic lipid peroxidation, increase the amount of GSH in the liver and clear ROS. Their function is manifested during liver damage caused by hepatotoxins, for example, ethanol. In addition, ebselen (an analogue of glutathione peroxidase) emerges as a therapeutic agent in early alcohol-induced liver injury. Studies have shown that vitamin E suppresses HBV replication and inhibits TGF beta gene expression in rat NASH models. It has been reported that vitamin E can also prevent the progression of NAFLD (99, 100). The best clinical evidence of successful antioxidant therapy in liver diseases is the use of vitamin E for NASH. However, despite numerous studies in human and animal models, it is extremely difficult to understand and describe the efficacy of antioxidative agents in hepatology (38,101). Oxidative stress biomarkers can provide important information about the efficacy of a treatment and thus provide guidance for the selection of the most effective drugs/dose regimens for patients. In addition, if a biomarker of oxidative stress is particularly relevant from a pathophysiological perspective, it may also be useful in research as a therapeutic target to identify new treatments with antioxidant properties (102). Antioxidants protect the body from the harmful effects of free radicals. The defense of endogenous antioxidants against reactive oxygen species is strengthened by natural antioxidants that strengthen them and restore optimal

balance by neutralizing ROS. Traditional medicine has used a number of natural and herbal products to treat various diseases, including malignant tumors. Many herbal plants have proven their ability to resist cancerous activities. Some previous studies have shown that herbal treatments can have antitumor effects by enhancing the immune system including cell differentiation, inducing apoptosis of cancer cells and inhibiting telomerase activities. The protective effects of natural antioxidants have received more attention against toxicities caused by free radicals (103).

Flavonoids play an important role in protecting against oxidative stress, especially in the case of cancer (104, 105). Flavonoids are commonly found in vegetables, red wine, fruit, cocoa, and tea (106, 107). Flavonoids are common in beverages and foods that have a wide variety of biological activities, the antioxidation of which has been extensively studied (108,109). The antioxidant activities of phenolics are related to a number of factors; They involve different mechanisms such as free radical scavenging, hydrogendonation, singlet oxygen quenching, metal ion chelation, and acting as a substrate for radicals such as superoxide and hydroxyl (110, 111). Green tea (Camellia sinensis L.) and parsley (Petroselinum crispum) produced an increase in the level of GSH, an antioxidant (112). It has been noted that application of Curcuma longa, Trigonella foenumgraecum, Allium sativum, Coffea Arabica, Petroselinum crispum, Olea europaea leaves and Mentha piperita showed remarkable liver protection against hepatotoxic agents. Natural antioxidants have various biochemical effects such as inhibition of ROS production and scavenging of free radicals (110).

In a study, it was suggested that the use of vitamin C together with the anthrax vaccine may be beneficial in terms of reducing oxidative stress (113). In mice, diabetes has been reported to cause a decrease in catalase enzyme level (114). 7,12-Dimethylbenz[a]Anthracene (DMBA) levels were formed; The liver has been reported to contain significantly higher levels of DMBA compared to the kidney (115). It has been reported that melatonin increases the glutathione peroxidase (GPx 1) enzyme, which has a protective effect in case of oxidative stress (116). Ethyl alcohol and carbon tetrachloride caused a decrease in Glutathione-S-Taransferase enzyme levels (117). It has been reported that the consumption of fenugreek, curcumin, mint, parsley, rosemary, garlic, pomegranate, sesame and propolis has protective effects against kidney diseases and renal dysfunction of nephrotoxic agents in experimental animals and humans. The nephroprotective effect may be due to inhibition of tissue lipid peroxidation and increased antioxidant activity. Therefore, the study suggested that these antioxidants may be beneficial for people exposed to nephrotoxic

agents and for patients with kidney disease. These protective effects may be due to the presence of benzoquinones, flavonoids, flavonol glycosides, alkaloids, carotenoids, catechols, glycosides, steroid glycosides, terpenoids, glycoalkaloids, mono, di and triterpenes, saponin (118, 119).

In this review, an extensive current literature review has been brought together and the effects of the liver in terms of oxidative stress antioxidant interaction and the ways in which it is affected have been tried to be revealed.

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CHAPTER 4

THE CONCEPT OF OXIDATIVE STRESS BY THE YEAST *SACCHAROMYCES CEREVISIAE* PERSPECTIVE

Irem ULUIŞIK YILMAZ
(Assistant Prof Dr), İskenderun Technical University,
e-mail: irem.uluisik@iste.edu.tr
Orcid: 0000-0001-8880-4078

1. Introduction

Photosynthetic activities of *Cyanobacteria* resulted in releasing molecular oxygen to the atmosphere approximately 3,5 billion years ago. While molecular oxygen provided great progress in the evolution of life on earth, it can also be highly reactive when partially reduced (1,2). Reactive oxygen species (ROS) including superoxide anions (O_2^-), hydroxyl radical (HO^-) and hydrogen peroxide (H_2O_2) are generated as intermediates of cellular metabolism. When the production of reactive oxygen species increases, cellular defense mechanisms are induced to protect cellular components. However in the cases in which the balance between existing ROS and antioxidant defences is disturbed, oxidative stress occurs. Thus all organisms living in an oxygenated environment suffer from oxidative stress (3,4,5). Reactive nitrogen species (RNS) including nitric oxide ($^{\bullet}NO$), nitric peroxide (peroxynitrite, $OONO^{\bullet-}$) and their derivatives have also regulatory roles (6,7).

Oxidative stress was defined in 1985 for the first time by Helmut Sies (8,9). Since then many researches were conducted and the concept was accepted and used widely in many fields of life sciences.

The origin of nearly 90% of ROS in organisms is mitochondrial respiratory chain, other endogenous metabolic sources are electron transport chains in membranes and phosynthesis reactions. ROS can also be generated by exposure to ionizing radiation, heavy metals or some redox enxymes (10,11,12,13,14).

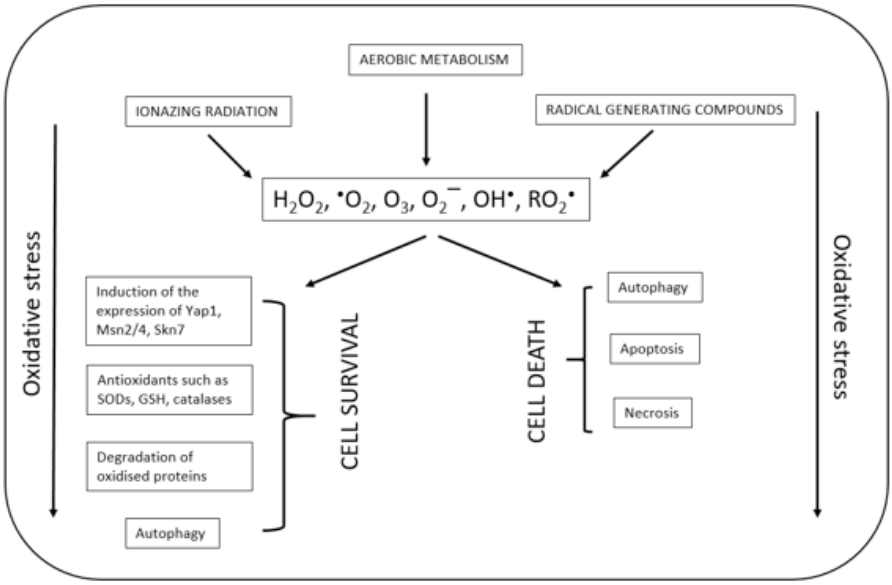

Figure 1: Oxidative stress responses in *Saccharomyces cerevisiae*. Almost all organisms encounter ROS- driven oxidative stress following aerobic metabolism, ionizing radiation and exposure to radical generators. Signals arised from the accumulation of ROS can activate the upregulation of some transcription factors which then regulate the expression of antioxidant genes. All these mechanisms help cell to cope with the damage caused by ROS. Oxidative stress can result in lipid peroxidation, protein oxidation and damage to genetic material. Oxidized proteins can be removed by the ubiquitin dependent proteosome system. In addition cells can remove oxidized macromolecules or organelles that do not function properly by autophagic pathways (15,16).

The budding yeast *Saccharomyces cerevisiae* can also grow both aerobically and fermentatively. Adaptive stress response mechanisms and molecules are common in both *S.cerevisiae* and higher eukaryotes including mammals. Additionally, yeast have proteins exhibiting homology to human proteins. This conservation between yeast and mammals induces yeast to be extensively used as a model in understanding the responses to oxidative stress so far.

Cells develop defence systems to protect their intracellular redox state. Glutathione (GSH) which is a tripeptide □-L-glutamyl-L-cystinylglycine, acts as a radical scavenger in the cell. This important antioxidant reacts with oxidants and produces reduced glutathione (GSSG) (17,12). Analogous to GSH, phytochelatins plays roles mainly in fungi and plants (18). Ascorbic acid is a well-known antioxidant in eukaryotes (12).

Membrane lipids are also important for the oxidative stress resistance. When yeast cells having higher levels of saturated fatty acid in their membranes compared with the cells having membranes with unsaturated fatty acids it was shown that cells having membranes with high amounts of saturated fatty acids were more resistant to oxidative stress (12,19,20). There is an undeniable link between metal ions and oxidative stress. Some metal ions such as copper and iron are known as redox active metals and they produce hydroxyl radicals. Metallothionein proteins exhibit antioxidant properties by binding different metal ions (4,12). Thioredoxin and glutaredoxin belong to a class of small proteins which have reductive activities for ribonucleotide reductase in yeast (21).

Cellular defences against oxidative stress also involve enzyme systems in addition to abovementioned non-enzymatic defences. Catalase is one of the enzymes that catalyses the breakdown of H_2O_2 to O_2 and H_2O. In *S.cerevisiae* there are two types of catalases. One of them is located in peroxisome while the other remains in the cytoplasm (22,23). Superoxide dismutases (SOD) catalyses the ransformation of superoxide anion to H_2O_2 and O_2. *S.cerevisiae* superoxide dismutases are in the cytoplasm and mitochondria as in other eukaryotes protecting mitochondria from the toxic effects of superoxides generated during fermentative growth. Pentose phosphate pathway enzymes are crucial for the production of NADPH which then will be used as reductant by glutathione reductase and thioredoxin reductase to generate antioxidants (24,25,26).

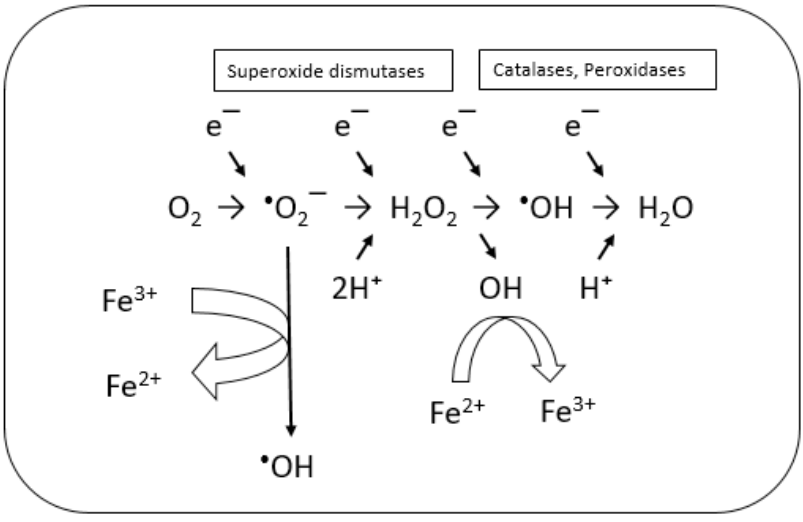

Figure 2: Unpaired electrons of oxygen can be transferred in order to yield superoxide radical ($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$)

and water (H_2O). Superoxide radical can be generated as a by-product of the activity of mitochondrial electron transport chains. The activity of superoxide dismutases results in the formation of hydrogen peroxide. Hydrogen peroxide can then be reduced by (Fe^{2+}) Fenton reaction and produce hydroxyl radical. Hydrogen peroxide can also be further reduced to hydroxyl radical by Haber-Weiss reaction in which Fe^{2+} occurs after donation of an alectron to Fe^{3+}. Antioxidant enzymes such as catalases and peroxidases catalyse the detoxification of hydrogen peroxide (16).

2. Oxidative damages

Oxidative stress occurs when the amounts of reactive oxygen species exceed its limits, the antioxidant system of cells do not cope with them. This unbalanced condition turns out cellular damages and in more severe situations may result in cell death.

Protein oxidation is one of the common oxidative damages. All of the reactive oxygen species can oxidize proteins directly. For example superoxide radicals oxidize 4Fe-4S clusters in enzymes resulting the inactivation of the enzyme. Mitochondiral enzymes are subjected such an inactivation so that mitochondrial respiration is impaired and ageing is accelerated. During the modification of proteins by reactive oxygen species, iron is released and accumulates in the vacuole causing oxidative damage to vacuoles this time (4,27,28). Hydrogen peroxide can oxidize thiol groups of cysteine residues at the active sites of some enzymes. It can also oxidize methionine residues to methionine sulfoxide or sulfone (29,30). Protein carbonylation that is the oxidation of aminoacids to carbonyl derivatives causes toxicity. These oxidized residues can then be reduced and activated by the help of enzymes belong to the antioxidant system in the cell or they can be inactivated irreversibly. The components of proteolytic degradation system in *Saccharomyces cerevisiae* were shown to be induced by hydrogen peroxide (30,31). As one of the results, unrepaired proteins are targeted to proteolytic degradation. In addition, hydrogen peroxide is reduced to the hydroxyl radicals by Fenton reaction where hydrogen peroxide reacts with Fe^{+2} or Cu and produces hydroxide (OH^-) and hydroxyl radical ($\cdot OH$) (32). By damaging proteins irreversibly, hydroxyl radicals are responsible most of the oxidative damages.

The oxidative damage to nucleic acids is another aspect of the damage. It leads to base modifications, DNA strand breaks and sugar damages. These damages can be lethal or mutagenic causing ageing or even cancer if they cannot be removed by the repair systems in the nucleus as secondary antioxidant

defences. Mitochondrial DNA can also be subjected to oxidation and its repair is essential for the cell survival (33,34).

Lipids also face oxidative damages. Polyunsaturated fatty acids are oxidixed and fatty acid hydroxyperoxides are produced. These lipid peroxides are fragmented and can generate epoxides, aldehydes and alkanes. Yeast however cannot synthetise polyunsaturated fatty acids by itself. It uses fatty acids which are added to the growth media exogenously. Coenzyme Q and vitamin E are known oto protect cells lipid peroxidation by reducing lipid peroxyl radicals (35). Lipid peroxidation products can also damage proteins oxidatively (27).

3. Molecular mechanisms underlying oxidative stress response

Aerobic conditions, increase in mitochondrial respiratory chain, exposure to drugs that generate reactive oxygen species induces an oxidative stress response in yeast for survival. Early and late responses include different trancription factors. Basically the transcription factors Msn2p, Msn4p mediate general oxidative stress response. Msn2/4 which resides normally in the cytoplasm is transported to the nucleus in response to environmental stress conditions including oxidative stress and induces the expression of stress response element (STRE) regulated genes (36,37,38,39).

Additionally Yap1, Skn7 and Hsf1 transcription factors regulate some specific responses. Yap1 protein is a member of AP-1 type transcription factors. The AP1 family is conserved among eukaryotes and play roles in the regulation of response to different stresses (40,41,42,43). Yeast has eight Yap proteins acting against oxidative stress response (44,45,46). Some of the important genes that are activated in response to oxidative stress by Yap1 are *TRX2* (thioredoxin), *GSH1* (γ-glutamylcysteine synthase) (47), *GSH2* (glutathione synthase) (48), *TRR1* (thioredoxin reductase 1) (49), *GPX2* (glutathione peroxidase 2) (50), *TSA1* (thioredoxin peroxidases 1) (51,52), *AHP1* (alkylhydroperoxide reductase 1) (30,53). Yap1 also regulates the genes that are involved in membrane transport such as *YCF1* (54), *ATR1* and *FLR1* (55,56). Yap1 becomes oxidixed when H_2O_2 is sensed by a glutathione dependent peroxidase Gpx3 so that it cannot bind to Crm1 protein which provides Yap1 to localize in cytoplasm. This oxidation causes Yap1 accumulating in the nucleus and regulating the expression of its target genes. Yap1 may then be reduced by thioredoxin and thus the cycle is completed (57). Yap1p levels is also affected by both cellular NADPH and carbohydrate metabolism. Yap1 homologs have been identified in fusion yeast *Schizosaccharomyces pombe* Pap1 and *Candida albicans* Cap1 (58,59). Yap1 is the key regulator of oxidative stress in yeast. Regulatory mechanisms include

the reversible phosphorylation events especially in Skn7, Msn2/4 and MAP-kinase pathway in response to oxidative stress. This is an example mechanism by which hydrogen peroxide is sensed and a defense mechanism is built up. Cells can activate various defensive signaling pathways against different stressors. However the molecular mechanisms of their regulation need to be clarified.

Table 1: Some of the antioxidant enzymes that are overexpressed by ROS in yeast *Saccharomyces cerevisiae* (16).

Enzymes	**Gene**	**Location**
Thioredoxin	*TRX1, TRX2* *TRX3*	Cytoplasm Mitochondria
Thioredoxin reductase	*TRR1* *TRR2*	Cytoplasm Mitochondria
Peroxiredoxin	*TSA1, TSA2, AHP1* *DOT5* *PRX1*	Cytoplasm Nucleus Mitochondria
GSH synthase	*GSH1, GSH2*	Cytoplasm
Glutathione reductase	*GLR1*	Cytoplasm, Mitochondria
Glutathione trasnferase	*GTT1* *GTT2* *GTO1* *GTO2, GTO3*	Endoplasmic reticulum Mitochondria Peroxisome Cytoplasm
Glutathione peroxidase	*GPX1, GPX2, GPX3*	Cytoplasm
Glutaredoxin	*GRX1* *GRX2* *GRX3, GRX4* *GRX5* *GRX6, GRX7* *GRX8*	Cytoplasm Cytoplasm, Mitochondria Nucleus Mitochondria Golgi apparatus Cytoplasm
Superoxide dismutase	*SOD1* *SOD2*	Cytoplasm, Nucleus Mitochondria
Catalase	*CTT1* *CTA1*	Cytoplasm Peroxisome
Methionine sulphoxide reductase	*MXR1 (MSRA)* *MXR2 (MSRB)* *fRMsr (YKL069W)*	Cytoplasm Mitochondria Cytoplasm
Erythroascorbate	*ALO1*	Mitochondria

The damages on DNA caused by oxidative stress induce antioxidant defences as well. With the activity of both base excision repair and nucleotide excision repair, bases that are oxidised are replaced which is mediated by Rad1p (60,61). Additionally, mitochondrial DNA suffer from oxidative damage (62). Mitochondria contain Ntg1p that is a glycosylase involved in excision repair mechanism. Yeast mitochondria has an additional repair enzyme Mgm101p (63). Since mitochondrial DNA does not have histone proteins that protect the integrity of the mitochondrial genome, there are different molecules that are important for the mtDNA integrity such as glutathione (GSH), Atm1p (64).

Another stress response mechanism involves the controlling of RNA metabolism. This idea has been confirmed by the discovery of tRNA and rRNA fragments in yeast during oxidative stress. Additionally, it is revealed that tRNA cleavage the exact function of which is not clear yet, is conserved among yeast, plants and mammals in response to oxidative stress (65).

4. Oxidative stress related diseases

Cells develop strategies to avoid damaging effects of oxidative stress. Four strategies that stand out among them are as follows: to prevent the stress condition, to pause the cell cycle, to make alteration in regulatory mechanisms, and to repair and eliminate the damage (7,65,66).

High levels of free radicals in cell cause oxidative damage to DNA, proteins and lipids. Unless the damage is repaired, it can lead to various diseases such as neurodegenerative disorders, diabetes, atherosclerosis, cancer, ageing, and cell death in more severe conditions (67).

The link between oxidative stress and age-related diseases causes new treatment strategies to arise including the use of dietary antioxidants to protect from oxidative damage (67).

Denham Harman was the first who described the free radical theory of ageing in 1950s. It proposes that accumulation of oxidative damage results in aging of the organism (68). The mitochondria is important in this sense since it produces most of the energy needed for the cellular metabolism. According to rate-of-living hypothesis; the more metabolic rate an organism has, the higher the production of reactive oxygen species and the shorter its life cycle. It is thought that the free-radical theory of aging and rate-of-living hypothesis act together.

There are a number of studies demonstrating that the accumulative roles of ROS and oxidative stress on ageing. Using different model organisms from yeast to mice, it has been claimed that decreased ROS levels extends the

lifespan. However in recent years there are other reports which are contradictory to this theory. Researchers are now starting to argue that ROS accumulation and oxidative damage is one of the causes in the aging process but not the only cause, there are other causes such as heterogeneity, imperfectness of biological processes (69,70,71).

Recently, reactive oxygen species has been proposed as signalling molecules in biological processes. Accumulation of ROS is inevitable and it is thought to have negative effects on cells. However in some conditions, the generation of ROS within certain limits is necessary for the homeostasis. It does not matter if it is of mitochondrial origin or not. An increase in ROS resulted in the activation of many stress signalling pathways which may damage or protect the cell (72,73).

Apoptosis is the main strategy for multicellular organisms to control diseases and to develop normally. It is a highly regulated event taht is regulated by anti-apoptotic and pro-apoptotic proteins (74,75). Experimental studies suggested that oxidative stress plays roles in yeast apoptotic pathway and reactive oxygen species seem to regulate the apoptosis in yeast (76). ROS are produced mainly in mitochondria in mammals as well as yeast. The accumulation of ROS may have roles in propagation of apoptotic signals.

Recently reactive oxygen species are associated with some diseases such as ALS, FRDA by using yeast as a model system. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease resulted in the degeneration of motor neurons. Since it is reported that CuZnSOD mutations are related to the disease's familial version (77), yeast has been used for revealing the effects of this mutation. Evidences point out a SOD1 mediated mechanism in disease formation (78).

The breakdown of iron homeostasis in the cell has shown to be connected some human diseases including Friedreich's ataxia (FRDA). It is a neurodegenerative disease that affect the nerveous system. Iron is an essential element for cells. However by Fenton reaction it can also cause the production of reactive oxygen species at high levels. For this reason iron transport in- and out of the cell needs to be regulated carefully. Mammals have iron sensor proteins and yeast has Aft1p, Aft2p transcription factors for managing iron homeostasis and oxidative stress (79). Frataxin (a mitochondrial protein) deficiency is the major cause of the disease. Yfh1p which is the frataxin homologue in yeast is an iron binding protein and provides oxidative stress resistance (80).

Additonally, in some cases increase in ROS levels does not reversible. It only reaches at some level and is stabilized which is known as "quasi-stationary

level". This condition is encountered in some diseases such as diabetes, atherosclerosis, cardiovascular and neurodegenerative diseases (81). Recently there are studies going on developing fluorescent nanodiamond biosensors that can sense oxidative stress in yeast. Nanodiamonds are carbon-nanomaterials and it is found in yeast that nanodiamonds act as ROS scavengers and protect cells from damage (82,83,84,85).

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CHAPTER 5

POTENTIAL THERAPEUTIC EFFECTS OF RESVERATROL

Aslı YAYLALI[1] & Nadire ESER[2] & Atila YOLDAŞ[3]

[1](Assoc. Prof. Dr.), Kahramanmaraş Sütçü Imam University,
Faculty of Medicine, Department of Histology&Embryology,
e-mail: aslierylmz@gmail.com
Orcid: 0000-0003-2360-5320
[2](Asst. Prof. Dr.), Kahramanmaraş Sütçü Imam University,
Faculty of Medicine, Department of Pharmacology,
e-mail: esernadire01@hotmail.com
Orcid: 0000-0003-1607-5114
[3](Prof. Dr.), Kahramanmaraş Sütçü Imam University,
Faculty of Medicine, Department of Anatomy,
e-mail: atillayoldas99@hotmail.com
Orcid: 0000-0002-7807-0661

1. Chemical Structure of Resveratrol

Resveratrol (RSV; 3,5,4′-trihydroxy-trans-stilbene) is found in a variety of plants, including grapes, peanuts, tea, blueberries, and mulberries. It is a compound belonging to the polyphenolic phytoalexin class, which is a class of antibiotic compounds that these plants naturally produce in order to establish the resistance mechanism when they encounter some external effects such as cold weather conditions, ultraviolet radiation, injury, bacterial and fungal infections at any stage of their growth and development (1). RSV has a molar weight of 228.25 g/mol and is a chemically flavonoid, polyphenolic, non-steroidal compound (2,3). Resveratrol has also cis and trans isomers (Fig. 1) (3). They are separated according to their UV-spectral properties. Grape extracts don't have cis form. Trans-resveratrol remains stable as long as it is protected from light.

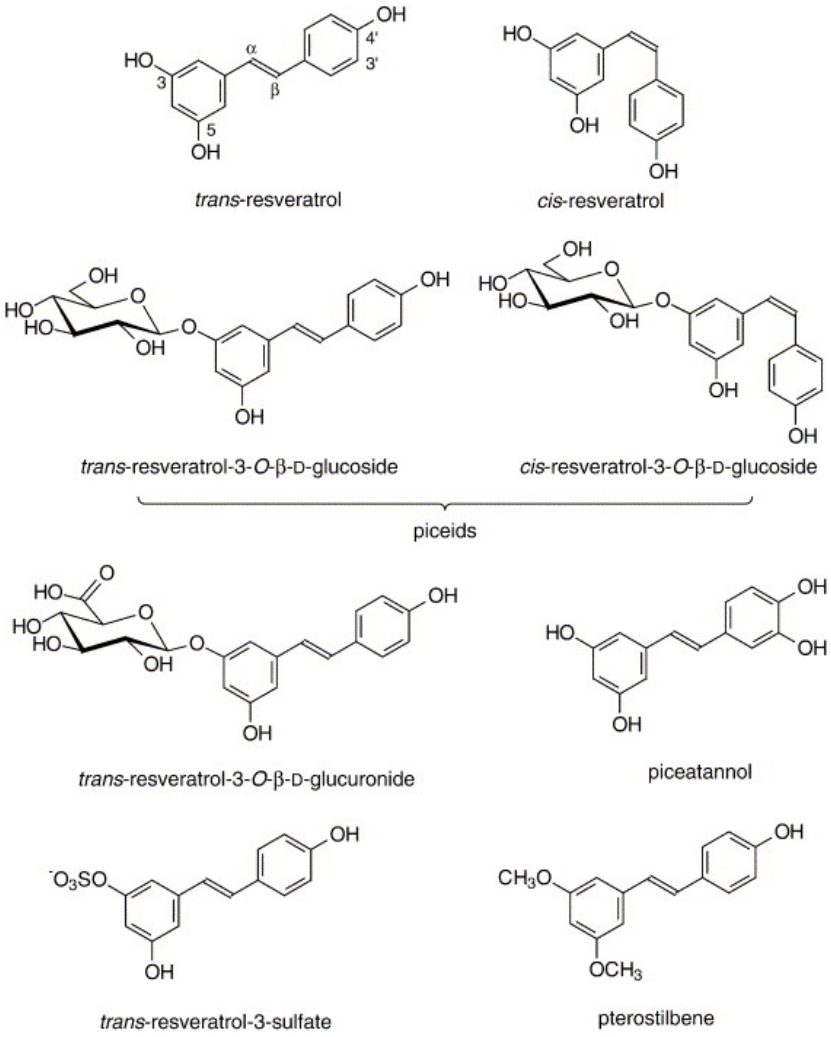

Figure 1: Structural formulas of resveratrol and its most common conjugates and analogues (3).

The emergence of resveratrol and the first studies related to it started with the discovery of *Vitis Vinifera* in grapevine by researchers named Langcake and Pryce in 1976 (4). In 1992, researchers named Siemann and Creasy showed for the first time that resveratrol was found in wine (5). Resveratrol has also been detected in the roots of Polygonum Cuspitatum, a natural medicine known as "Kojoto" in Japan (6). It has been shown that resveratrol in grapes is affected by environmental conditions such as room temperature, microbial infection, ultraviolet radiation, and ozone exposure, and its ratio changes (2).

2. The Effects of Resveratrol

Resveratrol has a wide range of biological activities, including enhancement of erectile function, free radical scavenging and antioxidant, inhibition of lipid peroxidation, inhibition of platelet aggregation, cardioprotective, vasodilator, anti-inflammatory, anti-carcinogenic, anti-diabetic, copper chelator, anti-aging, antiviral effects and also immunomodulator factor and estrogenic activity. Some of these effects have been proved by the researchers, some of them has been just supposed these important activities (7,8,17-19,9-16).

2.1. Enhancement of Erectile Function and Spermatogenesis Activity

Erectile dysfunction (ED), a widespread problem in men, is the inability to achieve or maintain an adequate penile erection to induce sexual activity (20). Various kind of risk factors identified for ED, including ageing, diabetes, psychological, coronary artery disease, obesity, smoking, depression, hypertension, and spinal cord injury (18,21). Although there are pharmacological [Phosphodiesterase type 5 inhibitors (PDE-5i), intracavernosal injection (phentolamine, atropine, alprostadil and papaverine) or intraurethral suppository (alprostadil)] and non-pharmacological (surgical methods, low-intensity extracorporeal shockwave therapy, lifestyle change, etc) methods in the treatment of diabetic impotence (21-24), preclinic studies on the useful effects of bioactive components for the treatment strategies of various diseases are widely gaining importance in recent years (22,25-27). One of the major bioactive components is Resveratrol, a plant polyphenol, has reported to improve endothelial function, and restore vascular eNOS activity of endothelial dysfunction in preclinical studies (12,13,28-30) smilarly with the other polyphenols, such as genistein, daidzein. It is well-known that the NO-cyclic guanosine monophosphate (cGMP) pathway takes part in retaining normal erectile function (31). RSV upregulate the NO–cGMP signaling pathway by involving various erectile mechanism (12,32). Furthermore, effectiveness of RSV has shown in the treatment of erectile dysfunction not only alone, but also sinergistically by using combination with PDE5 inhibitors (12,29,33).

On the other hand, testosterone, which is essential for promoting spermatogenesis activity, is well known to decrease in erectile dysfunction (24,34). In this sense, it is important that treatments provide both ameliorating ED and spermatogenesis activity, including testosterone levels. Polyphenols, including resveratrol, quercetin and genistein, have a significant role in the treatment of ED, testosteron levels and spermatogenesis damages (22,34,35). The enhancing effect of Trans-Resveratrol on testosterone levels and sperm quality

and corpus cavernosum relaxation has shown (34). In a study on Swiss albino mice, it was shown that RSV has an ameliorative effect on sperm parameters and histopathological damages (35).

2.2. Free Radical Scavenging and Antioxidant Effects

Resveratrol possess the feature of delaying and preventing the detrimental effects of free radical-containing reactive oxygen radicals (ROR) formed during the metabolism of macromolecules, reducing their cytotoxic effects and protecting the ROR-induced cell membrane damage (36).

In a study in which ischemia and reperfusion injury were created by coronary occlusion, resveratrol significantly decreased Malondialdehyde (MDA) levels, considered an indicator of lipid peroxidation in coronary perfusion (25). Resveratol prevents the dysfunction caused by oxidized low-density lipoprotein (LDL) in vascular smooth muscle and endothelial cells as a result of binding of LDL to its receptors on CHO-K1 cells. Thus, it has a protective effect against atherosclerotic diseases (37). It's known that Free radicals cause to DNA damage. In parallel, it was revealed in a study that resveratrol reduces DNA fragmentations by its potent hydroxyl radical scavenging effect (38). The high copper binding capacity of resveratrol has significant role in that the high copper binding property of LDL (39). Chanvitagaporgs et al. Demonstrated that resveratrol have a significant antioxidant effect than vitamins E and C in preventing LDL oxidation (40).

2.3. Effects on the Cardiovascular System

Cardiovascular diseases are the most widespread and leading causes of mortality and morbidity worldwide. Resveratrol has shown therapeutic potentials against cardiovascular diseases, in particular on animal studies (15,41), besides limited clinical studies (42,43). Resveratrol treatment is a promising therapeutic approach in cardiovascular diseases such as heart failure, myocardial ischemia/reperfusion (I/R) injury, diabetic cardiomyopathy and atherosclerosis by effecting various pathways (41,44-46). Adenosine monophosphate- (AMP-) activated kinase (AMPK), Sirtuin 1 (Sirt1) modulator, Sirt3, which is the receptor of sirtuin and tumor necrosis factor-α (TNF-α)/nuclear factor kappa B (NF-κB) signaling are the main target mechanisms of RSV (41,47,48). It is suggested that the most important reason for the low mortality rate due to cardiovascular diseases in France is the regular consumption of wine, despite the fact that people consume a large amount of red meat and are fed a fatty diet. This situation has been called the "French Paradox". After this determination, the number of studies on red

wine has increased. Grape skins contain approximately 50–100 μg/g resveratrol. It is found in the skin part of the grape rather than the flesh part, and it is found in very small amounts in other grapes compared to red grapes. It is thought that the 0.2–7 mg/L resveratrol it contains is responsible for the protective effect of red wine on heart (Sun et al., 2002). In a study conducted in rat aorta with intact endothelium, it was shown that resveratrol inhibited contractions caused by noradrenaline and phenylephrine (49). In another study, it was found that resveratrol has a stimulating effect on calcium-sensitive potassium channels in the blood vessels and this is a relaxing effect that occurs independently of the endothelium (50). Nitric oxide (NO) secreted from the endothelium has protective effects on ischemia reperfusion. NO concentration was significantly increased with administration of resveratrol 60 minutes before ischemia reperfusion. Accordingly, a decrease in the size of the infarct area was observed (51). Resveratrol has been shown to prevent cardiac fibrosis and cardiac muscle hypertrophy by inhibiting cardiac muscle fibroblast activity in proliferation and differentiation steps (52).

2.4. Antiplatelet Effects

Intravascular thrombosis may lead to many crucial cardiovascular diseases, including arterial disorders, strokes and myocardial infarction (53). RSV and its analogues inhibit the biological activity of blood platelets (54) by involving several signaling pathways, such as vascular Protection by Suppressing TLR4/Syk/NLRP3 Signaling, reducing thrombin-induced aggregation, decreasing platelet adhesion to type-I collagen and fibrinogen, inhibiting Ca^{+2} signal, reducing platelet aggregation and induced by collagen, ADP, and thrombin (54-60). Resveratrol has been shown to suppress thrombin and ADP-activated platelet adhesion by reducing the intracellular calcium concentrations that is increased during the aggregation phase. In addition, it has been reported that human platelet aggregation stimulated by collagen, thrombin and ADP is suppressed by resveratrol (59), and the amount of thromboxane A_2 formed is significantly reduced (61).

2.5. Anti-inflammatory Effects

Resveratrol, which has anti-inflammatory activity, targets the cyclooxygenase (COX), and 5-lipoxygenase (5-LOX) and protein kinase B, related with its ability to inhibit COX-1 and COX-2 activity (62). RSV has demonstrated to prevent inflammation by suppressing the formation of substances responsible for inflammation such as prostaglandins, complement system, proteases, NO,

bradykinins, adhesion molecules and cytokines (40). In the pathogenesis of atherosclerosis, sepsis, arthritis and diabetes, it has been revealed that NO, which is formed in excess, transforms into peroxynitrides and has a cytotoxic effect, and can cause mutagenic and carcinogenic effects by causing damage to DNA. RSV has demonstrated to ameliorate the ear oedema of mice, by inhibiting the production of NO, elevating the activity of SOD in serum, reducing the content of MDA and elevating the T-SOD activity in serum (17). RSV has also been shown in rabbits to suppress tumor necrosis factor (TNF-α), NF-kB activity, interleukin-6 (IL-6), macrophage inflammatory protein-2 and cyclooxygenase-2 activity levels, reactive oxygen species (ROS), caspase-3 and 9 (63). It was shown that trans-resveratrol reduces the cytotoxicity of natural apoptotic cells, decreases cytokine production in CD4, CD8 T lymphocytes and cellular toxicity, suppresses lymphocyte proliferation, and the production of lymphocyte-derived interleukins and TNF-α (19).

2.6. Anticancerogenic Effects

Apoptosis has a critical role in the balance between cell proliferation and death; It is known that many cytotoxic and cytostatic cancer drugs affect apoptosis for neoplastic cells. Resveratrol has a major role as a chemopreventive agent at four main stages of carcinogenesis: initiation, progression, progression, and metastasis, and has also demonstrated efficacy for cancer therapy *in vivo* and *in vitro* (64,65). To date, it has demonstrated efficacy against pancreas, liver, postmenopausal breast, colorectal, prostate, skin, lung and hematological malignancies (11,65-67).

It has been shown that RSV suppresses the proliferation of cells, activate apoptosis, prevents the increase of the antiapoptotic oncoprotein Bcl-2, and causes DNA fragmentation in human promyleocytic leukemia cells (68). It has been reported that the antitumor effect of RSV may be due to the suppression of ribonucleotide reductase, DNA polymerase, protein kinase C, cyclooxygenase-2 activities, suppression of carcinogenesis, and apoptotic cell activation (36).

One of the tumor suppressor proteins, p53, suppresses the apoptosis of cancer cells, cell cycle, cell proliferation. In this pathway, activation of RSV-stimulated p53 and apoptosis depend on the phosphorylation of ERKs and p38 kinase activation (69).

2.7. Antidiabetic Effect

Diabetes Mellitus (DM) is a multifactorial, metabolic disorder. Chronic hyperglycaemia leads to metabolic abnormalities and crucial complications. It

has been demonstrated that RSV has an important role in preventing diabetes and alleviating some diabetic complications, such as blindness, angiopathies, nephropathy, cardiomyopathy and retinopathy (70). Since the elevation in glycemia causes many diabetic complications, it is essential to keep blood glucose within physiological limits in treatment. Broad-spectrum anti-diabetic drugs are used in the pharmacological treatment of diabetes. Because of many adverse effects (hypoglycaemia, gastroistestinal problems, etc.) of the anti-diabetic drugs, new alternative therapies, particularly natural compounds, which has minimum side effects last decades. In that context, it has been suggested that RSV can be useful in type 1 and type 2 DM by reducing blood glucose levels even when administered for a long time without any side effects (16). The initial study on the effects of RSV on insulin was done in 2004 by Zhang et al. and it has demonstrated that RSV was ineffective on INS-1 cells (insulinoma cell line), on the other hand, it was also shown that insulin-secreting cells are significantly affected (71).

Some clinical trial studies revealed that Glycated hemoglobin (HbA1c) levels, which can be used as an indicator of type 2 DM-induced microvascular and macrovascular complications (43,72), have been demonstrated to be reduced with 3 months of RSV treatment. Besides HbA1c levels, RSV-treatment also improved systolic blood pressure, total cholesterol, and protein, and ameliorating glycemic control (73).

In previous studies conducted with diabetic rats, it has been suggested that RSV improves metabolic parameters, reduces plasma glucose, total cholesterol, and triglyceride concentrations, albeit partially, and, in parallel, reduces the effect of hyperinsulinemia (16). It is known that diabetes management consists of three main elements: lowering blood glucose, protecting pancreatic β cells, and restoring insulin activity in type 2 diabetes. It has been suggested in various studies that RSV has a significant role on regulating these main elements (16,74).

It was shown that RSV improved glycemia by increasing the levels of glucagon-like peptide-1 (GLP-1) and insulin in the portal vein by increasing the levels of colonic proglucagon mRNA transcription in obese diabetic mice (75). Some studies reveal that the antihyperglycemic effect of RSV in diabetic animals is because of increasing intracellular transport of glucose and that the expression of GLUT4, which provides insulin-dependent glucose transport, is increased (14,76).

In a previous study conducted on rats with diabetes induced by intravenous (i.v) injection of streptozocin (STZ), dose-dependent improvement in the side effects of RSV such as decrease in body weight, polyphagia and polydipsia,

which are the general symptoms of diabetes, were also enhanced (14). Another previous work reveal that the mechanisms involving to the dose-dependent hypoglycemic effect of RSV contain both the insulin-dependent/independent pathways, and also that phosphatidyl-3-kinase (PI3K-Akt) signaling is involved in the post mechanism in increasing glucose uptake 90 minutes after RSV administration in skeletal muscle (77).

Diabetic nephropathy is one of the common and serious complications of DM. Diabetes-induced renal diseases include many different mechanisms such as renal hemodynamic changes, oxidative stress, mitogen-activated and polyol protein kinase activation, lipid disorders, and enhace the nonenzymatic glycosylation of proteins signaling pathways (70). It was demonstrated that RSV ameliorates renal dysfunction in STZ-induced diabetic animals, involving different mechanisms (78). Another serious complication is retinopathy of the diabetic complications as well as diabetic nephropathy. In the treatment of retinopathy, the ameliorative effect of RSV has demonstrated in a longer lasting treatment, ranging from 4 to 7 months, by acting many different pathways, such as reduction in oxidative stress-mediated damage and NF-κB-mediated inflammation, reducing VEGF expression, and inducing stromal-derived factor-1α-mediated PI3K/Akt (70).

2.8. Anti-aging Effects

Aging is a biological process plays an important role in the etiopathogenesis of several diseases, causing to death of the organism (79). It has been reported that RSV has some significant role such as, a potent antioxidant, a genetic expression modulator, an inhibitor of inflammatory mediators, and have phyto-hormonal effects. All the biological and cosmetic properties make RSV an important candidate anti-aging potential agent (80).

Since the important biological activities, structure, and the crucial effects of RSV, various studies have evaluated the relationship or comparison with some of vitamins, stilbene derivatives. The major activities of RSV in platelets, inhibitory effect of the production of ROS and lipid peroxidation [80]. On the other hand, Silymarin (81), extracted from the milk thistle plant, has been found to share some features with RSV, such as antiapoptotic effect, modulation of signal transduction, and activation of sirtuin (10).

The ameliorative effects of RSV alone or in combination with different components/agents have been shown in many aging-related important diseases (9,82). One of the important study on age-related bone loss and inhibition of some gene expressions in heart, increasing effect of RSV alone on bone

microstructure and mechanical properties (83) and revert age-related changes in cardiac functions (79), respectively. Zhao et al. demonstrated that local administrations of RSV and Metformin promising agents in the preventing AMPK pathway suppression and angiogenic inhibition in wound beds (84).

2.9. Neuroprotective Effects

Neuroprotective effects of RSV have been demonstrated in neurodegenerative disorders, such as ischemic cerebral stroke, Parkinson disease, seizure, Alzheimer disease. RSV has been demonstrated to suppress the stimulation of microglia and reduction the production of pro-inflammatory factors (85). RSV has an important role in neuroprotection by decreasing mitochondrial dysfunction, oxidative damage and chronic inflammation (86).

Suppressing effect of RSV on brain-derived neurotrophic factor expression, cholinergic neurotransmission and oxidative stress (87). It has also indicated that RSV may be a new potential agent in the treatment of intracerebral hemorrhage by improving motor abilities and deactivating neuroinflammatory responses (87,88).

On the other hand, since RSV has some limitations, such as poor bioavailability and pharmalogical benefits, various nanotechnology strategies, including prolong the half-life and be able to cross the blood brain barrier of potential therapeutic drugs. In the last decades, current studies revealed that nanoencapsulation strategy enhances the bioavailability, prevents degradation and reduces the toxicities of RSV (89,90).

2.10. Antiviral Effects

Respiratory viral infections, a crucial public health problem, causes severe complications, involving acute respiratory distress syndrome (ARDS), respiratory failure, pneumonia, and multi-organ failure (7). Influenza virus, respiratory syncytial virus (RSV), human rhinovirus (HRV) and human metapneumovirus (hMPV) infections led to overexpression of pro-inflammatory cytokines and the overstimulation of immune factors may improve lung injury (91).

Investigations of alternative potential anti-viral therapeutics gain importance in the last decades, because the still unavailability of remarkable clinically validated anti-viral drugs against respiratory viruses, such as middle-east respiratory syndrome coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus (SARS-CoV) (92). Because of many important properties and effects of RSV, it has been focused on investigating the therapeutic potential of RSV in respiratory viral infections in recent years (7). It has been supposed

that the anti-viral activity of RSV and its analogues are both inhibition of viral replication directly and inflammation, causing by various pathogenic viruses (93).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes the current COVID-19 pandemic and various anti-viral drugs, such as ribavirin, remdesivir, chloroquine, and lopinavir/ritonavir, have been used in the potential treatment of SARS-CoV-2 infection (94). However, the use of the antiviral drugs has been restricted because they provide clinically unsuccessful results because of their side effects and low efficacy. It has been supposed that RSV acts on SARS-CoV-2 by the same mechanisms, affects other respiratory viruses and it may be a potential therapeutic agent against against SARS-CoV-2 infection in the next future (94-98).

3. Therapeutic Potential of Resveratrol

Since Resveratrol, a prominent polyphenol, l has potential therapeutic and preventive effects on various chronic diseases, *in vivo* and *in vitro* studies have been gain importance in the last decades (42,43,86,87). It was also supposed to increase the life span in a research (99).

One of the targeted mechanisms of RSV is to activate sirtuin-1 (SIRT1), a mammalian form of the sirtuin protein family, either directly or indirectly *in vivo* and *in vitro* (100). Therefore, SIRT1 activation by RSV may has a therapeutic intervention of a various chronic diseases, such as cardiovascular diseases, cancer, age-related and inflammatory diseases, oxidative stress, diabetes mellitus and its complications (8). Another targeted mechanism of RSV is inhibition and neutralization of ROS, chelation of metal cations and inhibition of lipid peroxidation for antioxidant activity (101). Another pathway of RSV is to provide anti-inflammmatory effects by suppressing of NF-kB and NOS expression and pro-inflammatory cytokines, such as IL-1β, TNF-α, COX-2 (102,103). Induction of apoptosis, suppression of NF-kB activation, protein kinase and GFR-mediated signal pathway is one of the significant target to exhibit anticancer activity of RSV (104). RSV also upregulates the NO–cGMP signaling pathway by involving various erectile mechanism (12). Last but not least, cardioprotective and immunomodulatory activities of RSV target to enhancement of NF-kB activity and inhibition of ANP and TGF- β1; also enhancement of formation of antibody cells and promotion of humoral immune response, respectively (105,106).

Despite the beneficial effects of RSV, it has some negative features such as poor oral bioavailability and toxicities that limit its clinical use. Rising

trends of nanotechnology-based applications may be a promising strategy to produce nanoencapsulations in order to increase the oral bioavailability and avoids the toxicity of RSV in the future (90,107). Besides that, RSV has been also encapsulated in different cyclodextrin complex, including α-, β-,γ-, 2-hydroxypropylβ-, and dimethyl-β-cyclodextrins, to improve the bioavailability and stability (108). Nanocarrier systems, including solid lipid nanoparticles and nanosuspension, have been formulated to bioavailability and stability as well as vesicular systems, delivering encapsulated drug and providing longer therapeutic activity (109-111).

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CHAPTER 6

THE IGF SYSTEM IN THE HUMAN PLACENTA

Seher KOÇ SALTAN

(PhD, Dr.), Kafkas University Department of Histology-Embryology, Kars, Turkey, E-mail: seherkoc36@hotmail.com
Orcid: 0000-0003-3795-5145

1. Placenta

The placenta is an extraembryonic tissue that forms between the fourth and twelfth weeks of embryonic life from the fusion of the chorion and the uterine mucosa (endometrium), providing the metabolic and hormonal relationship between the mother and the fetus. There are two parts in this tissue which belong to the mother and the fetus. These are the larger fetal part, which develops from the chorionic sac, and the smaller maternal part, which develops from the endometrium in the small maternal part (1).

Usually the placenta is round or oval-shaped (2). The fetal part, which belongs to the fetus, is covered with the amnion membrane and has a bluish-red color. Under the smooth and shiny amnion membrane, large branches belonging to the arteries and veins of the umbilical cord stand out. The maternal part, which belongs to the mother and is formed by cotyledons adjacent to the uterus, has a bumpy reddish appearance when it is separated from the uterus (3) Because there are more fetal and maternal tissues around the placenta, a surrounding protrusion occurs, which is called the Winkler-Waldeyer's ring (2).

The preembryon, which is now at the blastocyte stage with subsequent divisions from the zygote and has reached the uterine cavity, is very tightly or loosely attached to the uterine mucosa through the membranes covering it for further development. This attachment, which forms the placenta, is called implantation. Implantation is divided into 3 types according to the relationship between the maternal and fetal halves. These are the central (superficial), excentric and interstitial types (1).

2. Decidua

The decidua is the uterine endometrium during pregnancy and is so named because most of it is shed after birth. Depending on the increase in progesterone levels, connective tissue cells (stromal cells) in the endometrium grow and turn into polygonal or round decidua cells that are painted in a light color (1,4). Cellular and vascular changes that occur in the endometrium due to pregnancy are called decidual reactions. During implantation, arias-stella cells are formed by the multiplication and atrophy of uterine glands in the endometrium. It is believed that the onset of the appearance of these cells is related to the transformation of fibroblasts into decidua cells and the increase in the number of cells. In addition, it is accepted that the increased progesterone levels after implantation also initiate such changes of fibroblasts (5). Decidual cells contribute to fetal nutrition with their abundant glycogen and lipid content (4). Moreover, the decidua cells act on the endothelium of the blood vessels with their enzymes, allowing the blood to be released and creating a rich source for fetal nutrition. It also protects the developing creature from the negative effects of the immune reaction by stopping the proliferation of lymphocytes and the production of anti-fetal antibodies (5).

During pregnancy, the decidua thickens, reaching a size of 5-10 mm. According to their relationship with the implantation sites, there are three parts of the decidua. These are;

2.1. Decidua basalis: It is the part directly below the place of implantation (the maternal part of the placenta).

2.2. Decidua capsularis: It is the part that covers the developing ovum and faces the endometrium cavity.

2.3. Decidua vera (Decidua parietalis): It is the part that surrounds the rest of the uterine cavity (4). After the decidua capsularis disappears, decidua vera adheres to the non-placental parts of the chorion, allowing amniotic fluid to pass between the amnion and chorionic sacs (6).

3. Placental Development

Development of the placenta occurs on the 7th-8th day of pregnancy, with the implantation of the blastocyst into the endometrium. The blastocyst is a vesicular structure consisting of about 100-250 cells. The majority of these cells are trophoblastic cells that surround the blastocyst cavity and form the outer cellular layer. Several groups of cells on the inner surface of the blastocyst are responsible for the development of embryoblasts (7).

During the embedding of the embryo, the trophoblastic wall begins to differentiate to form a close relationship with the uterine vessels (8,9). In the second week of embryonic development, trophoblasts develop as primary chorionic villi (3). Some of the villi, which are initially located all over the chorion, later disappear and only those in the part where the embryo attaches to the uterus remain. The chorion section without villi is called the smooth chorion (chorion laeve), and the part with plenty of villi is called the chorion frondosum (10). For the formation of villi, trophoblast cells at the implantation site undergo rapid mitosis and differentiate into syncytiotrophoblast cells at the outer and cytotrophoblast cells at the inner sides (3). The primary chorionic villi formed at the end of the second week soon begin to branch. At the beginning of the third week, the mesoderm grows into the primary villi and forms a center of loose connective tissue inside each villus. Villi in this period are called secondary chorionic villi. Arteriocapillary venous networks form within the 3rd week, as some of the mesodermal cells differentiate into blood capillaries. With the formation of blood vessels in the villi, these are called tertiary chorionic villi. While the tertiary villi of the chorion frondosum continue to branch and develop in the decidua basalis, they melt the epithelial cells of the decidua basalis with their proteolytic enzymes, resulting in the formation of some gaps called clefts and lacunae (labyrinth). Later, the tertiary villi stretching out to these lacunae filled with maternal blood provide substance exchange from the maternal blood with their capillaries. At the end of the third week, blood flow begins in the vessels inside the villi. Lacunae that are developed in the syncytium expand and merge, forming structures called the intervillous space between the villi. Nutrients and oxygen diffuse from the maternal blood in the intervillous spaces into the villous vessels. Carbon dioxide and metabolites also undergo diffusion in the opposite direction. Some cytotrophoblastic cells in the tertiary villi proliferate and protrude into the syncytium. The layer formed by these cells is called the cytotrophoblastic shell. The cytotrophoblastic shell connects the chorionic sac to the endometrium. The villi that contact with the mother's tissue through this shell are called anchoring villi or main (stem) villi. The new villi that develop from the sides of the main villi are called branch villus. The pits that contain these clusters of villi are called cotyledons. Each cotyledon includes two or more stem villi, and branch villi which are separated from them. At the end of the fourth month, the decidua basalis is completely covered with cotyledons (3).

The part of the decidua that surrounds the embryo and the structures attached to it, the decidua capsularis, merges with the decidua parietalis as the pregnancy progresses and the fetus fills the uterine cavity. As a result, the

smooth chorion fuses with the uterine wall (decidua parietalis) on the opposite side. Thus, the uterine lumen closes. The chorion frondosum and the decidua basalis together form the placenta. With the enlargement of the chorionic cavity, the decidua capsularis gets under pressure, degenerates, and disappears (3,11).

4. Fetal and Placental Membranes

In humans, implantation occurs by layers of the uterine mucosa that participate in the formation of the placenta. These layers are the epithelial layer, connective tissue and vascular endothelium of the uterine mucosa. The fetus, on the other hand, participates in placentation with its villi (villi choriales), which form the chorionic frondosum. These are; the chorionic epithelium (trophoblast cells), the chorionic mesenchyme (embryonal connective tissue) and the vascular endothelium. These six layers, three of which belong to the mother and three to the offspring, form a barrier that allows the exchange of nutrients and gases between the offspring and the mother by diffusion between the blood vessels of both sides. This is called the blood-placental barrier (1). Maternal blood and fetal blood are separated from each other by the placenta membrane (10). Placental barrier consists of 4 layers until the 20th week. These are; syncytiotrophoblast, cytotrophoblast, connective tissue of chorionic villi and endothelium of fetal capillaries (3,12). After the twentieth week, structural changes occur in the villi. Cytotrophoblast cells in large areas in the villi become thinner and disappear and remain in small patches (3,13). Therefore, in most areas the placental barrier consists of 3 layers (13).

The chorion forms the amnion, the vitellus sac and the allantois fetal membranes. These structures develop from the zygote, except for the yolk sac and the allantois, and none of them contribute to the formation of the embryo and fetus. The vitellus sac joins the embryo as the intestinal primordium. Allantois, on the other hand, forms a fibrous cord called urachus in the fetus and continues to exist as a median umbilical ligament during adulthood (14). The placenta and fetal membranes perform the functions of protection, nutrition, respiration, secretion and hormone production. Shortly after the birth of the baby, the placenta and fetal membranes are expelled from the uterus by stretching and tearing with mechanical intervention (15).

5. Trophoblasts

The chorion wall is covered with an ectoderm on the outside and a mesoderm on the inside (1,10). The ectoderm covering the outer surface of the chorion gets

its name trophoblast (trophoectoderm) because it fuses with the uterine mucosa during the formation of the placenta and provides nutrition to the offspring. The outer surface of these chorionic villi, which participate in the structure of the placenta, is covered with trophoblast cells (1). Trophoblasts are examined under two groups; cyto and syncytiotrophoblast (16-18).

5.1. Cytotrophoblast: They are mononuclear trophoblastic cells, undifferentiated from the trophoblast cells from which they originated. They are located between the syncytiotrophoblasts and the villus basal membrane in the chorionic villi structure (16,19). Cytotrophoblast cells are connected to each other and to the syncytiotrophoblasts around them by desmosomes. These cells divide by mitosis and differentiate into syncytiotrophoblasts (12). Cytotroblasts decrease in number in the last period of pregnancy (16,19).

5.2. Syncytiotrophoblast: They are large and multinucleated cells and are the most differentiated form of trophoblasts (16,19). The syncytiotrophoblast cells are postmitotic (12). It is located in the outer cell layer of the villus. The presence of microvillus structures and cytoplasmic vacuoles on the apical surface, reflects the absorption and secretory activity of these cells. In addition, syncytiotrophoblasts are cells of abundant protein nature and contain small amounts of steroid hormones such as human chorionic gonadotropic hormone and human placental lactogen (16,19,20).

6. Umbilical Cord

The umbilical cord connecting the fetus to the placenta is often centrally connected to the fetal part of the placenta (3). With the development of the placenta, the umbilical cord begins to form at 3-4th weeks. Therefore, the primary mesoderm thickens at one pole of the embryoblast, this part is called the body stalk. The body stalk determines the lower parts of the embryo. At the beginning of the development of the umbilical cord, the stems of the vitellus and allantois sacs also vascularise and get closer. Over time, the extraembryonic coelom disappears between them and the stems of these sacs are surrounded by the amniotic membrane along with their vessels. At this time, capillaries appear in the chorionic villi. Together they form the umbilical cord, surrounded by the amniotic membrane. The umbilical cord is approximately 50 cm long and curves ventrally to the placenta. In the 4th week of fertilization, the embryo is fed from the mother's blood by connecting the developing placenta via the umbilical cord. Harmful substances formed in the embryo and fetus are again transported to the placenta by the umbilical cord and released to the maternal

blood. The umbilical cord contains the vessels arteria umbilicalis and vena umbilicalis. With arteria umbilicalis, harmful substances formed in the fetus are carried away. With vena umbilicalis, the substances necessary for the fetus are taken from the mother and carried to the offspring (10). These vessels have a jelly-like, lamellar structure, and are located in a mucous connective tissue called Wharton's jelly, which is composed of mesodermal cells (2). Wharton jelly, a tissue rich in proteoglycans, forms a protective layer around the vessels (11).

7. Insulin-Like Growth Factors (IGFs)

Growth hormone is a hormone that is necessary for normal growth and has an anabolic effect throughout life. However, it does not directly act on all body cells that have the ability to grow, especially bone, cartilage, and skeletal muscle, and acts with an intermediate called somatomedin (21). Somatomedin is a broad-spectrum growth factor that stimulates DNA synthesis in about 20 different cells originating from the ectoderm, endoderm, and mesoderm, which are secreted from the liver and other tissues, with the effect of growth hormone (22). Many effects of somatomedins on growth are similar to those of insulin. This is why they are called Somatomedins, as well as insulin-like growth factors (23).

The main circulating somatomedins are insulin-like growth factor–1 (IGF-1, Somatomedin C) and insulin-like growth factor–2 (IGF-2) (21,22). IGF-1 is a growth factor consisting of 70 amino acids with three disulfide bridges and weighs approximately 7500 daltons. IGF-2 in human plasma is a polypeptide consisting of 67 amino acids and weighs approximately 7471 daltons (21,24,25). IGF-2 is 65% similar to IGF-1 and 41% similar to insulin's A and B chains (26). It is difficult to distinguish the effects of insulin on cell growth and proliferation from the similar effects of IGF-1 and IGF-2. Insulin and IGFs interact with each other in growth and proliferation (27).

The IGF system consists of IGF-1, IGF-2, 4 IGF receptors (insulin receptor, Type-1 IGF receptor, mannose 6-phosphate/IGF-2 receptor and hybrid insulin/IGF-1 receptor), and IGF binding proteins (IGFBP) (28). Like all peptide hormones and growth factors, IGFs act by binding to receptors on the cell surface. Two types of specific receptors have been shown for IGFs (26). Type 1 IGF receptors are structurally very similar to insulin receptors. The IGF-1 receptor (IGF-1R), which is a member of the tyrosine kinase growth factor receptor, has a molecular weight of 400 kDa. The Type-1 IGF receptor is the critical determinant for the regulation of growth and metabolic response in the IGF system. In addition, the Type-1 IGF receptor also has a coordinating

role in the process of the cell cycle, cell differentiation and development (29,30). The IGF-2 receptor (IGF-2R) shows a different structure than both the IGF-1 receptor (IGF-1R) and the insulin receptor. The type-2 IGF receptor is a single-chain protein which weighs about 220 kDa (24,31,32). Although the functions of the IGF-2 receptor are not fully known, it has been reported that it is responsible for peptide metabolism and destruction (29).

IGFBPs (IGF binding proteins), which are important modulators in the biological functions of IGFs, bind up with them in the circulation to move them from one place to another and extend their half-lives (10,24,28). The binding affinities of IGFBPs to IGFs and the release of IGFs are controlled by the phosphorylation, glycosylation, and specific proteolysis of IGFBPs (24,30). IGFBPs are more than just transport proteins, they can increase and decrease the effects of IGFs at the cellular level (33,34). Therefore, fetal growth in terms of IGFs is considered to be due to IGF-IGFBP interactions at the local level rather than central endocrine control. A small proportion of IGFs circulate freely without binding to IGFBPs (33). In recent years, it has been reported that IGFBPs have 8 different forms with different molecular weights with their binding pattern, distribution, and amino acid compositions in biological fluids (35).

8. The Mechanism of Action of IGFs

Physiologically, local production of IGFs is important. Circulating IGFs have an endocrine effect, while those produced locally have an autocrine or paracrine effect (36). IGFs act by binding to specific receptors. However, in order for IGF to interact with the receptor, it must be separated from binding proteins. At this stage, proteases get involved (37). The proteolysis of IGFBPs in the circulation allows IGFs to circulate freely, and IGF passes into the extravascular space. In the extravascular space, IGFs also bind to various IGFBPs. They are broken up by various proteases at the tissue level and bind with their receptors (38).

9. Localization and Effects of the IGF System in the Human Placenta

In the human placenta, mRNAs of the IGF system other than IGF-2 are similarly expressed and are present in excess throughout pregnancy. Although IGF-1 and IGF-2 mRNAs show a similar tissue distribution, IGF-2 is higher every trimester of pregnancy (39). It has been reported that IGF-2 is abundantly synthesized by cytotrophoblast, extravillus trophoblast, fetal endothelial cells and fetal mesenchymal cells in villi (40). In addition, IGF–2 has also been reported to be

abundant in intermediary trophoblasts that invade the maternal spiral arterial wall (39). It has been reported that IGFBP-1, which is one of the IGFBPs, is also synthesized by the decidual stroma, and different amounts of IGFBP mRNAs, particularly IGFBP-1, are found in the maternal decidua basalis and parietalis (39,40).

It should be noted that, during pregnancy, IGF-1 and IGF-2 play a key role in feto-placental growth. They have been shown to have metabolic and mitogenic effects on the placenta in fetal tissues (41). In particular, it has been reported that IGF-1 is secreted by pleomorphic cells (Hofbauer cells) with vacuolated cytoplasm in chorionic villi, chorion fetal membranes, syncytiotrophoblasts, and cytotrophoblasts (42). Another study on the placenta reports that the presence of IGF-1 produced in decidua, syncytiotrophoblast, and cytotrophoblast cells may be the source of some IGF-1 in the fetal circulation or may play a paracrine role affecting cell proliferation in the placenta (43). It has been shown that IGF-1 has a mitogenic effect on placental stromal fibroblast cells and has metabolic effects like insulin in terms of causing an increase in amino acids while being transported in placental cells (44,45).

The presence of IGF receptors is of great importance, as insulin-like growth factors act by binding to receptors on cell surfaces (46,47). In the placenta, IGF receptors can be identified at the 6th week of pregnancy, and are expressed in both the cytotrophoblast and the syncytiotrophoblast at the 12th week (48,49). In a study on placentas from the 6th week to birth, Han et al (50) reported that the presence of IGF-1R mRNA was low in all cell types. However, it has been reported that maternal IGF levels increase rapidly throughout pregnancy (51). The distribution of IGF-1R in the placenta, especially the syncytiotrophoblasts in the villi of the maternal part, reveals that maternal IGF-1 plays a role in fetal development by regulating placental function. In their study, Hayati et al. (46) reported that there was a significant IGF-1R immunoreactivity in the placenta of normal pregnant women, in the decidua, trophoblast cells of the chorionic villi, and fetal vessel endothelium of the villi. In addition, in a study conducted to examine fetal growth retardation and the distribution of IGF-1R in normal human placentas, it has been reported that IGF-1R immunoreactivity is also present in the stroma of the chorionic villi in cases with normal human placentas (47). In their immunohistochemical study, Iwashita et al. (52) reported the presence of IGF receptors in different parts of the placenta. Regarding the physiological effect of locally produced IGF and maternal IGF on the placenta, *in vitro* data show that IGF is important in the growth, development and function of the placenta through IGF-1R. In addition to the proliferation of IGF-1 trophoblasts,

it enables the differentiation of trophoblasts into syncytiotrophoblasts, which produce hCG and hPL (53). It has been reported that maternal and placental IGF-1 may also affect fetal development by an indirect mechanism via increasing dose-dependent glucose and amino acid uptake in trophoblasts by acting on IGF-1R (54). The localization of IGF-1R in the microvillus membrane on the maternal part of syncytiotrophoblasts indicates that maternal IGF is important in placental function (55).

The fact that the IGF level decreases immediately after delivery, while it is high during pregnancy, shows the importance of its effect on the placenta. The effect of the IGF-IGFR system on placental function and development is a complex event due to various factors. The effects of IGF, which is in the maternal circulation and locally produced in the placenta, can be summarized as; increase in dose-dependent amino acid and glucose transport from trophoblasts, enabling the trophoblast proliferation and differentiation, hCG and hPL release from trophoblasts (53).

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CHAPTER 7

BIOCHEMICAL AND HISTOPATHOLOGICAL PROFILE OF THE LIVER IN CHEMICAL POISONING

Aysel GUVEN[1] & Gökhan NUR[2] & Haci Ahmet DEVECİ[3]

[1] (Assoc. Prof..Dr.), Başkent University, Vocational School, Pathology Laboratory Techniques, Ankara, Turkey,
E-mail: aguven@baskent.edu.tr
Orcid: 0000-0001-7511-7105,
[2] (Assoc. Prof..Dr.) Iskenderun Technical University, Faculty of Engineering and Natural Sciences, Department of Biomedical Engineering, Hatay, Turkey,
E-mail: gokhan.nur@iste.edu.tr
Orcid: 0000-0002-5861-8538,
[3] (Assoc. Prof..Dr.) Gaziantep University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Gaziantep, Turkey,
E-mail: h.ahmet_deveci@hotmail.com
Orcid: 0000-0002-3862-1991

1. Intraduction

Poisoning is a disease that occurs either sporadically or epidemically, either directly by pathogenic microorganisms, toxins, chemicals or by consuming food or water contaminated with them. Metals (lead, cadmium, mercury, copper) polychlorinated biphenyls, pesticide residues, radionuclides, contaminants carried from packaging materials to food, detergents/disinfectant residues, improperly used additives, pollutants formed during the cooking process (polycyclic aromatic hydrocarbons, heterocyclic amines, acrylamide, N-Nitroso compounds) cause chemical toxicity (1). The rapid development in science and technology, especially in the last 30-40 years, has also helped to determine the damage caused by chemicals in the organism, which we call toxicity mechanisms at cellular, biochemical and molecular levels. It has been

observed that it causes damage to many toxic tissues used in experimental animals. Using toxicity tests, the toxicity of a chemical and the biochemical and histopathological changes caused by its toxicity are comprehensively examined. Due to its anatomical location and important functions, the liver is the organ that is most exposed to toxic substances and can be damaged by many factors (2). Liver damege due to toxic factors varies. I addition to the fact that there are more date on liver damege due to drugs and viral factors, intoxication due to chemical substances and radioactive substances and radioactive substances is also substantial (3). With each passimg day, studies on nutritional and biological methods continue increasingly on the prevention of diseases caused by these toxins and treatment of the damage that has occurred. In addition to the rapid ivrease in the use of chemicals, the tragic events (cancer, heart attack) caused by chemicals brought about a development that we can call social chemophobia. For this reason, the mechanism of the damages that chemicals can cause in the body should be well enlightened. Especially the liver is an organ that plays a very important role in the detoxification and metabolism of many drugs and xenobiotics. Biological toxins and medical agents cause an increas in the production of free oxygen radicals (ROS) during their hepatic metabolism and cause degradation in the biochemical functions of hepatic cells by damaging proteins, lipids and carbon-hidrates secondary to this. Therefore, it causes hepatotoxicity subclinical disorder to severe necroinflammatory hepatitis (4). The degree of hepatotoxixity that occurs of hepatotoxicity that occurs varies depending on the duration of exposure to xenobiotics, döşe and antioxidant mechanisms, this the most obvious reason fort he effects of ROS's on hepatocytes (5). In this study, the physiological, histological, biochemical and pathological findings of living tissues obtained by surgical intervention as a result of the application of various chemical substances (carbon tetra chloride, ethyl alcohol, cadmium, dichlorvos) to various animal groups (goose, sheep, rabbit, rat and Mouse) it was aimed to present the changes comparatively and collectively and to determine the effects of different doses of all chemicals on lipid peroxidation, defense system and tissue degeneration in various tissues.

2. Chemical Toxins and Hepatotoxicity

2.1. Hepatotoxicity of carbon tetrachloride (CCl_4)

Carbon tetrachloride is obtained by chlorination of carbon disulfide or by reacting the same compound or by reacting the same comound with sülfür monochloride (6). This substance is absorbed through the respiratory, skin and gastrointestinal

tract. Carbon tetrachloride and other liquid halogenated hydrocarbons have been used as cleaning and degreasing agents (detergents) for a long time. They are also used in veterinary medicine for parastic control against antheln-mintics. Carbon tetrachloride (CCl_4) is a xenobiotic widely used experimentally to induce liver damage and is metabolized by the mitochondrial monooxygenase (P450 2E1) system (7). During metabolism, the initial unstable metabolite trichloromethyl (CCl_3) forms covalent bonds with lipids and proteins after the free radical is formed and rapidly transforms into trichlormethyl peroxide (Cl_3 COO^-) or chloroform from, which has lost hydrogen atoms. Later, secondary structures such as conjugated diene, lipid hydroperoxide and malondialdeyde and short-chain carbohydrates are formed. These free radicals cause cell destruction by causing the peroxidation of fatty acids found in phospholipids in cell membranes. Lipid peroxidation that occurs in liver damege due to CCl_4 is very important (6, 8). Because, depending on this damege, liver fibrosis and cirrhosis may ocur at the end of the progressive process. Liver fibrosis is a dynamic process characterized by an icrease in extracellular matrix components. Blance between the construction and destruction of the extracellular and destruction of the extracellular matrix; depending on the toxic oxygen radicals formed, it is degraded inthe direction of continuous matrix formstion with the activation of potent profibrogenic mediators. Taking CCl_4 in toxic doses orally or by inhalation causes liver disorders in a short time. The first symptoms of intoxication due to these substances are increased liver enzyme (AST, ALT, LDH) levels in plasma and fat formation in the liver. Events seen in CCl_4 metabolism cause triglyceride accumulation, polyribosomal disaggregation, depression in protein synthesis, cell membrane destruction and ultimately cell death (9, 10). CCl_4, which is known to cause damage especially on the liver, has also been reported to directly cause oxidative damage to erythrocytes (11). CCl_4, which takes place in veterinary practice as an anthelmintic, causes acute or delayed liver toxicities. In the histopathology of acute intoxications, liver damage, fatty tissue and necrosis are observed. CCl_4 is converted into trichloromethyl and trichloromethyperoxy radicals by the cytochrome p-450 monooxygenase system. These radicals are very active anda re responsible for the necrosis caused by CCl_4, in the liver, especially in the centrolobular region. The trichloromethyl radical forms a durable adduct with macromolecules and combines with oxygen to form the more active metabolite, the trichloromethylperoxy radical. This radical is the main initiator of lipid peeroxidation. Lipid peroxidation helps hepatic lipidosis by damaging the structures necessary for lipoprotein synthesis. SAs a result of excessive lipid accumulation in the liver, organ dysfunction and changes progressing to

cirrhosis ocur. In the study conducted by Ekebaş et al. (12) , intense macro and microvesicular fatigue in CCl_4 group hepatocytes was observed in the portal and parenchyna mostly in mononuclear cell infiltration areas and in necrotic pads. In experimental CCl_4 poisoning in gees, MDA levels, which are indicative of lipid peroxidation, were found to be significantly higher than controls,and macroscopically diffuse fat formation was observed in the liver (13, 14).

In another study conduced similarly, a large number of positive cells were detected in hepatocytes cells were detected in hepatocytes in the periacinar regions of the livers in the CCl_4 group. According to histopathological and immunohistochemical findings, CCl_4 has been shown to cause oxidative stress and lipid peroxidation (15). Güven et al. (16) to measure glutathione peroxidase (GSH-Px) and catalase (CAT) activity in the erythrocytes of mice given CCl_4, Cadmium (Cd), CCl_4-kefir, and Cd-kefir, to determine reduced glutathione (GSH) levels and in their study to determine the levels of thiobarbituric acid-reactive substances (TAARS), which is an indicator of lipid peroxidation, plasma TBARS and GSH levels were significantly higher ($P<0.001$) in the group given CCl_4 compared to the control group. Phosphorus and trihalomethanes (bromotrichloromethane, bromoform, iodoform and dibromochloromethane) have also been found to be hepatotoxic by lipid peroxidation. The 3,4-epoxide metabolite of bromobenzene, one of the other xenobiotics, has been shown to cause cell necrosis and hepatotoxic effect (17, 18).

2.2. Hepatotoxicity of ethyl alcohol

Ethyl alchol (C_2H_5OH); It is a mixture of 95.57% alcohol and 4.43% water by weight. 100% or 99.99% ethanol is called “absolute ethanol” or absolute alcohol. If absolute alcohol is left in the open air, it absorbs moisture from the air and turns into 95.57% alcohol after a while. After alcohol consumption, ethanol is distributed throughout the body. Stomach mucous cells and red blood cells are cells affected by alcohol. The most important part of alcohol metabolism occurs in thee liver. This pathway causes the spread of acetaldehyde in the bloo, abnormality and fragility of erythrocytes (19).

An insignificant portion of alcohol is excreated unchanged from the lungs (2-3%) and kidneys (1-2%), while a significant portion is metabolized in the body. Ethanol can also fill the biological membranes, expanding them and increasing their fluidity. When affected membranes are in an excitable state, this results in a change in action potential. Transport is damaged and neurotransmitter release is also affected. All of these suppress the cerebral function andi if developed in sufficient weight, can cause come and death due to repiratory paralysis. In

addition, NADH, which is formed during the metabolism of ethanol, competes with reducing equivalents from other substrates fort he respiratory chain, preventing their oxidation. Increased NADH/NAD^+ ratio may reduce the activity of the citric acid cycle by causing a leftward shift in the malate-oxaasetate balance (20, 21). Alcohol can be metabolized in the microsomal cytochrome P_{450} 2E1 (CYP2E1) in the liver and lungs. CYP2E1 occurs especially during chronic alcohol use is responsible fort he production of ROS (22). The net effeeect of fatty acid oxidation inhibition is the increase in the esterification of the fatty acid in triacylglycerol. This is shown to cause fatty liver. Alcohol intake increases the production of free radicals or reactive oxygen species (ROS) and causees oxidative stress by reducing the antioxidant defense system (23). In alcoholis, fat accumulation in the liver is called hyperlipidemia and cirrhosis is formed with its progression.

2.3. Hepatotoxicity of Cadmium

The main sources of cadmium in the environment are mines, refineries, industrial wastes, phosphate fertilizers, some pesticides, shellfish and motor oils. One of the important sources of cadmium is cigarettes. In poisoning with cadmium, many organs and systems such as especially the liver and kidney, respiratory system, circulatory system, stomach and intestines, bone tissue, blood production, testis, pancreas are damage (24, 25).

Radical production of cadmium occurs through some indirect mechanisms. One of the defense mechanisms against cadmium is the binding of cadmium to thiol groups and the consumption of high levels of glutathione stores in the liver (26, 27). In this case, liver sensitivity and radical production increase. Defense system disruption by cellular glution is the main pathway in oxidative stress caused by cadmium in the liver. Radical oxygen species and radical generation are shown as a mechanism for acute cadmium toxicity. Exposure of hapatocyte cultures to cadmium causes lipid peroxidation. In culture cells, cadmium produces superoxide anion, nitric oxide and H_2O_2, and these cause oxidative damage and lipid peroxidation in the plasma, brain, lung, liver and kidneys of animals exposed to cadmium in various ways. Given vit E and beta-carotene reduce the harmful effects of $CdCl_2$ (28, 29).

It was observed that the blood and liver malondialdehyde and nitric oxide levels of the group given cadmium to cause liver damage in rats increased compared to the control group, while it was observes that superoxide dismutase, catalase and glutathione peroxidase activities and glutathione levels decreased in the same group compared to the control group (30). Cadmium is a carcinogen

for many tissues/organs. Active oxygen group are formed in acute cadmium exposure. Acute poisoning mechanisms with cadmium are in the form of depletion of glutathione, binding to sulfhydryl groups in the proein structure, formation of superoxide ionsi increase of ROS such as hydrogen peroxide and hydroxyl radicals. Cadmium also binds to metallothione by increasing syntesis in the liver and increases hepatocellular damage. It accelerates the expression of stress genes that disrupt the activity of various enzymes that protect the liver from oxidative stress. The increase in oxidative stress leads to lipid peroxidation, DNA destruction, and protein carboxylation (31). Cadmium plays a rol in ROS mediated harmful effects. Cadmium cases tissue damage by oxidative stress, inceases lipid peroxidation, disrupts the antioxidant defense system, makes epigenetic changes in DNA expression, depletes glutation stores by binding to sulfhydryl groups, disrupts calcium balance and mitochondrial functions, and initiates the process leading to apoptosis (32, 33).

2.4. Hepatotoxicity of Fluorine

In histopathological studies on fluorine toxicity, it has been reported that fluorine has toxic effects, especially on liver tissue. Fluorine, which has important effects on bone structure as well as tooth structure, is one of the important inorganic elements that should be taken from outside (34). Two types of fluorine poisoning, acute and chronic are observed due to the excessive intake of fluorine (35). Acute fluorine poisoning is rarely seen with the ingestion of rodenticide poisons such as high doses of sodium fluorosilicate, sodium fluoride and water, plants and foods that are heavily contaminated with fluoride. Sodium fluoride at the level of 5 g is a toxic dose for humans (36). High doses of fluorine intake, increase the production of H_2O_2, superoxide, and hydroxyl. Excessive intake of fluorine increases respiratory burst and thus causes more production of superoxide (37). H_2O_2 causes lipid peroxidation in membrane lipids, inactivation of superoxide dismutase, DNA damage (38, 39). After high dose and long-term fluorine treatment mitochondrial swelling at the level of fine structure in rabbit liver cells, dilatation in the endoplasmic reticulum cisternae and decrease in RNA granules were found. It was determined that fluorine increase H_2O_2, superoxide and hydroxyl radicals and H_2O_2 inhabits SOD activity in Tuj sheep with experimental fluorosis (40). Reductions in Ca^{+2} levels, which are shaped by the irritating effects of fluorine, binding to Ca^{+2}, and inhibiting many enzyme systems in the loos tşssues of living beings with fluorisis, reduced the use of oxygen in cells, and as a result, various impairments may ocur (41). In chronic fluorosis, defects in the bone, kidney, thyroid gland, pituitary, hypothalamus,

testicles and teeth can be seen together with disorders that ocur in loose tissues (acute fluorosis) (42). Fidancı and Sel (43) stated that the serum lipid profile was affected in sheep fluorisis, and triglyceride, total cholesterol, VLDL and LDL cholesterol levels increased. Shanthakumari et al., (44) reported that in rats receiving fluride at a dose of 25 ppm/rat/day with drinking water for 16 weeks, lipid peroxidation increased, SOD, CAT, GSH-Px activities and GSH concentrations decreased. Lipid peroxidation levels significantly increased in blood samples taken during the week. These changes were attributed to the inccreaded production of free radicals and it was stated that could cause lipid peroxidation in the presence of fluorosis.

2.5. Hepetotoxicity of Dichlorvos

Dichlorvos is an organophosphate insecticide widely used in pest control. Dichlorvos is one of such chemical toxic substances. There are strong evidences indicating that oxidative stress arising from metabolic changes developing based on dichlorvos contribute the developmen of complications of metabolic syndrome (45-47).

Dichlorvos; active oxygen formsa re formed during the normal processes of the body' use of oxygen in living metabolism and due to some chemical factors. The active oxygen forms formed caused structural deformations in protein, carbohydrate and lipids. This disrups both the structure and functions of the cell, causeing many diseases (48). It has been reported that a decrease Paraoxonease (PON) activity, an antioxidant enzyme assoviated with high-density lipoprotein (HDL), may cause some degenerative impairments in the liver (49). In the study conducted by Nur et al. (48), they observed that the intake of dichlorvos increased oxidative stress and strained the antioxidant level in their study on rats administered dichlorvos. Another study investigated effects of non-lethal dichlorvos concentrations on the hematological component (red blood cells, White blood cells (WBC), mean cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet counts, hemoglobin and hematocrit levels). Their results indicate once again that subacute DIC applications induce damage marker enzymes and leukocytosis levels (50).

2.6. Toxicologically Free Radicals

Topics such as free radicals, their sources and effects, their mechanism of action, the damage they cause, their consequences and their prevention are the most frequently discusses and studdied topics lately. Free radicals are short-lived, unstabil, low molecules with one or more unpaired electrons. Free radicals have

different chemical structures such as hydroxyl, superoxide, nitric oxide and lipid peroxide radicals (13, 51, 52). Singlet oxygen, which is formes rapidly by superoxide groups, reacts with unsaturated fatty acids in the cell membranes in the structure of phospolipid, glycolipi, glyceride and sterol to form various lipid peroxidation products such as peroxides, alchols, aldehydes, hydroxy fatty acids, ethan and pentane. The most important product of lipid peroxidation is malondialdehyde (MDA). MDA occurs in the peroxidation of fatty acids containing three or more double bonds. The resulting MDA causes cross-linking of compounds in the membrane by affecting the ion exchange through cell membranes and causes negative consequences such as changes in ion permeablity and enzyme activity, while reacting with nitrogen bases of DNA may be mutagenic, genotoxic and carcinogenic for cell cultures (53, 54). In a study with metals, it suggested that radicals can produce lipid peroxides formed by the attack of polyunsaturated fatty acid residues of phospholipids, redox metals and finally mutagenic and carcinogenic malondialdehyde, 4-hydroxynonenal and other exocyclic DNA inserts (eteno and/ or propano additions) (55). In many cases free radical production is part of the pathological-mechanism and the toxicity of many xenobiotics is related to free radical production. In some professions, prolonged exposure to some pollutants sch as carbon tetra chloride, cadmium, lead and alcohol causes oxidative stress (56).

Free radicals are formed in cells due to endogenous and exogenous factors. Among exogenous factors, free radicals, which are formed as a result of consumption or exposure of substances such as ethyl alcohol, carbon tetrachloride, cadmium, radiation, phytochmicals, cigarette smoke, solvents, drugs, are important in terms of liver toxicology (9, 57, 58).

For example, with the application of carbon tetrachloride, it is stated that all biochemical parameters (ALT, AST, ALP, glucose, triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, total protein, albümin) change and MDA and NO processing increase (10). Again, exposure of hapatocyte cultures to cadmium causes lipid peroxidation (28). Likewise, while excessive ethyl alcohol intoxication increases lipid peroxidtion in rats, it has been reported that it causes a decreases in enzymes that destroy free radicals (57).

3. Antioxidants

Oxidative stress can simply be defined as the imbalace between the antioxidant defenses of the body and the production of free radicals that cause peroxidation of the lipid layer of the cells. Antioxidants are substances that protect cells against the undesirable effects xenobiotics, drugs, carcinogenes and toxic radical

reactions, both directly and indirectly. It activates antioxidant systems against cell oxidative stres. Intracellular defense systems are sometimes not enough. In these situations, reactive oxygen species (ROS) become dominant and oxidative stress occurs. As a result, cellular macromolecules such as DNA, lipids, proteins, carbohydrates are damaged (48, 59, 60).

GSH, glutathione peroxidase, catalase, superoxide dismutase, thioredoxin reductase, nitric oxide synthase, both oxygenase-L and eosinophil peroxidase are included in this group (28, 51). GSH plays a role in the first stage against cadmium poisonin. It consumes glutation and protein-bound sulfhydryl groups and then increases ROS production, ultimately causing lipid proxidation, ultimately causing lipid peroxidation and oxidative DNA damage. The binding of cadmium to sulfhydtyl (SH) groups affects many enzymes and other SH-containing molecules (61, 62). Again liver metallothionin and GSH levels have an important role in the extent of liver damage (63). Amara et al. (64) showed an increase in LDL and MDA level/activ of cadmium chloride given to rats at a dose of 40 ppm; they repored that it caused a decreasevin GSH-Px, CAT and SOD activity. This indicates that cadmium causes liver damage.

4. Effects of Chemical Toxins on Liver Cell Pathology

Liver has a unique structure since it provides homeostasis, functions as a filter detoxifying the portal blood, does a great majority of metabolic events, delivers arterial blood to systematic circulation and is an important organ fighting against infections with its lymphoid tissue characteristics. It acquires characteristics of as an indispensable organ as a result of the great number of functions it has, and when considering the dependency of other organs on the liver by means of its metabolic functions, functional disorders likely to occur especially on this organ and the disorders likely to occur in the end are assessed to be crucial (65).

Liver functions as a principle organ for the metabolism of many medications or chemical agents. The reasons of toxic hepatitis are generally examined under three titles; medications, natural toxic agents and chemical agents. More than 1100 substances being harmful to liver and regarded as toxic can be listed. It is possible to group the toxic mechanisms causing liver damage under several titles. The first of these mechanisms is the cell swelling, breakdown of the membrane and cell lysis occurring as a result of disruption in the intracellular ion balance. The other one is that the transport proteins are affected as a result of the canalicular injury or cholestasis occurs as a result of the obstruction of gall bladder secretion. The immune system stimulated as a consequence of cell damage activates the cytokines and causes the activation of caspases and in

the end, the formation of apoptosis, the other mechanism. Another significant mechanism causing damage is the immune response. Some foreign formations that enter the body or some formations that develop in the body can be considered as antigens by the immune system elements. Antibodies responsible for humoral immunity form against them, and T cell response occurs as a result of this immunity. Due to the strong response of the immune system, cytolysis occurs.. Lastly, mitochondrial dysfunction arising from the beta oxidation of fatty acids, inhibition of the respiratory chain enzymes or the effects over mitochondrial DNA can be given as the final mechanism. In mitochondrial dysfunction, aerobic metabolism starts to experience problems in the cell, and steatosis in cells takes place as a result of lactic acidosis and the accumulation of triglycerides with the anaerobic metabolism (3, 66-70).

The concentration of enzymes involved in the metabolism of a chemical substance reaching its active site is the most important factor affecting the rate and path of biotransformation. This concentration depends on the lipophilicity of the substance exposed, its protein binding property, the dose and the route of administration (route of exposure). The most important organ in the biotransformation of xenobiotics is the liver (71, 72).

Liver damage, fatty tissues and necrosis are seen in the histopathology of acute intoxications. CCl_4 is converted into trichloromethyl and trichloromethyl peroxy radicals through the cytochrome P-450 monooxygenase system. These radicals are very active and are responsible for the necrosis caused by CCl_4 in the liver, especially in the central region. Fat dissolves in sections stained with Haematoxylin-Eosin as a result of alcohol and xylol processes during tissue follow-up. Therefore, fatty tissue is seen as hollow, roundish transparent areas. The nuclei of the parenchyma cells seem pushed to the edge of the cell. Sometimes, when the lipid accumulates rapidly and in large amounts, the fat-loaded cells rupture or merge with each other and turn into fat cyst. If the accumulated fat itself is to be seen, the severity of degeneration can be understood by making special fat paints (Sudan III-IV, Sharlack Red, Osmic acid) (65, 73, 74).

Carbon tetrachloride (CCl_4) is a toxic substance having a wide area of use ranging from the dry cleaning sector to the fight against insects (75-78). Contact with this chemical agent is known to induce oxidative stress and cause tissue damage along with the formation of free radicals (75, 79). A study where hepatotoxicity was induced by carbon tetrachloride reported cellular infiltration, necrotic areas, degeneration, sinusoidal haemorrhage foci and congestion in the liver and revealed histopathologically that green tea (*Camellia sinensis*) and parsley (*Petroselinum crispum*) diets did not provide an apparent

improvement in the lesion severity and frequency occurring in the liver (80). In a study investigating the effect of chokeberry plant juice and silymarin against hepatoxicity induced by CCI_4, it was observed that fibrosis severity and prevalence in the CCI_4 and CCI_4+chokeberry groups were similar; however, despite this, an apparent decrease in the number of vacuolated hepatocytes and a significant increase in the infiltration of lymphocytes were observed in the CCI_4+chokeberry group. This result may be arising from the immune system activating quality of chokeberry in regard to the antioxidant effect. In the CCI_4+ silymarin group, however, a decrease was reported in the severity of fibrosis, but an apparent decrease was not seen in the number of vacuolated hepatocytes (Figure 1) (81).

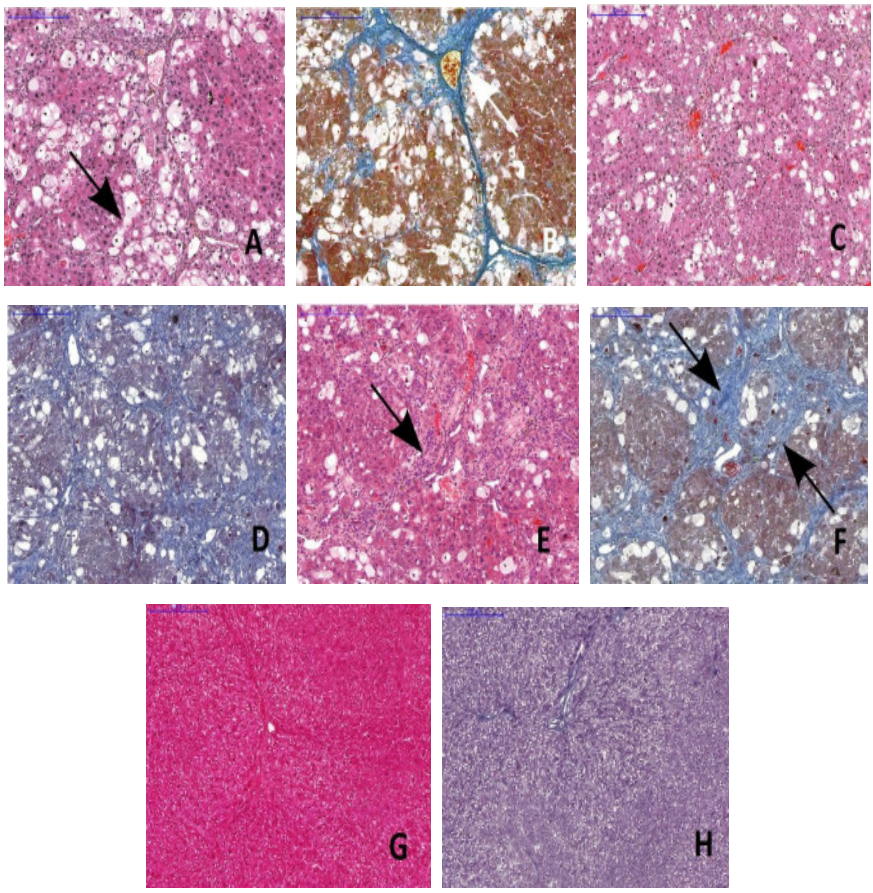

Figure 1. Histological assessment of the changes occurring in the liver as a result of carbon tetrachloride toxicity. A-B. CCI_4 group, C-D. CCI_4+ silymarin group, E-F. CCI_4+chokeberry group, G-H. Control group. In the CCI_4 and CCI_4+chokeberry groups fibrosis (arrows on A, B, E and F) and vacuolated

hepatocytes. H&E staining (A, C, E, G), Masson staining (B, D, F, H). Scale bar: 200 μm (81).

Alcohol is regarded as one of the main reasons of tissue pathogens due to its widespread use in the world and its potential of causing damage on the tissues, and for this reason, the mortality rate of consumers in the society are considered to be high. Alcohol abuse can affect adversely the immune system, organs such as the brain, liver, pancreas and heart and even the foetus depending on the alcohol consumption of the mother. The studies have reported that consumption of alcohol is one of the significant reasons for development of cancer, which is a multifactorial disease (82-84). In the oxidative stress induced by ethyl alcohol (20%), hydropic degeneration was observed in the cytoplasms of the hepatocytes in the portal regions of the liver. In addition, irregularity in the remark cords of the hepatocytes in the area and sinusoidal obstruction took place. These lesions were observed more rarely around the vena centralis (85). As a result of alcohol use disorder developing based on excessive alcohol consumption, many effects involve neurotransmitter systems and also impairments in hippocampal neurogenesis and neural connections and synaptic plasticity are observed (86).

It is known that heavy metals, which are commonly used in the industry, have negative effects posing threats for the living organisms as a result of their accumulation in the nature. Especially, cadmium, copper, zinc, arsenic, nickel and chromium can be primarily mentioned among the heavy metals found commonly in waste water. These metals lead to degradation in the protein structures and cellular damage by moving the essential elements or metals away from the regions they are biologically bound to. The oxidative deterioration of the biological macromolecules initially arises from the binding of heavy metals to DNA and nuclear proteins (87, 88). Cadmium accumulates particularly in the liver, pancreas, kidneys and lungs. The cadmium accumulating in the renal cortex has a half-life of 20-30 years. Cadmium (Cd) indirectly leads to the formation of kinds of reactive oxygen and nitrogen (such as superoxide, hydroxyl, nitric oxide radical) (89, 90). It especially increases the metallothionein synthesis in the liver as well as the hepatocellular damage by binding to it. It accelerates the expression of the stress genes that disrupt the activity of various enzymes protecting the liver from oxidative stress. Therefore, as a result of the disruption of antioxidant/oxidant balance in favour of oxidative stress, the DNA damage, lipid peroxidation and protein carboxylation are triggered (31, 91). In a study investigating the effect of Ginkgo biloba against the hepatotoxicity induced by cadmium and fluoride in a rat liver were researched, it was observed that cadmium and fluoride led to inflammatory cell swelling in the kupffer cells,

thus causing an increase in quantity. Moreover, a sinusoidal dilatation and inflammation case was reported. However, it was observed that the severity and the frequency of the lesions occurring in the liver decreased significantly in treatment with Ginkgo biloba and sinusoidal dilatation occurred with mild inflammation (Figure 2) (92). There is a great number of studies analysing the effects of natural nutritional sources having rich antioxidant quality against heavy metal poisoning cases. In the hepatotoxicity induced by cadmium (5 mg kg^{-1}), pericellular fibrosis, hepatocyte ballooning, macro and microvesicular steatosis, and inflammatory cell infiltration were observed in the liver.

In the group administered with 20 mg kg-1 resveratrol and cadmium; mild hypertrophy, mild expansion in the portal veins, inflammatory cell infiltration and binuclear hepatocytes were observed; however, a decrease was found in the severity and frequency of the lesions (93).

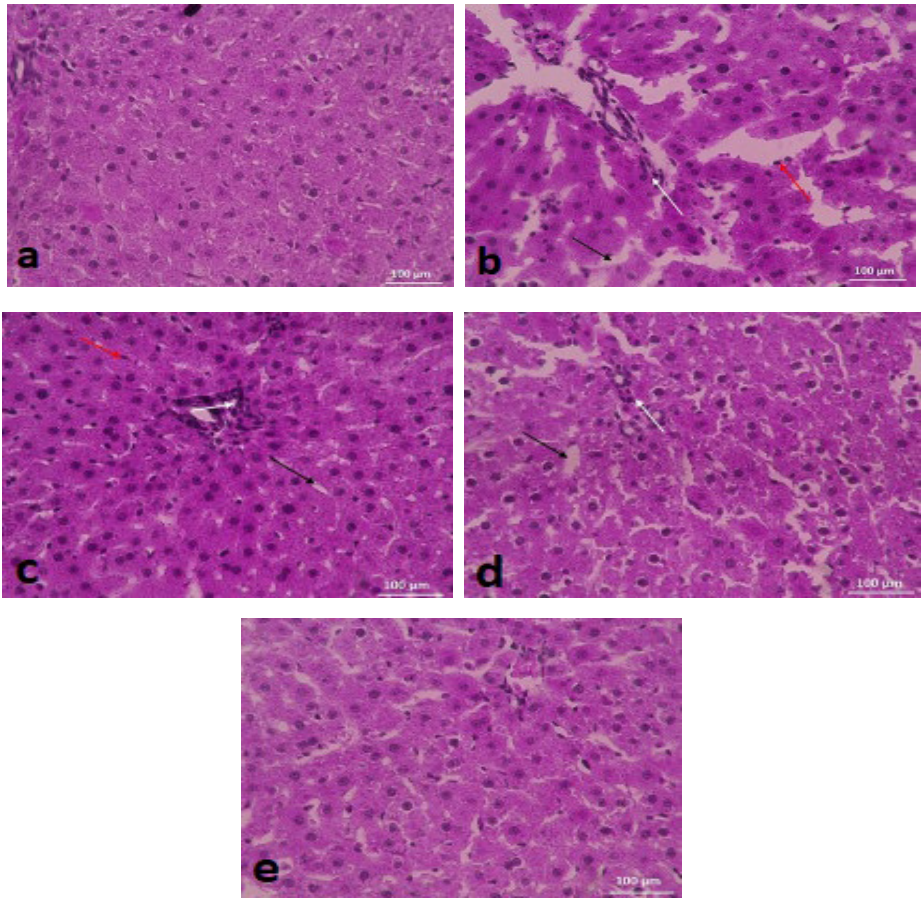

Figure 2. Light microscopic analysis of cadmium-fluoride toxicity in the liver tissue. a. Control group, b. F+Cd group, c. Ginkgo biloba (100 mg kg^{-1})+F+Cd

d. Ginkgo biloba (100 mg kg^{-1})+F+Cd, e. Vit-C+F+Cd. Proliferation of the Kupffer cell (red arrow), swelling of inflammatory cells (white arrow), sinusoidal dilatation (black arrow), H&E staining, X40. (92).

Fluoride is a moderate level of contaminant that does not go through biodegradation and has the potential to lead to significant health problems. Sodium fluoride (NaF), the compound mostly taken into the organism, affects and targets liver by triggering patomorphologic or metabolic changes. Due to its widespread use in many industrial sectors, it is released to the environment at a high rate (94-96). While it can mix into the aquatic ecosystems through many ways, the concentration of fluoride is 1.3 mg l^{-1} in the sea water and can be 10 mg l^{-1} in the well water rich in fluoride (94, 97). As a result of exposure to sodium fluoride at different doses from 15 days to 16 weeks (5, 30 mg kg^{-1}), severe necrosis in hepatocytes, nuclear degenerative changes, congestion in the central vein, pyknotic nucleus and degeneration were observed in the rabbit liver (98). In albino rabbits exposed to NaF at different doses (5, 10, 20, 50 mg kg^{-1}) through chronic and acute ways, increasing degenerative changes, vacuolisation in hepatocytes, hepatocellular necrosis, hepatic hyperplasia, centrilobular necrosis, dilatation in the central vein and congestion were encountered in the liver in parallel with the increase in dosage (99). NaF at the doses of 5, 10, 15 and 20 ppm was added into water and, hyperplasia, changes in the fat composition, necrosis and vacuolisation were reported in the livers of albino rat consuming this water (100). After it was added into drinking water at higher doses (50 ppm NaF), cytoplasmic vacuolisation, necrosis, and irregular hepatic cords were observed (101). It was reported that subchronic exposure to NaF at doses of 12, 24, and 48 mg kg^{-1} caused oxidative stress and apoptosis in the rat liver (Figure 3) (102). It was stated that the administration of NaF at a high dose (100 ppm) in drinking water for 21 and 42 days caused congestion, mononuclear cell infiltration in the periphery of bile duct, degenerative changes and necrosis in the rats (103). Another study revealed that NaF caused vacuolar degeneration in the rat liver (104).

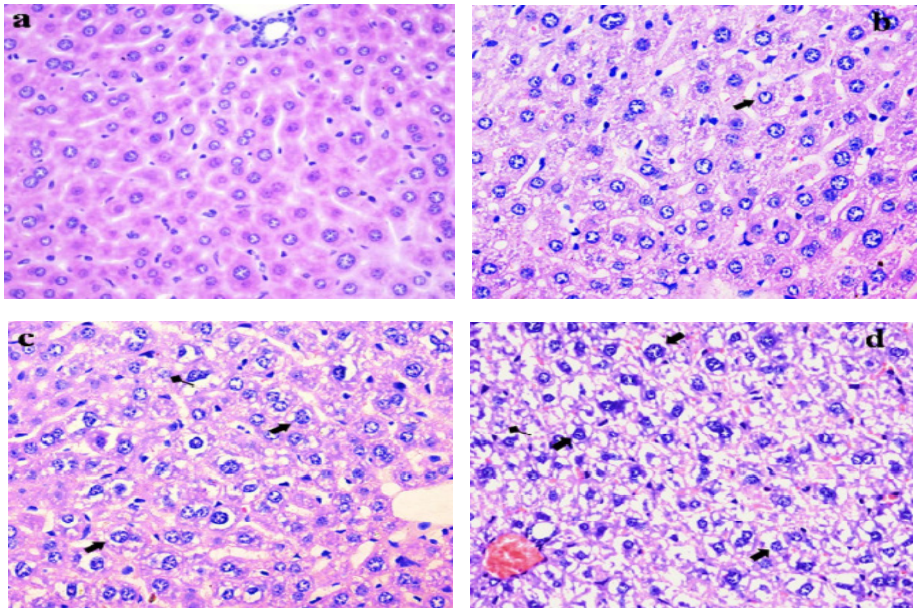

Figure 3. Liver tissue following subchronic NaF administration. a. Control group, b. 12 mg kg^{-1} group, c. 24 mg kg^{-1} group, d. 48 mg kg^{-1} group. Granular and vacuolar degeneration in the hepatocytes (↑), necrotic hepatocytes (🡅), widespread irregular hepatic cords. Magnification: x400, H&E staining. (102).

Pesticides having a wide area of use in the agriculture have a toxic effect on many non-target creatures through different ways, especially by mixing into the other ecosystems which enable to contact with aquatic and other creatures in addition to their effects on target creatures they are designed for. They appear themselves as hematologic, dermal, immunotoxic, biochemical, pathologic, genotoxic, teratogenic and mutagenic effects depending on exposure, contact time, and the rate of accumulation and excretion in the organism. Due to these common effects, there are many studies focusing on pesticides and the target and non-target creatures affected by them. In a study, it was observed that subacute exposure of clothianidin caused sinusoidal dilatation and congestion, hepatocellular degeneration, focal necrosis areas, mild steatosis, pyknosis, fibrosis and vacuole in the liver tissue depending on the increase in the dose of the substance (105). In a study investigating the effects of green tea (*Camellia sinensis*) and parsley (*Petroselinum crispum*) diets on the carbon tetrachloride hepatoxicity in albino rats, degeneration, sinusoidal haemorrhage foci, cellular infiltration, necrotic areas and congestion were reported in the liver in the group administered with CCI_4. Either parsley or green tea did not significantly decrease the severity and frequency of the lesions against CCI_4 toxicity (80).Vascular and degenerative

changes, focal necrosis in the hepatocytes, and polymorphonuclear cell infiltration were observed in the liver tissue of mice exposed to Deltamethrin of 0.1 and 0.05 mg kg^{-1} (106). Dichlorvos from the organophosphate class is a pesticide used in the domestic pest control. Its toxic character inhibits acetyl cholinesterase in the cholinergic junction region in the nervous system. (107). After albino rats were exposed to oral inhalation of the fumigants dichlorvos and cypermethrin (for 2, 4 and 6 weeks and for 4 hours per day) average level of lymphocytic infiltration and hepatocytic steatosis was found in the liver (through the dilution of dichlorvos and cypermethrin, with distilled water at the ratio of 1:1 from the 1000 mg L^{-1} stock) (Figure 4) (108). In the exposure of experimental aflatoxin carried out on broilers in the dose of 2 mg kg^{-1} for 21 days, it was found that aflatoxin had a highly toxic character for the liver tissue and there were congestion, fibrosis in the portal region, bile duct proliferation, and hepatocyte degeneration (109).

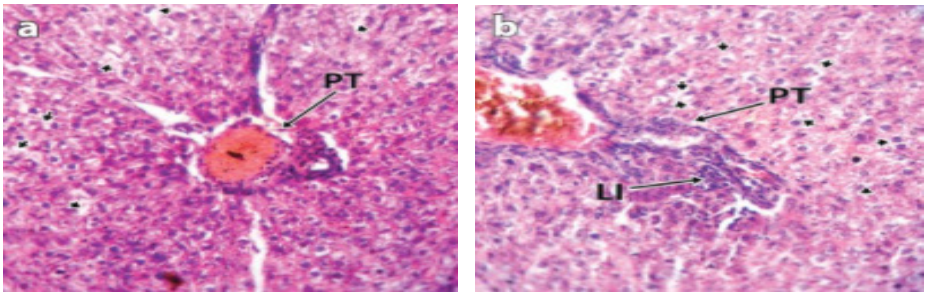

Figure 4. Liver tissue exposed to cypermethrin and dichlorvos for 2 weeks. a. Cypermethrin, mild hepatocyte steatosis (short arrows) around the portal region (PT) b. Dichlorvos, moderate hepatocyte steatosis (short arrows) and mild lymphocytic infiltration (LI) in the portal region (PT). Magnification:x400, H&E staining) (108).

In a study in which two non-lethal doses of chlorpyrifos as much as one fifth and one tenth of lethal 50 doses, were used on the freshwater fish *Pseudetroplus maculatus*, hepatocytic and cytoplasmic degeneration, necrosis and vacuolization were observed on the liver tissue in 15- and 30-day treatments (110). In a study where different doses of glyphosphate (5 and 50 mg L^{-1}) were administered in the juvenile carps, it was observed in the pathologic evaluations on tissue sections taken at the end of 15, 30 and 45 days that hepatocyte swelling, cytoplasmic vacuolization, changes in the increasing fatty tissues and ballooning degeneration were observed in parallel with the increase in the exposure days (111). Among diabetic rats which were exposed to subacute administration of 150 mg kg^{-1} malathion, hypertrophy in the hepatocytes in the liver tissue; mild degenerative deteriorations in the hepatocytes of centrilobular region, severe degenerative deteriorations in the hepatocytes of portal region, degenerative

deteriorations around bile duct, and mononuclear cell infiltration were observed. As a result of administration of malathion to rats in the diabetic group, an increase was detected in the severity and frequency of pathological findings in the liver tissue (112). Obstruction in the central veins, irregular cell cords, focal necrosis areas and mononuclear cell infiltration were observed in liver tissue of rats administered with Dichlorvos (5 mg kg^{-1}). On the other hand, in the group administered with CAPE (caffeic acid phenethyl ester) together with dichlorvos, congestion and cell infiltration were observed in the central and interlobular vein. It was observed that exposure of CAPE decreased the oxidative stress induced by dichlorvos and the severity and frequency of pathological cases decreased. However, in groups using solvents of the substances studied for effect (corn oil and ethanol), cell infiltration and a mild congestion were found around the central and portal regions (Figure 5) (48).

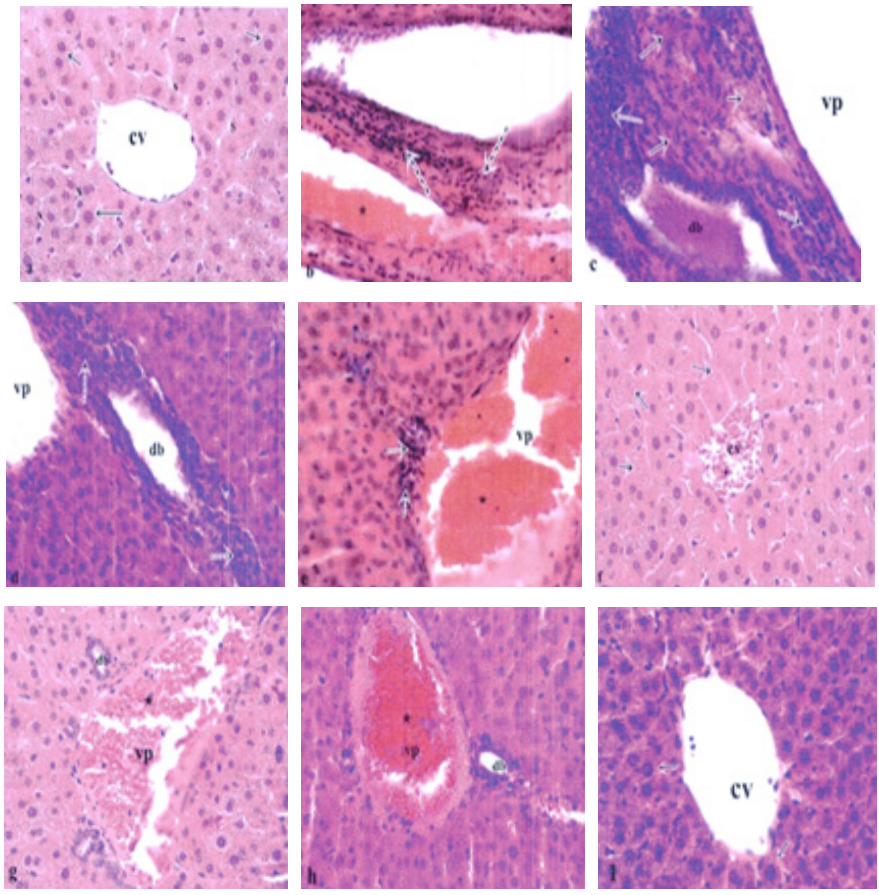

Figure 5. Liver sections obtained from the groups administered with Dichlorvos (5 mg kg^{-1}), CAPE (10 μM kg^{-1}) and solvent. **a.** Control 1 group (corn oil, 5 ml kg^{-1}). ı. Control 2 group (10% ethanol 0.5 ml), s: sinusoid, vp: portal vein,

hepatocytes (arrows), cv: central vein. **b, c, d.** Dichlorvos group, degenerative areas and focal necrosis (arrows), congestion in the central and portal veins, cell infiltrations (segmented arrows), db: ductus biliferus. e, f, g, h. Dichlorvos+CAPE group, severe congestion in the portal and central veins (asterisk). H&E staining (48).

5. Conclusion

Liver tissue hosts primarily many events such as the metabolic decomposition of substances having toxic characterization in the organism, their detoxification and evacuation. Numerous factors such as the amount of toxic substances, their level of accumulation in the organism and the duration of exposure reveals their level of effect namely damage on the liver tissue. For this reason, it is necessary that they are not mixed into the ecosystems of other creatures for the purpose of not damaging the sectors using toxic substances or the non-target groups. Here the ecological way that many toxic substances reach to the non-target creatures is their mixing into the aquatic habitats. Accumulation takes place with the natural food chain here and in the end humans are also affected negatively. As a result of the application of toxic substances, pathological lesions are seen in the tissues and blood biochemical values can go beyond the normal limits. Many studies have reported that pathological lesions reduced and biochemical data approached normal values with the administration of substances which have a protective potential against these toxic chemical substances or whose potential has been investigated. Substances having such potential in general have an antioxidant character and oxidant scavenger characteristics. Substances with high antioxidant capacity increase lymphocyte cell infiltration by stimulating especially the immune system in damaged areas.. The findings on which this therapeutic effect was firstly reflected are biochemical data and thus the protective potential profile is put forward. However, in the event that this therapeutic level does not affect the expression of genes, the improvement in the pathological findings remains insufficient. Decreases in the severity and frequency of lesions in pathological findings are observed as a result of having positive and sustainable changes in the gene expression significant for especially the immune and antioxidant system to improve the pathological findings emerging due to toxic substance exposure.

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CHAPTER 8

DETERMINATION OF RADIOACTIVITY DISTRIBUTION AND RADIOLOGICAL EFFECTS IN SOILS OF NORTHEASTERN ANATOLIAN REGION OF TURKEY

Gülçin BİLGİCİ CENGİZ

(Doç. Dr.), Kafkas University, Faculty of Arts and Sciences,
Department of Physics, Kars, Turkey
gulcincengiz@kafkas.edu.tr
Orcid: 0000-0002-6164-3232

1. Introduction

People are exposed to radiation from both natural and artificial sources in the environments where they live in Knowing the natural radioactivity levels in any area is of great importance for humans not only to know the natural radioactivity levels of the habitats they live in, but also to detect possible changes in natural radioactivity levels in the future. Radioactive elements such as $^{238}U(^{226}Ra)$, ^{232}Th, ^{40}K and ^{137}Cs make a considerable contribution to environmental radiation (1). The associated external exposure due to natural environmental radioactivity and gamma radiation is highly dependent on n geologic and geographic state of affairs and appears at dissimilar levels in soils in any part of the world (2). Studies to determine the radiation doses that people receive from natural sources are carried out by international and national organizations such as the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR), the International Committee on Radiological Protection (ICRP), the European Atomic Energy Community (EURATOM) and the Turkish Atomic Energy Agency (TAEA). Estimated average values of ^{226}Ra, ^{232}Th and ^{40}K activity concentrations in soil recommended by UNSCEAR for the world are given as 35 Bq kg^{-1}, 30 Bq kg^{-1} and 400 Bq kg^{-1}, respectively. The limit values for radiological hazard risk parameters such as radium equivalent activity (Ra_{eq}), absorbed dose rate (ADR), annual effective dose rate (AED) and lifetime

fetal cancer risk (LCR) are set by UNSCEAR as 370 Bq kg^{-1}, 60 nGy h^{-1}, 70 μSv y^{-1} and 0.29x10^{-3}, respectively (3).

Many researchers around the world and in Turkey are conducting scientific investigation to determine terrestrial radiation from radionuclides in the soil and to study the influences of radiation on living things.

In a study conducted by Singh et al. (2003) in Hamirpur district of Himachal Pradesh, India, uranium, thorium and potassium analyzes in soil samples were carried out using gamma ray spectrometry. Natural radioactivity levels in soil samples were measured using a NaI(Tl) scintillation detector. In the analyzed soil samples, the ^{40}K activity concentration varies between 143.7-228.9 Bq kg^{-1} with an average value of 174.48 Bq kg^{-1}, ^{232}Th activity concentrations varies between 35.2-122.8 Bq kg^{-1} with an average value of 93.10 Bq kg^{-1}, and ^{226}Ra activity concentrations varies between 25.1-75.7 Bq kg^{-1} the mean activity concentration was found to be 44.21 Bq kg^{-1}. In addition, the average radium equivalent activity was calculated as 192.09 Bq kg^{-1}, although it varies between 90.88-275.33 Bq kg^{-1}. It has been reported that the radium equivalent activity values are lower than the internationally recommended value of 370 Bq kg^{-1} and the radioactivity levels in the study area are within safe limits (4).

Radioactivity concentrations in soil samples in Tamil Nadu, India were investigated by Ravisankar et al in 2012 using gamma-ray spectrometry with NaI(Tl) scintillation detector. In the analyzes, the ^{40}K activity concentration was determined by using the gamma peak at 1460 keV, the ^{238}U activity concentration, the gamma peak of ^{214}Bi at 1764.5 keV, and the ^{232}Th activity concentration using the peak of ^{208}TI at 2614.5 keV. The radioactivity concentration values of ^{238}U were found to be in the range of 2.17 Bq kg^{-1} and 53.23 Bq kg^{-1} (mean value 19.16 Bq kg^{-1}), the radioactivity concentration values of ^{232}Th were found to be in the range of 13.54 Bq kg^{-1} and 89.89 Bq kg^{-1} (mean value 48.56 Bq kg^{-1}) and the radioactivity concentration values of ^{40}K were found to be between 625.09 Bq kg^{-1} and 2207.3 Bq kg^{-1} values (mean value 1146.88 Bq kg^{-1}). The obtained radioactivity concentrations were compared with the world average values, it was reported that the ^{232}Th and ^{40}K activity concentrations were 1.38 and 2.86 times higher than the world average values, respectively, and the average activity concentration of ^{238}U was below the world average activity concentration. Again in this study, radium equivalent activity, absorbed gamma dose rate, annual effective dose equivalent and external hazard index were calculated in order to evaluate the radiation hazards arising from natural radioactivity and the average values of these radiation hazard parameters were found to be as 168.8 Bq kg^{-1}, 86.62 nGy h^{-1}, 0.106 mSv y^{-1} and 0.478, respectively. It has been reported that the

radium equivalent activity is less than the internationally recommended value of 370 Bq kg^{-1}, the absorbed gamma dose rate is slightly above the world average value, and the annual effective dose rate is below the permissible limits. It was also stated that the external hazard index was smaller than the internationally recommended value (5).

The gamma spectrometric analyzes of soil samples and also food products used by local people in their daily diet were carried out using a high resolution germanium (HpGe) detector by Khan et al. in Pakistan (2010). In soil samples the activity concentration for ^{226}Ra, ^{232}Th and ^{40}K ranged from 30.0 Bq kg^{-1} to 81.2 Bq kg^{-1}, 31.4 Bq kg^{-1} to 78.25 Bqkg^{-1} and 308.8 Bq $kg^{-1}$to 2177.6 Bq kg^{-1}, respectively and with mean values of 56.2, 58.5 and 851.9 Bq kg^{-1}, respectively. ^{137}Cs activity concentrations varied from 1.3 to 46.8 Bq kg^{-1}, with an average value of 13.39 Bq kg^{-1}. It was observed that the average activity concentrations of ^{226}Ra, ^{232}Th and ^{40}K in the soil samples examined in the study were above the world average values. In this study, internal and external hazard indices were calculated and their average values were found to be 0.70 and 0.55, respectively. As a result of the dose calculations, it has been reported that the activity concentrations of the natural radionuclides in the study area are at nominal values and will not pose a health risk for the people of the region (6).

The analyzes of ^{238}U, ^{232}Th and ^{40}K natural radionuclides in soil samples collected from the Delta region of Nigeria in 2012 were carried out by Agbalagba et al. by using a NaI(Tl) scintillation detector in gamma spectrometric analyzes. The activity concentration of ^{40}K was evaluated using the gamma peak at 1460 keV, the activity concentration of ^{238}U using the gamma peak of ^{214}Bi at 609 keV, and the activity concentration of ^{232}Th using the peak of ^{228}Ac at 911 keV. As a result of the analyzes made, the ^{266}Ra activity concentration in the soil samples was between 19.2 ± 5.6 Bq $kg^{-1}$and 94.2 ± 7.7 Bq kg^{-1} values, with an average value as 41.0±5.0 Bq kg^{-1}; the ^{232}Th activity concentration was between 17.1 ± 3.0 Bq kg^{-1} and 47.5 ± 5.3 Bq kg^{-1}values, with an average value as 29.7 ± 4.0 Bq kg^{-1}; and the ^{40}K activity concentration was between 107.0 ± 38.5 Bq kg^{-1} and 712.4 ± 38.9 Bq kg^{-1} values, with an average value as 412.5 ± 20 Bq kg^{-1}. In this study, some radiation hazard indexes were also calculated. The mean value of the radium equivalent activity was reported as 98.5±12.3 Bq kg^{-1}, the mean value of the absorbed dose rate was 54.6 nGy h^{-1}, the mean value of the annual effective dose equivalent was 0.07 mSv y^{-1}, and the mean value of the external hazard index was 0.3. It has been reported that the concentrations of natural radioactive nuclei in the study area are compatible with the results of studies carried out in different parts of the world, the radiation hazard indexes calculated to evaluate

the radiological health risks are within the internationally permissible limits and do not pose any health risks for the people of the region (1).

Natural and artificial radioactivity levels were investigated in soils in Chamba and Dhoremshale region of India by Bara et al. in 2012. The ^{226}Ra, ^{232}Th and ^{137}Cs distributions in the soil samples were determined using in gamma spectrometry with high resolution germanium (HpGe) detector. The average activity concentrations of ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs in soil samples taken from Chamba region were measured as 32.3, 58.4, 588.3 and 10.9 Bq kg^{-1}, respectively. The average activity concentrations of ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs in soil samples taken from Dharamshale region were measured as 35.7, 61.3, 594.9 and 10.0 Bq kg^{-1}, respectively. In addition, the absorbed gamma dose rate, radium equivalent activity and activity concentration index were calculated in the study. The absorbed gamma dose rate has been reported to range from 45.2 nGy h^{-1} to 1038 nGy h^{-1}. Radium equivalent activity calculated for the soil ranged from 95.5 to 234.2 Bq kg^{-1} with average of 171.0 Bq kg^{-1}. The calculated activity concentration index was ranged from 0.34 to 0.85 with an average value of 0.64. The contributions of ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs to the calculated mean absorbed gamma dose rate for the study area were reported to be 22%, 46%, 32%, and 2%, respectively (7)

In a study by Srilatha et al. in 2015, activity concentrations of ^{226}Ra, ^{232}Th and ^{40}K natural radionuclides in soil samples collected from Ramanagara and Tumkur districts of Karnataka city, India were measured using gamma ray spectrometry with high resolution germanium (HpGe) detector. The ^{226}Ra activity concentration in the examined soil samples was between 14.38 ± 1.16 Bq kg^{-1} and 50.49 ± 2.31 Bq kg^{-1} values, and the average value was found to be 33.78 ± 1.99 Bq kg^{-1}. ^{232}Th activity concentration ranged from 42.2 ± 2.67 Bq kg^{-1} to 116.12 ± 3.23 Bq kg^{-1} values with mean of 77.44 ± 2.37 Bq kg^{-1} and ^{40}K activity concentration ranged from 388.98 ± 3.34 Bq kg^{-1} to 1563.64 ± 7.68 Bq kg^{-1} values with mean of 791.58 ± 5.78 Bq kg^{-1}. In addition, in this study, radium equivalent activity, absorbed gamma dose rate, annual effective dose equivalent and external hazard index were calculated in order to evaluate the radiation hazards arising from natural radioactivity, and their average values were found to be 205.47 Bq kg^{-1}, 95.07 nGyh^{-1}, 0.116 mSv y^{-1} and 0.554, respectively (8).

Gamma spectrometric analysis of the collected 36 different agricultural soil samples taken from a depth of 30 cm around the port of Aden in the south of Yemen were determined with a study by Harb et al. using a NaI(Tl) scintillation detector. The average concentrations determined radionuclides ^{226}Ra, ^{232}Th and ^{40}K were found to be 48.01±3.84, 58.11±4.65 and 624.80±49.98 Bq kg^{-1}

for Beer Ahmed- Beer Fadle area farm soil. For Daar-saad -Al-Masabian area farm soil the corresponding values were found to be 70.78±5.66, 84.75±6.78 and 771.53±61.72 Bq kg^{-1}, respectively. In addition, it was stated in the study that the activity concentrations of ^{26}Ra, ^{232}Th and ^{40}K radionuclides were higher than the world average values. It has been reported that this difference is due to the fertilizers used in agricultural soils and that the use of phosphate fertilizers should be controlled. Again in this study, radium equivalent activity, absorbed gamma dose rate, annual effective dose equivalent and external hazard index were calculated in order to evaluate the radiation hazards arising from natural radionuclides in soil samples. The mean values of radium equivalent activity, absorbed gamma dose rate, annual effective dose equivalent and external hazard index were found 179.23 Bq kg^{-1}, 82.98 nGy h^{-1}, 0.100 mSv y^{-1} and 0.48 for Beer Ahmed- Beer Fadle area farm soil. For Daar-saad -Al-Masabian area farm soil the corresponding values were found to be 251.38 Bq kg^{-1}, 114.07 nGy h^{-1}, 0.14 mSv y^{-1} and 0.68, respectively (9).

A comprehensive study was conducted by Oto et al. in 2017 to research natural radionuclides of ^{238}U, ^{232}Th and ^{40}K in 73 soil samples collected from Adır, Çarpanak and Akdamar islands on Lake Van (Turkey). The radioactivity concentrations of these natural radionuclides were measured ranging from 4.11 to 77.1 Bq kg^{-1}, 6.0 to 70.0 Bq kg^{-1} and 133.05 to 749.77 Bq kg^{-1} using a gamma spectrometer with a NaI(Tl) detector, respectively. The average activity values of ^{238}U, ^{232}Th and ^{40}K radionuclides were calculated as 33.33 Bq kg^{-1}, 36.68 Bq kg^{-1} and 478.19 Bq kg^{-1}, respectively. Moreover, they found the gamma dose rate (ADR) and annual effective dose equivalent (AED) absorbed in air to be 57.5 nGy h^{-1} and 70.5 μSvy^{-1}, respectively. They compared their results with some published results in Turkey and the world, which were within acceptable limits (10).

In this chapter, studies evaluating the radiation hazard for humans of natural and artificial radionuclide concentrations of soil samples in Kars, Ardahan and Iğdır are compiled. The activity concentrations of natural and artificial radionuclides in a total of 428 soil samples from undisturbed and uncultivated areas around the provinces of Kars, Ardahan and Iğdır in the Northeastern Anatolian Region of Turkey were measured by NaI(Tl) gamma spectrometry. Previous reported results of radium equivalent activity (Ra_{eq}), outdoor gamma absorbed dose rate (ADR), annual effective dose rate in air due to presence of radionuclides (AED), and risk of cancer formation over an average human lifetime (LCR) were tabulated. The consequences of this compilation may be enable as a reference for future evaluations.

2. Materials and Methods

2.1 Survey Areas

Within the aim of the environmental radioactivity monitoring, the results of the studies comparing the radioactivity concentrations of the surface soils collected from the provincial centers, districts and villages of Kars, Ardahan and Iğdır provinces in the Northeastern Anatolia region between 2013 and 2019 with international values were compiled. The map of Kars, Ardahan and Iğdır provinces where the samples were collected is shown in Figure 1.

Figure 1. Map of Kars, Ardahan and Iğdır cities.

2.2 Sampling

A total of 428 soil samples were gathered from places where no cultivation was made in the provinces of Kars, Ardahan and Iğdır. Each soil sample was taken from 6 distinct points in an area of approximately 1.2 m^2 and at a depth of 0-20 cm. After removing foreign materials such as stones, grass and tree pieces in the soil samples taken from each point, the soil samples were mixed appropriately and embed in tagged plastic sample bags. After the made uniform soil samples were dehydrated at room temperature, they were powdered with a grinder to be passed through a 10 mesh sieve. Samples packed in airtight cylindrical plastic containers and weighed dry were retained in these containers for a period of approximately 45 days to ensure the secular equilibrium between ^{238}U and ^{232}Th and short-lived degradation products (1).

2.3 Gamma-ray detection system

Natural radioactivity concentrations in soil samples picked up from designated study areas were assigned using a scintillation detector with a 76 mm x 76 mm NaI(Tl) detector based on a gamma ray spectrometer system manufactured by Ortec. In conjunction with a personal computer, the multi-channel analyzer emulation software (MAESTRO-32) is used to analyze the acquired spectral data. Samples were counted for 86400 seconds with a NaI(Tl) detector encircled by a 50 mm thick lead shield to reduce background gamma radiation (11).

The calibration of energy and relative efficiency of gamma spectrometer were made usage of standard reference material (IAEA-375). To calculate the net count rates of radium and thorium daughter peaks below the most significant photographic peaks, the background spectrum obtained for the same count with a blank plastic cup under the identical circumstances was subtracted from the corresponding count rate (12). To calculate the ^{40}K and ^{137}Cs activity concentrations, 1461 keV and 662 keV gamma lines were examined, respectively. The activity concentration of ^{226}Ra was determined by measuring 609, 1120 and 1764 keV gamma rays from ^{214}Bi and also 583 keV and 2614 keV from ^{208}Tl were exploited to identify the activity concentration of ^{232}Th (13).

3.3.1 Activity determination

The net counting rate below the best significant photo peaks of entire radionuclides daughter peaks were specified by removing the background spectrum correspondent to the same counting time. By using equation 1, the activity values of the radionuclides acquired as a consequence of the measurements were computed (14);

$$Activity = \frac{N_{area}}{\varepsilon_{effc} \cdot t_{count} \cdot M_{sample} \cdot I_{\gamma}} \quad (1)$$

where N_{area} is the net area of a peak at energy, M_{samle} is the mass of the sample in kg, e_{effc} is the detector efficiency of the specific gamma-ray line, t_{count} is the counting time, and I_g is the intensity of the gamma-ray line in a radionuclide.

3. Gamma Radiation Parameters

3.1. Radium Equivalent (Ra_{eq})

The primary goal of specifying radioactivity is to prognosticate the contingent radiation dose to be transferred to living creatures. Exposure to radiation can be clarified by manifold parameters. Radium equivalent activity (Ra_{eq}) is a

frequently used hazard indicator. It was reckoned using equation 2, assuming that 4810 Bq kg^{-1} of ^{40}K, 259 Bq kg^{-1} of ^{232}Th, and 370 Bq kg^{-1} of ^{226}Ra create the equivalent gamma dose rate (15).

$$Ra_{eq}\ (Bq\ kg^{-1}) = C_{226Ra} + 1.43C_{232Th} + 0.077C_{40K} \quad (2)$$

where C_{226Ra}, C_{232Th} and C_{40K} are the activity concentration of ^{226}Ra, ^{232}Th and ^{40}K in Bq kg^{-1}, respectively. The maximum recommended value for radium equivalent activity by UNSCEAR is 370 Bq kg^{-1} (3).

3.2. Absorbed Dose rate (ADR)

Respecting the biological effect, the most basic action for assessing the health risk to humans would be to calculate the dose ratio, as the radiological and clinical effects depend on the ratio of the dose absorbed. The calculated activity concentrations of ^{40}K, ^{232}Th and ^{226}Ra are converted to absorbed dose rate by exploiting 0.0417, 0.604 and 0.462 conversion factors for potassium, thorium and uranium, respectively. These factors are implemented to compute the outdoor dose rate (nGy h^{-1}) using equation 3 (16);

$$ADR\ (nGy\ h^{-1}) = 0.462\ C_{226Ra} + 0.604\ C_{232Th} + 0.0417\ C_{40K} \quad (3)$$

where C_{226Ra}, C_{232Th} and C_{40K} are the activity concentrations (Bq kg^{-1}) for ^{226}Ra, ^{232}Th and ^{40}K in soil samples, respectively. The international recommended value for ADR is 60 nGy h^{-1} (3).

3.3. Annual Effective Dose Equivalent (AED)

The average annual evaluated effective dose equivalent (AED) received by an individual was calculated with the aid of equation 4 using a transformation factor of 7×10^{-1} Sv Gy^{-1}. In this equation, the outdoor occupancy factor is taken as 0.2, which means that people spend their time of 20% in open air in one year (8760 hours y^{-1}). The world average value for the annual effective dose equivalent (AED) from external gamma radiation is 70 μSv y^{-1} (3, 17).

$$AED\ (\mu Sv y^{-1}) = ADR\ (nGy\ h^{-1}) \times 0.7\ Sv\ Gy^{-1} \times 8760\text{-hour} \times 0.2 \times 10^{-3} \quad (4)$$

3.4. Lifetime Cancer Risk (LCR)

Different kinds of health difficulties may occur at dissimilar times in different parts of the body of people who are exposed to varying doses of radiation. If a

person is exposed to a dose of 5 Gy or more, left untreated they can die from bone marrow or digestive system damage. However, it is not possible for people exposed to a dose of 50 Gy to survive even after medical treatment. If the dose is low or the dose is exposed over a long period of time, the effects on injured tissues, and the effects that occur in later years of life or in future generations are called stochastic effects (18). The probability of these effects occurring depends on the amount of radiation that the irradiated person receives from natural or artificial radiation sources. Cancer, the most important of the stochastic effects, is always serious and often fatal. The lifetime risk of fetal cancer relates to the probability of developing cancer over a lifetime at a given exposure level. The LCR is calculated using equation 5, where AED is the annual effective dose (Svy^{-1}), LE is the average life expectancy (about 70 years), and FR is a fatal cancer risk factor (Sv^{-1}) (19).

$$LCR = AED \times LE \times FR \quad (5)$$

For stochastic impacts, ICRP exploits FR as 0.05 Sv^{-1} for the community. The world's permissible standard of LCR is 0.29×10^{-3} (3).

4. Results

4.1 Kars Province

The Kars region, which consists of plateaus, mountains and green areas, is located between 40° 34' - 40° 38' North and 43° 01' - 43° 08' East longitudes, and the area of the region is 10.193 km^2 and an average altitude is 2000 m. It has 7 districts and 384 villages. In Kars province, 34.7% of its land is agricultural land, mainly cereals (wheat, barley) production, fodder crops and industrial crops are grown. The climate structure, landforms and geographical location of the province have a great impact on this. Apart from these products, fruit and vegetable production is also carried out, especially in Digor and Kağızman districts. The most basic economic sector in the rural areas of Kars is animal husbandry. Meadow and pasture lands are wider than agricultural land with 39.2%. The fact that this ratio is large makes a great contribution to the development of especially small and bovine animal husbandry in the province. Apart from this, poultry farming is also carried out, especially beekeeping. Produced cheddar, honey and other animal products are marketed throughout the country.

4.1.1 Kars City Centrum

Cengiz and Reşitoğlu (2014) assigned the distribution of natural radionuclides in 38 soil samples collected at different depths ranging from 0 to 10 cm from different uncultivated areas around the city center of Kars. Gamma spectroscopic measurements were performed using a NaI(Tl) detector based on the gamma spectrometry system. The declared average activity concentrations of ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs in soil samples from Kars city center were 47.8 ± 5.3 (6.85 ± 1 – 74.5 ± 7), 31.2 ± 3 (7 ± 3 – 90.3 ± 15), 536 ± 52 (224 ± 51 - 1068 ± 82) and 18.3 ± 2.7 (5.2±3.6 – 43.3 ± 3.6) Bq kg^{-1}, respectively. In the study, radiation hazard parameters such as radium equivalent activity, absorbed dose rate, annual effective dose and lifetime cancer risk were calculated and the mean values of the aforementioned gamma radiation parameters were found to be 135.0 Bq kg^{-1}, 44.7 nGy h^{-1}, 54.9 µSv y^{-1} and 0.27, respectively. (20). The results of ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs activity concentrations in soil samples collected from different sampling stations of the Kars Center are given in Table 1 and the results of the radiation hazard parameters calculated using these activity concentrations are given in Table 2.

4.1.2 Digor

The dispersion of natural radioactivity in soil gathered from virgin areas in Digor districts of Kars was ascertained by Bilgici Cengiz and Öztanriöver in 2018 using NaI(Tl) gamma-ray spectrometry. It has been reported that the concentrations of ^{226}Ra ^{232}Th, ^{40}K and ^{137}Cs natural radionuclides in 55 soil samples taken from their study areas ranged from 21.6 ± 7.0 to 55.7 ± 8.2 Bq kg^{-1}, 45.0 ± 14.7 to 94.7 ± 15.3 Bq kg^{-1},474.5 ± 0.0 to 666.5 ± 9.2 Bq kg^{-1}, and BDL (Below Detection Limit) to 13.3 ± 1 Bq kg^{-1}, respectively (Table 1). The specified average values of activity concentrations of ^{226}Ra, ^{232}Th and ^{40}K were used to figure out the radiation hazard indexes in soil samples. In the perused domain, the radium equivalent activity (Ra_{eq}) ranged from 131.3 to 195.1 Bq kg^{-1}, with the mean value of 171.5 Bq kg^{-1}, the gamma dose rate (ADR) absorbed in open air ranged from 61.7 to 88.0 nGy h^{-1}, with the mean value of 79.0 nGy h^{-1}, the annual effective air dose rate (AED) ranged from 75.7 to 107.9 µSv, with an average value of 96.8 µSv and the average risk value for cancer genesis (LCR) was reported as 0.34x10^{-3} over an average human lifespan (Table 2) (21).

4.1.3 Selim

The activity concentrations of 99 soil samples collected from different locations in the Selim district of Kars were determined by Bilgici Cengiz in 2017, using

NaI(Tl) gamma spectrometry. The concentrations of natural radionuclide ^{40}K, ^{226}Ra and ^{232}Th in soil samples were found to be ranged from 290.9 ± 58.8 to 913.9 ± 184.7 Bq kg^{-1}, 9.4 ± 6.9 to 27.9 ± 8.0 Bq kg^{-1} and 30.6 ± 14.2 to 76.9 ±15.7 Bq kg^{-1}, respectively. Also relatively low amount of ^{137}Cs were found in the investigated area, where the activity concentrations ranged from 1.0 ± 0.9 to 29.6 ± 3.6 Bq kg^{-1} (Table 1). As seen in the Table 2, the radium equivalent (Ra_{eq}) ranged from 99.2 to 188.5 Bq kg^{-1}, with the mean value of 146.0 Bq kg^{-1}, the open air absorbed gamma dose rates (ADR) ranged from 49.5 to 92.1 nGy h^{-1}, with the mean value of 71.0 nGy h^{-1} and an annual effective dose value (AED) ranged from 60.7 to 112.0 μSv with the mean value of 81.7 μSv. The reported average value of lifetime fetal cancer risk (LCR) was found as $0.30x10^{-3}$ ($0.21x10^{-3}$ - $0.39x10^{-3}$) which is similar with the world average value of $0.29x10^{-3}$ given in UNSCEAR 2000. It was stated that there was no indication of the possibility of developing cancer cases on human beings (22).

4.1.4 Sarıkamış

The activity concentrations of 121 soil samples agglomerated from diverse places of Sarıkamış of Kars were found out make use of NaI (Tl) gamma spectrometry. The concentration of the natural radionuclides ^{40}K, ^{226}Ra and ^{232}Th in the soil samples were observed altered from 148.0±31.2 to 909.2±38.4 Bq kg^{-1}, BDL to 38.1±8.9 Bq $kg^{-1}$and 7.6±0.7 to 53.0±7.4 Bq kg^{-1}, respectively. In addition, relatively low ^{137}Cs residues were found in the studied area, where activity concentrations ranged from BDL to 21.0±1.1 Bq kg^{-1}. In order to calculate the radiation hazard indexes in soil samples, the determined average values of ^{226}Ra, ^{232}Th and ^{40}K activity concentrations given in Table 1 were used. The observed total dose rate in the Sarıkamış study area was found to vary between 18.4 and 87.7 $nGyh^{-1}$, with a mean value of 46.9 $nGyh^{-1}$, and also an annual effective dose value between 22.6 and 107.5 μSv and an average value of 57.5 μSv. The radium equivalent value was between 36.7 and 177.9 Bq kg^{-1}, the mean value was 95.5 Bq kg^{-1}, and the mean lifetime fetal cancer risk value was reported as $0.20x10^{-3}$ ($0.08x10^{-3}$ - $0.38x10^{-3}$). When these dose values are compared with the permissible world values, the obtained values given in Table 2, were below the standard limits recommended for the environment (13).

4.1.5 Akyaka, Arpaçay and Susuz

In this study, the activity concentrations in 17 soil samples collected from different sample stations in Akyaka, Arpaçay and Susuz regions were measured

using NaI(Tl) gamma spectrometry. As seen in Table 1, the concentration of the natural radio nuclides ^{40}K, ^{226}Ra and ^{232}Th in the soil samples of Ardahan city varied from294.9±17.4 (Arpaçay) to 712.5±23.1 (Susuz) Bq kg^{-1}; 15.6±7.5 (Susuz) to 48.4±7.4 (Akyaka) Bq kg^{-1}; and 16.3±4.8 (Arpaçay) to 45.0±5.1 (Susuz) Bq kg^{-1}, respectively. Also relatively low deposits of ^{137}Cs were found in the investigated area, where the activity concentrations ranged from BDL to 29.6±3.6 (Arpaçay) Bq kg^{-1}. The averaged outdoor gamma absorbed dose (terrestrial and cosmic), the average annual effective dose and the average radium equivalent activity in air due to the presence of radionuclides in 17 soil samples were obtained as 52.3 $nGyh^{-1}$, 64.17 μSv y^{-1} and 107.3 Bq kg^{-1}, respectively. The mean life time cancer risk was determined as $0.22x10^{-3}$ for the residents of Akyaka, Arpaçay and Susuz regions. The results obtained in this study are presented in Table 2 (23).

4.2 Ardahan Province

Neighboring the Georgian border in the Northeastern part of Turkey, Ardahan is surrounded by Göle in the south, Hanak, Damal and Posof in the North, and Çıldır in the East. The coordinates of Ardahan are between 41º 11' 30" latitude and 42º 70' 23" longitude and it has an area of 5.661 m^2. Ardahan Plateau, formed by the lava that emerged as a result of Neogene volcanism, is at an altitude of 1800-2000 m. Livestock farming is an important source of subsistence in this basin due to the fact that landforms and climatic conditions limit agricultural activities to a great extent. Natural radionuclides taken from the soil by plants used as human food or animal feed are redistributed directly into food chains. In order to provide adequate protection for all living beings, it is necessary to investigate the dispersion term behavior of radionuclides such as mobility, transfers and displacement. (9).

Bilgici Cengiz (2017) collected 35 soil samples from undisturbed and uncultivated areas to determine the terrestrial radiation from radionuclides in the soil of the region and examine the influences of radiation on living systems. Soil samples were taken from 6 different points up to 15-20 cm depth in an area of approximately 1.2 m^2 in Ardahan province and its surrounding districts. The reported results of the measurements of ^{238}U, ^{232}Th and ^{40}K and ^{137}Cs concentrations in soil samples are outlined in Table 1. Activity concentrations of ^{228} U were between 7.9 ± 4.8 Bq kg^{-1} (Posof region) and 48.6 ± 7.4 Bq kg^{-1} (Damal region), and 232Th activity concentrations varied between 11.8 ± 7.8 Bq kg^{-1} (Posof region) and 55.5 ± 9.0 Bq kg^{-1} (Damal region). In addition, the ^{40}K activity concentration range was between 256.2 ±

26.4 Bq kg^{-1} (Ardahan Centrum) and 667.6 ± 27.0 Bq kg^{-1} (Damal district), and the ^{137}Cs activity concentration range was between 1.3 ± 0.6 Bq kg-1 (Ardahan Centrum) to 39.50 ± 1.2 Bq kg^{-1} (Hanak district). The average activities of the samples were determined to be 29.9 ± 6.2, 36.7 ± 6.8 and 435.1 ± 23.9 Bq kg^{-1} for the natural radionuclides ^{238}U, ^{232}Th and ^{40}K, respectively. The mean activity of the samples for the ^{137}Cs fission product was determined as 15.5 ± 0.8 Bq kg^{-1}. The mean concentrations of ^{228}U in soil of studied areas is inferior than the world of mean value (35 Bq kg^{-1}) and the Turkey mean value (34.7 Bq kg^{-1}) but similar with the East Anatolia mean value (29.1 Bq kg^{-1}). (3, 24, 25). The mean activity concentrations of ^{232}Th and ^{40}K for Ardahan province were found to be lower than some other reported results as indicated in Table 3, but higher than the world mean activity concentrations of 30 Bq kg^{-1}, 400 Bq kg^{-1}, respectively (3). Owing to the entity of radionuclides in soil samples, the mean values of radium equivalent activity, outdoor gamma dose rate, annual effective dose rate in air and life time cancer risk were obtained as 115.0 ± 9.2 Bq kg^{-1}, 56.3 ± 8.7 nGy h^{-1}, 69.0 ± 6.7 μSv y^{-1}, and 0.23x10^{-3}, respectively (Table 2). These obtained values are compatible with the world average values of 370 Bq kg^{-1}, 60 nGy h^{-1}, 70 μSv y^{-1} and 0.29 x10^{-3}, respectively, for each indicated radionuclide (3).

4.3 Iğdır Province

Domiciled in the easternmost piece of Turkey, Iğdır is the only provincial border that borders the three countries. One third of the area of Mount Ararat, Turkey's highest mountain, is within the borders of the Suveren village of the city center. There is the Nakhchivan Autonomous Republic border of Turkey-Azerbaijan in the east and Turkey-Iran border in the southeast. The Aras River in the north and northeast of Iğdır also forms the Turkey-Armenia border and flows along the borders of Iğdır after joining with Arpaçay. This river is a kind of lifeblood of the people of Iğdır. On the plain irrigated by the Aras River, it is one of the most important plant production areas in the Eastern Anatolia Region. Iğdır lowland is suitable for agriculture and various fruits and vegetables such as sugar beet, cotton, watermelon and tomatoes are grown. In and around the Iğdır plain, firstly sheep and secondly cattle are raised. Herds of sheep are taken to the wet meadows in the highlands and grazed in summer. After the sugar beet production became widespread, cattle breeding developed. Butter and cheddar cheese are produced in dairy farms where milk from animals is evaluated. In addition, apiculture is made with the "Caucasian Bee Breed", which produces 20-25 kg of honey per hive in the region.

People are exposed to radiation due to the industrial or medical applications of artificial radioactive materials. In addition, radioactive materials emitted into the atmosphere as a result of nuclear tests and nuclear reactor accidents contaminate soil, water and vegetation through dry fallout and precipitation. Radioactive materials accumulated in soil, water and plants affect local and regional radioactivity significantly.

Due to the presence of the Metsamor Nuclear Power Plant located on Iğdır province border, radioactivity measurements were made in the soil in order to determine the radiation dose to which animals and people who consume the plants grown in the province's territory may be exposed. Gamma spectrometric analyzes were carried out to determine the activity concentrations of 8 soil samples taken from various points along the banks of the Aras river, and 3 soil samples consisting of the Meteor pit in Mount Ararat and the volcanic structures taken from its surroundings. The average activity concentration of soil samples in the investigated area were determined as 15.7, 18.3 and 332.6 Bq kg^{-1} for the natural radionuclides (^{238}U, ^{232}Th and ^{40}K), respectively. In addition to these, the fission product of ^{137}Cs concentration was determined 17.7 Bq kg^{-1}. The average activity concentrations of ^{40}K, ^{232}Th and ^{238}U obtained in this study were below the world averages given in the UNSCEAR 2000 report (3). As shown in Table 1, the ^{238}U, ^{232}Th, ^{40}K and ^{137}Cs activity concentrations of the soil samples taken from and around the Meteor pit on Mount Ararat are higher than the natural radioactivity concentrations of the soil samples taken from other stations.

Assessed the acquired results in terms of the radiological risk, the mean values of the absorbed gamma dose rate (ADR), the annual effective dose equivalent (AED), radium equivalent activity (Ra_{eq}) and lifetime cancer risk (LCR) have been predicted and found to have mean value of 31.0 nGy h^{-1}, 38.0 μSv y^{-1}, 65.5 Bq kg^{-1} and $0.13x10^{-3}$, respectively (Table 2). When the results obtained in this study are compared with the limit values recommended by national and international organizations, it is understood that no individual in Iğdır province is exposed to an extra risk in terms of natural radioactivity in the environment (26).

5. Discussion

Table 3 lists the comparison of natural radioactivity levels and absorbed gamma dose rate, annual effective dose equivalent, radium equivalent activity and lifetime cancer risk in soil samples from the investigated stations of Kars, Ardahan and Iğdır, with values reported in the published works. As an outcome of this study,

it was observed that the average gamma dose rate values monitored in the open air ranged between 31.0 nGy h^{-1} (Iğdır) and 58.8 nGy h^{-1} (Kars) values, and the average value was 48.2 nGy h^{-1}. This average value is compatible with the mean ADR values obtained in similar studies conducted in Turkey (Mersin, Yalova, Edirne and Kırklareli) and in the world (Bulgaria, Greece, Nigeria and the United States) (3, 27-29). Annual effective dose rates are given in column 8 of Table 3 of this report and range from 38.9 μSvy^{-1} (Iğdır) to 71.4 μSvy^{-1} (Kars). The AED average value was found to be 59.1 μSv y^{-1}, which is lower than the average values reported in Adana, Rize and Trabzon cities in Turkey and in Nigeria, Malaysia and Pakistan in the world (3, 30-34). The average values of radium equivalent radioactivity varied from 65.5 to 122.5 Bq kg^{-1} with an average value of 101.1 Bq kg^{-1}. The estimated average radium equivalent radioactivity value given in this report is higher than the average radium equivalent radioactivity value in Palestinian soil samples, but lower than the mean radium equivalent radioactivity value in Malaysia, Edirne and Kırklareli (Turkey) soil samples (29, 33, 35). Lifetime cancer risk values range from 0.13 $x10^{-3}$ to 0.25 $x10^{-3}$, with an average value of 0.20$x10^{-3}$. As seen in Table 3, the reported average value of LCR in İndia, Pakistan, Palestine, Rize (Turkey) and Yalova (Turkey) are higher than the calculated average LCR values in Northeastern Anatolia (Turkey) (28, 31, 34-36).

Table 1 The assessed activity concentrations of ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs for soil samples in Kars, Ardahan and Iğdır.

Regions	Locations	Activity Concentrations (Bq kg^{-1})							
		$^{226}Ra(^{238}U)$		^{232}Th		^{40}K		^{137}Cs	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
Kars	Centrum	47.8±5.4	6.8 ± 1 74.5 ± 7	31.2 ± 3	7.0 ± 3 90.3 ± 15	536.0 ± 52	224 ± 51 1068 ± 82	18.3 ± 2.7	5.2 ± 3.6 43.3 ± 3.6
	Digor	31.3±8.4	21.6±7 55.7±8	67.1 ± 13.8	45.0 ± 14.7 94.7 ± 15.3	574.7±7.4	474.5 ± 0.0 666.5 ± 9.2	8.1 ± 1.4	BDL 13.3 ± 1.5
	Selim	19.9±7.5	9.4±6.9 27.9±8.0	57.9 ± 14.6	30.6 ± 14.2 76.9 ± 15.7	562.0±132	290.9 ± 58.8 913.9±184.7	6.1±1.2	1.0±0.9 29.6±3.6
	Sarıkamış	17.9±7.7	BDL 38.1±8.9	30.7 ± 6.8	7.6 ± 0.7 53.0±7.4	448.7±34.6	148.0±31.2 909.2±38.4	5.8±1.0	BDL 21.0±1.1
	Akyaka	37.7±6.8	29.3±6.4 48.4±7.4	29.9 ± 4.8	22.1 ± 4.8 36.7±4.8	483.8±20.5	408.0±20.1 609.7±22	8.0±2.8	BDL 27.9±2.9
	Arpaçay	28.9±6.8	25.0±6.5 35.0±6.8	23.3 ± 4.8	16.3 ± 4.8 31.8±5.1	383.5±19.3	294.9±17.4 465.6±20.4	21.7±2.8	18.6±2.4 29.6±3.6
	Susuz	31.3±7.4	15.6±7.5 37.1±6.4	29.5 ± 5.0	19.2 ± 5.1 45.0±5.1	508.1±21.2	372.8±20.8 712.5±23.1	14.4±2.6	BDL 28.6±3.2
Ardahan	Centrum	32.6±6.4	19.8±5.9 41.8±6.4	33.7±5.6	24.1 ± 5.5 42.8±5.3	438.2±22.0	256.2±26.4 523.9±21.4	14.1±0.7	1.3±0.6 35.7±1.1
	Çıldır	27.6±6.2	23.6±6.6 32.9±6.1	44.8±7.7	33.8 ± 8.2 52.0±7.1	410.7±21.8	298.7±28.6 511.9±23.1	10.5±0.7	7.7±0.7 15.7±0.9
	Damal	36.6±7.3	25.4±7.8 48.6±7.4	48.3±8.9	39.7±9.4 55.5±9.0	441.9±28.4	285.7±25.9 667.0±27.0	21.5±1.0	19.8±0.9 25.0±1.0
	Göle	23.9±6.2	11.5±5.1 36.5±6.7	42.1±7.5	40.4±6.6 44.5±7.8	397.2±21.8	359.3±19.3 424.5±22.7	22.2±0.9	18.0±0.9 27.7±0.9
	Hanak	30.7±6.7	29.3±7.2 38.7±7.1	38.0±8.3	33.5±9.1 46.1±7.6	421.9±25.2	329.5±23.0 499.5±25.3	18.1±0.9	4.7±0.8 39.5±1.2
	Posof	14.0±6.1	7.9±4.8 22.8±6.3	21.9±8.2	11.8±7.8 27.6±7.9	490.0±20.0	401.8±28.1 621.3±25.3	11.0±1.1	3.0±0.9 21.7±1.6
Iğdır	Aras river littoral	13±3	11±2 16±4	16±3	13±4 18±3	303±31	265±28 359±35	7±2	1±1 17±3
	Mount Ararat	22±4	20±4 25±5	26±5	20±4 30±6	411±41	354±31 444±46	46±7	15±4 69±9

Table 2 Calculated values of radium equivalent activity (Ra_{eq}), absorbed dose rate (ADR), annual effective dose (AED), lifetime cancer risk (LCR) in Kars, Ardahan and Iğdır soils.

Region	Location	Ra_{eq} Bqkg^{-1})	ADR nGyh^{-1}	AED μSvy^{-1}	LCR (10^{-3})	References
Kars	Centrum	91.0	44.7	54.9	0.27	(20)
	Digor	171.5	79.0	96.8	0.34	(21)
	Selim	146.0	71.0	81.7	0.30	(22)
	Sarıkamış	95.5	46.9	57.5	0.20	(13)
	Akyaka	117.7	56.9	69.8	0.24	(23)
	Arpaçay	91.7	44.9	55.1	0.19	
	Susuz	112.5	55.1	67.6	0.23	
Ardahan	Centrum	114.5	55.5	68.1	0.23	(11)
	Çıldır	123.2	59.4	72.9	0.24	
	Damal	137.3	67.3	82.5	0.27	
	Göle	114.7	55.8	68.5	0.23	
	Hanak	117.5	57.0	69.9	0.23	
	Posof	83.0	41.9	51.4	0.17	
Iğdır	Aras River Littoral	56.0	26.5	32.6	0.11	(26)
	Mount Ararat	82.0	38.9	47.6	0.17	

Table 3 Comparison of natural radioactivity levels, Raeq, ADR, AED and LCR values in soil samples of Kars, Ardahan and Iğdır with values reported in the literature

Country (Region)	Activity concentration (Bq kg^{-1})				Ra_{eq}	ADR	AED	LCR	References
	^{226}Ra	^{232}Th	^{40}K	^{137}Cs	Bqkg^{-1})	nGyh^{-1}	μSvy^{-1}	10^{-3}	
Turkey (Kars)	30.9	38.6	499.4	12.4	122.5	58.8	71.4	0.25	**This study**
Turkey(Ardahan)	29.9	36.7	435.1	15.5	115.0	56.3	69.0	0.23	**This study**
Turkey (Iğdır)	15.7	18.3	332.6	17.7	65.5	31.0	38.0	0.13	**This study**
India	19.1	48.5	1146.8					0.70	(36)
China	38.5	54.6	584.0			122.0	150.0		(37)
Nigeria	41.0	29.7	412.5			54.6	200.0		(1)
United States	40.0	35.0	370.0			47.0			(3)
Greece	25.0	21.0	360.0			56.0			(3)
Bulgaria	45.0	30.0	400.0			45.0			(3)
Palestine	41.4	19.5	113.3	2.8	77.6	35.3	40.0	1.02	(35)
Pakistan	31.2	44.1	575.0	15.0		89.0	164.0	0.54	(34)
Turkey (Kilis)	16.1	15.0	206.0	9.5		25.0	31.0		(38)
Turkey (Mersin)	27.1	34.3	370.5	18.6		51.0	62.0	0.22	(27)
Turkey (Rize)	85.7	51.0	771.5	236.3		110.6	136.0	0.48	(31)
Turkey (Edirne and Kırklareli)	19.6	34.1	566.1		108.1	53.3	65.4		(29)
Turkey (Adana)	17.6	21.1	297.5	6.8		67.0	82.0		(30)
Turkey (Yalova)	22.3	26.8	419.3	2.5		48.8	59.9	0.42	(28)
Turkey (Trabzon)	41.0	35.0	437.0	21.0		59.0	72.0		(32)
Turkey	34.7	35.4	450.0	11.6		54.6	70.0		(24)
Worldwide	35.0	30.0	400.0		370.0	60.0	70.0	0.29	(3)

6. Conclusion

The activity concentrations of ^{226}Ra, ^{232}Th, ^{40}K and $_{137}Cs$ radioisotopes and their associated radiation hazard levels were specified by gamma ray spectrometry in 428 soil samples taken from investigation areas in different locations of Kars, Ardahan and Iğdır provinces. The average concentrations of naturally occurring radioisotopes and ^{137}Cs in soil samples from the Northeastern Anatolia Region of Turkey were 25.5, 31.2, 422.4 and 15.2 Bq kg^{-1}, respectively. The average activity concentrations of ^{226}Ra, ^{232}Th and ^{40}K in the surface soil samples reported in this study are compatible with values reported by TAEA as 34.7, 35.4 and 450.0 Bq kg^{-1}, respectively, and also population-weighted world average values given by UNSCEAR (35.0, 30.0 and 400.0 Bq kg^{-1} for ^{226}Ra, ^{232}Th and ^{40}K, respectively). Based on these concentrations, the average values of radiological hazard indices such as the radium equivalent activity, outdoor absorbed dose rate, annual effective dose equivalent and lifetime cancer risk for adult person living in the region were calculated as 101.1 Bq kg^{-1}, 48.2 nGy h^{-1}, 59.1 μSv y^{-1} and $0.20x10^{-3}$, respectively. The results acquired showed that the radiological hazard indexes owing to the natural radioactivity of the soil samples were very similar to the advised world value and the values declared in the literature. Hence, it was concluded from this study that the radionuclide concentrations found in soil samples of the Northeastern Anatolian Region do not pose any potential health hazards to the general public. However, these data can provide a general background level for the studied area and can also serve as a guide for future measurement and evaluation of radionuclides in any radiological emergency.

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CHAPTER 9

DEEP VEIN THROMBOSIS AND OXIDATIVE DAMAGE

Ayse MALBORA[1]

[1] *(Assist. Prof.) Ege University Odemis Vocational School Laboratory and Veterinary Health Ödemiş / İzmir / Turkey, aysedogan2003@yahoo.com*
Orcid: 0000-0002-4235-3942

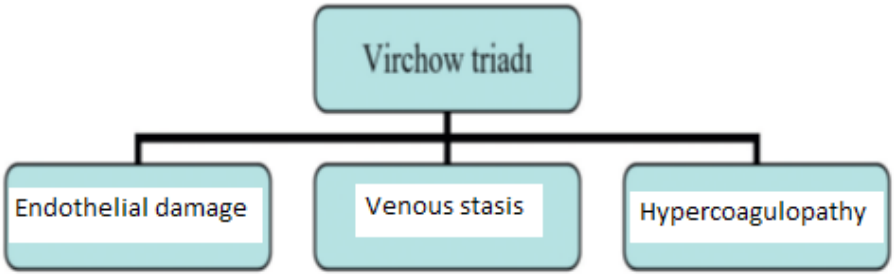
1. Introduction

Deep vein thrombosis (DVT) is a definition to describe the thrombosis which could appear in deep veins of upper and lower extremities also in cerebral and visceral veins or in vena cava. DVT can be asymptomatic and exhibit several degrees of clinical findings and due to reason for pulmonary embolism, it is an important disorder. It is part of thromoembolism together with pulmonary embolism that is the third frequent reason for cardiovascular disease- induced mortality after heart attack and stroke. Recurent thrombosis and post-thrombotic syndrome recurrent in DVT are most important causes of morbidity (2, 2). DVT is generally seen in deep veins of lower extremities and proximal veins of ilio-femoral segment. Rate of its incidence in deep veins of upper extremities, cerebral visceral veins and vena cava is about 10% (3). Lower extremity DVTs are classified as proximal and distal thromboses, which is important for treatment to be planned. Venous thromboses in posterior tibial, peroneal and anterior tibial veins of popliteal vein distal are called distal DVTs. However, venous thromboses in popliteal vein and its proximal superficial femoral, deep femoral, common femoral and external iliac veins are named proximal DVTs (3). It is important to know DVT etiology well in terms of prevention of morbity and mortality which it could lead to by providing related prophilaxis or performing its efficient treatment. Conduction of studies has consequently allowed numerous risk factors to be established (Table 1) (4), which indeed behave in a cumulative way thus leading to appearance of DVT (5).

Table 1. DVT related risk factors (4)

Acquired Risk Factors	**Hereditary Risk Factors**
Old age	Factor V Leiden mutation
Immobilization	Deficiency of Antithrombine III
Obesity	Protein C and S deficiency
Surgery	Prothrombin gene mutation (G2020210A)
Trauma	Dysfibrinogenemia
History of venous thrombosis	Hyperhomocysteinemia
Inflammatory diseases	Plasminogene and plasminogene activation disorder
Oral contraceptive use	
Hormon replacement treatment	
Cancer	
Varicose veins	
Antiphospholipid syndrome	
Nephrotic syndrome	
Central venous catheters	
Intravenous medication	

The main three pathogenetic mechanisms to cause DVT were defined by Rudolph Virchow in 1856, which is called the Virchow triad composed of 1- venous endothelial damage, 2- decrease in venous blood flow (venous stasis) and 3- hypercoagulopathy (Figure 1) (4).



Şekil 1. Virchow triadı (4)

Endothelial cells play an important role in provision of vascular homeostasis. Thanks to their surface structures and products which they secrete, they create anticoagulant, antithrombocytic and fibrinolythic effects. However, when a damage to cellular structure, expression of cellular adhesion molecules increase, anticagulant property disappears and their procoagulant aspects appear accounting for local coagulation (6).

Venous return normally occurs thanks to the fact that venous valves and calf muscles have contracted. Venous return fails due to long distance travels, immobility in post operative periods, tumors, pregnancy and obesity -associated venous compressions, congestive heart failure cardiac deficiency, acute myocardial infarction and left heart deficiency caused by cardiomyopathy.

When blood has long been in contact with vascular endothelium, coagulation factor inhibitors are prevented to be secreted followed by formation of thrombus in pouches caused by venous valves (venous valvular sinuses) where blood flow is the slowest and even returned by adenosine diphosphate secretion (7). DVT could develop as coagulation has been increased by hereditary factors such as antithrombin III deficiency, Factor V Leiden mutation, Protein C and S deficiency, prothrombin gene mutation and active protein C resistance and by acquired thrombophilic defects such as experienced DVT, pregnancy, use of oral contraceptives, malignancies, nephrotic syndrome and systemic lupus erythematosus.

Disturbance of the balance in biological systems between free radicals and anti oxidants which have an effect of sweepers against them is described as oxidative stress (Figure 2) (9). Types of reactive oxygen molecules with high reactivity are produced as the result of normal metabolism occuring in cellular organelles especially in mitocondrium or due to iscemia-reperfusion, aging, high oxygen pressure, radiation, inflammation and chemical agents (10-12).Oxidative stress is responsible for pathogenesis of various disorders such as diabetus mellitus, cardiovascular and neurologic diseases atherosclerosis and inflammatory disturbances (13-16).

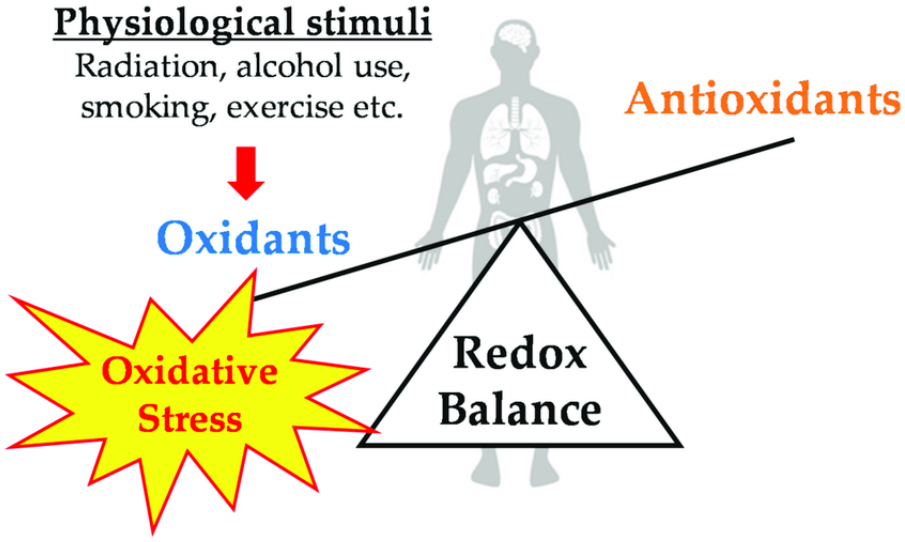


Figure 2. Oxidative balance

Oxygen in atmosphere is called molecular oxygen (O_2) or dioxgen. Little of normal oxygen is reduced to reactive oxygen species (ROS) during metabolism in the cellular compartments especially in mitachondria. Important reactive oxygen species are superoxyde radical ($O_2^{\cdot-}$), hydroxyle radical (OH•) and hydrogen peroxide (H_2O_2) ,the former two being free radicals and the latter prooxidant (17).

Biological systems have defense mechanisms which are called antioxidant systems or briefly antioxidants that are classified into endogenous (antioxidant enzymes etc) and exogenous (vitamins) antioxidants to prevent damages which reactive oxygen species (ROS) could cause in cellular structures (18).

Action mechanisms of antioxidants;

a) Repel oxygen from the medium or locally decrease oxygen concentration where it is present.
b) Repulse metal ions from where they are present.
c) Repel ROS from the medium or turn them into weaker molecules.
d) Prevent beginning of chain reactions likely to cause damage from free radicals.
e) Show effects to repair damages caused by free radicals.

Exogenous and endogenous antioxidant types/ species are exhibited in Tables 2 and 3 (19).

Table 2. Exogenous antioxidants (9)

Antioxidant	**Action Mechanism**
Ascorbic Acid	Hydroxyl radicals (OH•) removes
β-Carotene	Singlet oxygen with oil-soluble radicals cleans
Vitamin E	It is oil soluble and has a chain breaking effect.

Table 3. Endogenous Antioxidants (9)

Non enzymes	
Albumin	Binds Cu and Hem groups and removes HOCl from the medium binds Cu ions and enables reoxydation of Cu using H_2O_2
Seruloplasmin	Binds iron ions in ferric forms (Fe^{3+})
Transferrin	Binds iron ions in ferric forms (Fe^{3+}) at low pH values
Lactoferrin	Binds hemoglobin
Haptoglobin	Binds Hem group
Hemopexin	Clears peroxyl radicals
Bilirubin	Scavenges hydroxyl radicals (OH•)
Glucose	Clears radicals and binds metals
Urate	Clears hydroxyl radicals (OH•)
Melatonin	Clears hydroxyl radicals (OH•)
Mucus	

Those in enzymatic structure	Clears/removes superoxide radicals (O^{2-})
Superoxide dismutase (SOD)	$O_2- + 2H^+ \rightarrow H_2O_2 + O_2$
Catalase (CAT)	Removes Hydrogen peroxide from the medium if it is at high concentration $2H_2O_2 + O_2 \rightarrow 2H_2O + O_2$
Glutathione peroxidase (GPx)	Hydrogen removes from the medium if it is at low concentration $H_2O_2+2GSH \rightarrow GSSG+2H_2O$
Cytochrome oxidase	Prevents active oxygen from releasing into the medium during reduction of oxygen to water thus inhibiting formation of ROS (H_2O_2, OH•, O^{2-})

Increase of ROS for the reasons above or decrease in antioxidants due to pathological processes results in impairment of oxidative balance (20). The increase in amount of ROS consequently causes damage to cellular membranes, distortion in structures and functions of intracellular proteins and structural harm in DNA with inevitable injury to the cell.

Oxidative stress is a pathology which appears due to ROS caused by cellular metabolism and can be seen in all aerobic cells. Excessive proliferation of ROS for various reasons or decelaration in detoxifications based on deficiency of antioxidant systems lead to accumulation of such radicals and toxic effects on DNA with lipid and protein-structured molecules present in the cell. Oxidative damage occurs in lipid structures present in cellular membranes with resultant compounds in aldehydic structure such as 4- Hydroxynonenal (HNE) and hexenal ones, particularly MDA, the latter of which is a biochemical determinant used as the criterion of lipid damage to tissues. The effects of ROS on intracellular proteins cause protein carbonyl derivatives (PCO) to appear. Consequently, structural and functional losses emerge in protein-structured compounds (for instance enzymes, cellular skeletal proteins etc) as the final damage to cell. Protein carbonyl groups can be measured spectrophotometrically and preferred as the cellular criterion of oxidative damage since they have a longer live than aldehydes due to lipid damage. Free radicals caused by by oxidative stress lead to a modification similar to that of proteins in double bonds of bases on DNA, exhibit a wide variety of toxic efects followed by DNA protein cross bonds, breakage of chain and mutagenic effects and thus play an important role in pathogenesis of carcinogenesis (9).

Vein thromboembolism (VTE) is a complicated vascular disorder which could be accounted for by various etiologic factors (21). There are some elements such as flow of blood venous walls and and blood coagulant constituents/ compounds which are all included in and known as Virchow triad in terms of their influence on formation of thrombosis (22). Venous and arterial thromboses are different in nature of mechanism (23). DVT is the most frequently seen clinical form of vein thrombolism. A variety of etiologic factors play in DVT that is a very complex and the third most frequent major cardiovascular illness after ischemic heart disease and stroke (24). Incidence of DVT increases in proportionately to aging and similarly its related complications tend to rise based on older ages. DVT is known to cause mortality among lower income social groups of western nations (25).

Among risk factors of DVT are enviromental factors such as air pollution genetic factors and deficient and unbalanced way of living such as poor

diet/malnutrition, physical activity, smoking and alcohol consumption (29). Deficiency of essential elements as the result of unbalanced diet or malnutrition is effectively determinant. Copper (Cu), mangenese (Mn) and zink (Zn) are essential elements and important for protein to function in enzymes such as piruvate dicarboxylase and superoxide dysmutase (SOD) (30). On the other hand, lead (Pb), iron (Fe), chrome (Cr) and cobalt (Co) can be effective in proliferation of ROS in biological systems. Therefore, homeostasis of metal ions is effective in oxidative stress, which has effects on damage to DNA, lipid peroxidation and protein modification on damage to DNA, lipid peroxidation and protein modification. In this case, their influences on such diseases as various disorders, cancer, cardiovascular illnesses, diabetus mellitus, atherosclerosis, venous thromboembolism, neurologic diseases chronic inflamnation and others have been determined (31). A related study showed that DVT cases had a signigicant decrease in Mn level as compared to healthy individuals (32). Thus, Mn is an essential element in activation of SOD enzyme. The present study exhibited that SOD activity in DVT cases decreased as well as drops in Mn level as compared to healthy indivuduals (32). Lead (Pb) was determined to cause SOD and reduced glutathione (GSH) inhibition in erythrocytes (33). Atmospheric pollution causes exposure to Pb (exposure to Pb in atmospheric pollution) ,which leads to cardiovascular diseases such as inflammation, thrombosis, vascular dysfunction and atherosclerosis (34). Pulmonary embolism as a complication of DVT is a significant reason for DVT-associated mortality. Venous reflux in DVT - damaged veins is an important manifestation in DVT- related morbidity, causing pain, swelling and chronic skin ulcers as well in severe cases. Therefore, DVT has significantly contributed to present global disease load (25).

Anticoagulants is the most general modality of treatment used in DVT. However, these agents increase risk of hemorrhage since they prevent thrombus propagation. They increase dissolution of natural thrombus development. This requires their long term use, nevertheless (35). New treatment modalities for anticoagulation causes side effects even if they prevent thrombus formation or increase thrombus dissolution (36).

This compilation article involves various studies to discuss that anticoagulants could be regulated by ROS both in formation and dissolution of thrombosis. Intracellular ROS (superoxide radical, H_2O_2) have physiological functions which play a role as second messengers. Increase of intra and extra cellular ROS (oxidative stress) caused by pathological increase of ROS or weakening of antioxidant defense damages DNA lipids and proteins resulting in cell death (37). ROS are produced in mitochondria during aerobic respiration

(38). NADPH oxidase (NOX) enzymes has been found to be the most important source for ROS in the process of DVT. Depending on isotypes and type of cell, these membrane-based enzymes causes O_2 to interact with intra and extracellular compounds (39). Superoxide anion later turns into H_2O_2 spontaeously or with the influence of SOD and consequently H_2O_2 through fenton reaction leads to hydroxyl radical and then to oxidative damage (37). Various antioxidant enzymes and molecules such as SOD (40), catalase (CAT), glutathione peroxidase (GPx), heme oxidase (43), thioredoxine system (44) and glutathione (45) ,vitamins A-C-E (46) are proliferated to remove ROS and thus more limited damages appear (46). This redox mechanism is essential for normal physiological homeostasis but their imbalance could account for pathological consequences (46).

2. Vein thrombus Formation

Development of venous thrombosis; activation of endothelium, platelets and sterile inflammation (neutrophil, mast cells and monocytes) has influence on development of venous thrombosis which is stimulated by at least the two of the Virchow triad (1-decreased blood flow, 2-endothelial dysfunction and 3-hypercoagulation of blood). This process induces activation of coagulation phases by proliferating great amount of thrombins and transforming fibrinogen into fibrin (47).

Leukocytes (netrophils) are bound with endothelium in the sites where thrombus formation occurs, flow of blood is generally scarce and vein pouches and widened sinuses occur (48, 49). Activated neutrophils secrete neutrophilic extracellular traps (NETs) which increase coagulation by directly inducing platelet aggregation and erythrocyte reception fibrin accumulation (50) and also by endothelial activation. Mast cells on the vein walls contribute to venous thrombus formation(52). Modulation of some of these contributing factors may inhibit formation of thrombosis which depends on more activation than in the coagulation phases that involves DVT. In the regulation of various processes present in this phenomenon, ROS has been concluded to be an important regulator (52).

3. ROS and coagulation

ROS stimulate coagulation by increasing expression of tissue factors (TFs) in endothelial cells (53), monocytes (54) and vascular smooth muscles (55) and ROS-proliferated NOX enzymes are an important coagulation factor as well

(54, 56). Stimulation of protease-activated receptors (PARS) causes induction of endothelial tissue factors (TFs) by means of mitochondrial ROS signal (57). ROS can be an important procoagulant in oxidative modification of proteins in coagulation. Tissue Factor Inhibitor (TFPI) to physiologically regulate TFs could be inhibited by oxidative stress (58). ROS can directly inactivate important anticoagulant proteins which are protein C (PC) (59) and thrombomodulin (TM) ,PC's agonist. ROS appears in a prothrombic role by means of oxidated fibrinogen which later transforms into fibrin and interaction decreases between fibrin and protein C (PC), antithrombin III-heparine complex and thrombomodulin anticoagulants (60, 61). Heparin-binding capacity of antithrombine is reduced by oxidation caused by H_2O_2 (62) or lipid peroxide (63). Evidence has increased that platelets have an important role in formation of venous thrombosis (64) and changes in reaction of platelets have impacts on the risk of DVT formation (65). Platelet functions are regulated by ROS and disorders in this process could be responsible for negative results in the cases with risk of DVT development. ROS are effective in the expression of P- selectin associated with increased risk of venous thromboembolism (VTE) (66, 67). Expression of P-selectin and CD4OL (effective in platelet surface activation) is ROS-related. Platelet NOX 2,soluble P-selectin and soluble CD4OL plasma levels increase in obese indivuduals who have DVT risk increased by oxidative stress (68). Increase in soluble CD4OL can increase platelet activation and aggregation, platelet- leukocyte conjugation and ROS proliferation later (69). ROS can negatively effect endogenous mechanisms effective in platelet inhibition and for example remove NO which prevents platelet aggression synthesized by endothelial cells with the result that it can indirectly be effective in platelet activity (70). ROS was found to lead to decrease in activation of such antioxidants as CAT (71), N- acetylcysteine (72), polyphenols (73), vitamin C (73) and vitamin E (74) and increase in activation of ROS donors. In platelet- based thrombosis in mice, glutathione peroxidase-3 (GPx-3) antioxidant enzyme remains ineffective (75) but excessively produced GPx-1 antioxidant in mice protects them from platelet hyper activity (76).

Erythrocytes are basic cellular compounds in venous thrombosis. Although effects of erythrocytes on DVT are ignored as major active agents, some studies suggest that they have passive contributions by oxidative mechanism. Erythrocytes are rich in iron having O_2-carrying hemoglobin in their structures. In increased oxidative stress, oxidated Fe^{+2} including hemoglobin triggers Fe^{+3} secretion reaction chain, erythrocyte is thus lysed and oxidative stress and thrombus form in venous thrombosis sites (77). Hemoglobin simultaneously regulates expression of functional tissue factor (TF) in macrophages and makes

TF insensitive to effects of antioxidants (78). Therefore, erythrocytes passively shows actions by oxidative mechanism in the beginning of DVT formation. The process starts later and erythrocytes are involved in progressive thrombosis (78).

Mast cells are on the vein walls and become active in hypoxia (79). It is reported that number of mast cells increase in vein walls of cases of DVT as an indicative of profibrinolitic phenotype. It is generally admitted that mast cells proliferate ROS and are activated by them. Inhibition of activation of mast cells decreases proliferation of ROS. It was reported that mice increased sensitivity to DVT by means of H_2O_2 but were protected by excessive proliferation of GPx-1 (76). The most important source of ROS is NOX 2 and ROS activate redox-sensitive Ca^{++} pathways (83). In this process, Ca^{++}-ROS relationship has an important role in leukocytes and contribute to DVT formation/development. As for neutrophils, NOX 2 remains essential for the role of neutrophils in DVT. Activation of this enzyme in neutrophils is based on Ca^{++} signal pathway/channel/process (84). ROS in mast cells activate redox-sensitive Ca^{++} pathways/channels/processes (83).

A variety of factors increase risk of DVT development. The major factor in arterial and venous thromboses is endosomal redox signal which is an important pathophysiologic mechanism related to thrombosis formation (85). For example, in an auto immune disease, Behçet disorder, vein thrombosis is shaped and influence of oxidative damage on thrombosis has been found and its relationship with neutrophil activation in fibrinogen change and increased NOX-based ROS production relationship were determined (86). Factors such as air pollution, metabolic syndrome, chronic stress and age as well as Behcet disease are also effective in thrombosis due to oxidative stress mechanism (87).

4. Conclusion/Result

In conclusion, ROS are an important process in the development of thrombosis in which coagulation, platelet activation and inflammation are present. Disorder in redox control is important in increase of venous thrombosis risk.

Although numerous studies have been made concerning ROS and its mechanisms of action on cellular structures, the issue still remains on agenda since it has been correlated with pathogeneses of innumarable diseases. We therefore believe that further comprehensive studies would play an important role in explanation of clinics of many disorders in future.

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