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Investigation of Oxidative Stress, Lipid Peroxidation and DNA Damage in Muscle Tissue of Young Trout Treated with Arsenic

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ABSTRACT

Arsenic, metalloid and metal-based compounds commonly found in the earth's crust, dissolves through underground waters and causes toxic effects by spreading into industrial processes or the environment. In this study, modifies in the levels of antioxidant enzymes and some parameters (GSH-Px, CAT, MDA, SOD, and 8-OHdG) in the muscle tissues of juvenile trout (*Oncorhynchus mykiss*) after arsenic exposure were examined and the data obtained after the study were evaluated.

In our study, juvenile trout were divided into three groups and arsenic was applied to these three groups at saturation of 25, 50 and 75 mg/L. 96 hours after the application, the muscle tissues of the young trout were taken and homogenized. 8-OHdG levels in muscle tissue were determined using an ELISA kit. SOD, CAT, GSH-Px, and MDA levels were determined by spectrophotometric methods.

DNA damage was observed in juvenile trout with arsenic accumulation in muscle tissue, and accordingly, the 8-OHdG concentration level increased. Deterioration occurred in the membrane structure of the cell and lipid peroxidation occurred. When the muscle tissue of young trout treated with arsenic was examined, a significant decrease was observed in the concentrations of CAT, GSH-Px and SOD. It is thought that this situation is caused by oxidative stress caused by free radicals accumulating in the muscle tissue of fish. These changes were caused after the application of arsenic. The presence of heavy metals in large amounts in the living spaces of aquatic creatures negatively affects the life stage and developmental stages of the living creatures, and the presence of these creatures in the food chain and the use of these creatures as food by other species can cause significant toxicity in living creatures.

***Keywords**: Arsenic, MDA, 8-OHdG, Antioxidants, Oxidative stress, Juvenile Rainbow Trout*

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INTRODUCTION

Arsenic, metalloid and metal-based compounds commonly found in the earth's crust, dissolve through underground waters and cause toxic effects by spreading into industrial processes or the environment [1,2] Although it is used as raw material in agriculture, pharmaceutical and other industrial sectors, it has toxic effects on different organisms, including humans. Arsenic is found in water, especially after groundwater has dissolved certain compounds and minerals (for example, after arsenic has passed through soil and rocks) [3,4].

Toxicity due to heavy metals in living things is generally related to the formation of reactive oxygen species (ROS). After experiencing toxicity, the amount of ROS increases, leading to oxidative stress. Oxidative stress causes changes in the electron transport system and cellular damage in tissues and organs [5]. Hydroxyl radicals and hydrogen peroxides are among the free oxygen radicals with the highest activity in ROS. In order to minimize the destructive effects of ROS produced as a result of various effects, living organisms have developed an antioxidant self-defense system with two different classes. These systems are enzymatic antioxidant systems that contain enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione reductase (GR) and catalase (CAT); and non-enzyme antioxidants such as glutathione and thioredoxin [6]. CAT, SOD and GSH-Px are the most important antioxidant defense systems in living cells. And these enzymes always fight against free radicals and oxidative stress [7].

Low amounts of ROS do not cause destruction, but high amounts of ROS cause negative effects on intracellular components such as proteins, lipids and DNA, as said before. In particular, ROS can trigger lipid peroxidation (LPO) of polyunsaturated fatty acids, resulting in disruption of the cell membrane and changes in membrane structure and permeability. [8]. In addition to excessive production of reactive oxygen species, lipid peroxidation is also observed in aquatic creatures exposed to heavy metals. The increase in the amount of malondialdehyde (MDA) among the degradation products of polyunsaturated fatty acids is an indicator of the occurrence of oxidative stress. One of the most important indicators of lipid peroxidation is MDA. As a result of all these effects, free radicals cause lipid peroxidation and serious damage to the membrane with an autocatalytic effect [9].

The presence of intracellular reactive oxygen species affects more than twenty species. Among these damaged bases, 8-hydroxy-2'-deoxyguanosine (8-OHdG) is the most investigated base. An increase in the concentration of 8-hydroxy-2'-deoxyguanosine is an indicator of DNA damage [10]. It is known that intracellular oxidative stress disrupts base and sugar modifications on DNA through different mechanisms, causes single and double strand breaks, causes many problems such as abasic regions, DNA-protein cross-linking, and causes various damages. DNA damage that occurs in the cell for various reasons is one of the most common destructive events throughout the life of the cell. DNA damage can lead to mutation, cancer, aging and, as a result, cell death in living organisms. DNA is subjected to many changes by cellular metabolites (ROS) and exogenous agents throughout its life. These changes in DNA can lead to cellular death in single-celled organisms. It can also lead to wear and tear and aging in multicellular organisms [11].

MATERIALS AND METHODS

Materials

Juvenile trout (*Oncorhynchus mykiss*) were purchased from trout production facilities operating in Van-Çatak district. 60 juvenile trout (80-100 g) taken from this facility were distributed into four fiberglass aquariums (300 L) with an equal number of fish in each aquarium. The fish were waited for a week to adapt to the environment. Young trout were fed twice a day, in the morning and evening, using commercial feed. Fish were kept in tanks at a temperature of 18.0 ± 1 ℃, ventilated with an air stone.

Fish in one of the aquariums were used as the control group, and fish in the other three aquariums were used as the As concentration application groups. Arsenic-containing $NaAsO_2$ concentrations of 25, 50 and 75 mg/L were added to the aquariums to be treated with arsenic, respectively, and the concentrations were kept unchanged for 96 hours. Lethal dose determination according to the work of Buhl and Hamilton [12]. 96 hours after arsenic application, the fish were anesthetized with MS222 (0.1 g/L) and their muscle tissues were separated. The separated muscle tissues were homogenized and frozen at -83 ℃ for analysis.

Method

Preparation of tissue homogenate

The muscle tissues of the fish are weighed, homogenate buffer solution prepared with EDTA and KH_2PO_4 is added and broken down with a homogenizer. This resulting mixture was centrifuged and placed in Eppendorf tubes and used in analyses.

Determination of the concentrations of the mentioned antioxidant enzymes and apoptosis markers

SOD and GSH-Px levels in homogenized muscle tissues of young trout were determined by UV kit, and CAT and MDA levels were determined by using spectrophotometric methods. Randox-Ransel enzyme kit was used to detect SOD and GSH-Px levels and absorbance values were measured on the spectrophotometer device. To determine SOD enzyme activity, xanthine and xanthine oxidase were used together, and the solution took on a red appearance with the superoxide radical 2-(-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) formed at the end of the reaction. The apparent absorbance value of the resulting red solution was measured on the spectrophotometer at 505 nm. To determine the activity of the GSH-Px enzyme, the reagents were pipetted into the cuvette and the absorbance values of the samples at 340 nm wavelength were measured and recorded on a spectrophotometer with the Randox-Ransel enzyme kit.
8-OHdG level was measured using an ELISA kit specially produced for fish. The solutions required for analysis and standard solutions were added to each well of the plates in the ELISA kit, and the numerical data obtained as a result of the analysis were read in a microplate reader at a wavelength of 450 nm and calculations were made.

Statistical analyses

SPSS v25 package program was used to interpret the results accurately. Significance levels were determined at the .05 level. The multiple comparison test that Duncan used was performed for applications that appeared to be significantly biased.

RESULTS

When the data obtained is examined, it is seen that significant changes occur in the levels of antioxidant enzymes in the muscle tissues of juvenile rainbow trout applied with a certain concentration of Arsenic. Statistically, it was observed that there was a significant decrease in the levels of SOD, CAT and GSH-Px in the muscle tissue of juvenile trout. A statistically significant increase was observed in MDA and 8-OHdG levels due to oxidative stress in the muscle tissues of fish treated with arsenic (Figure1-2).

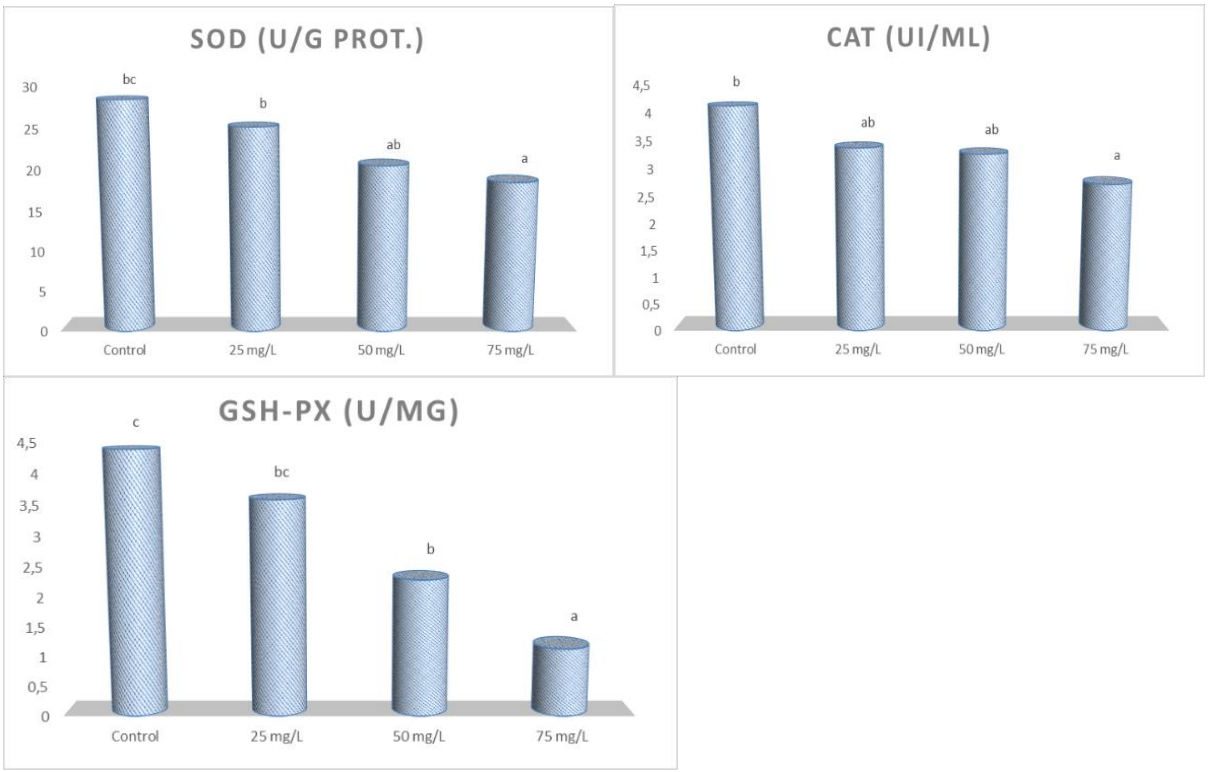

Figure 1. Changes in CAT, SOD and GSH-Px activity levels in rainbow trout muscle tissue.

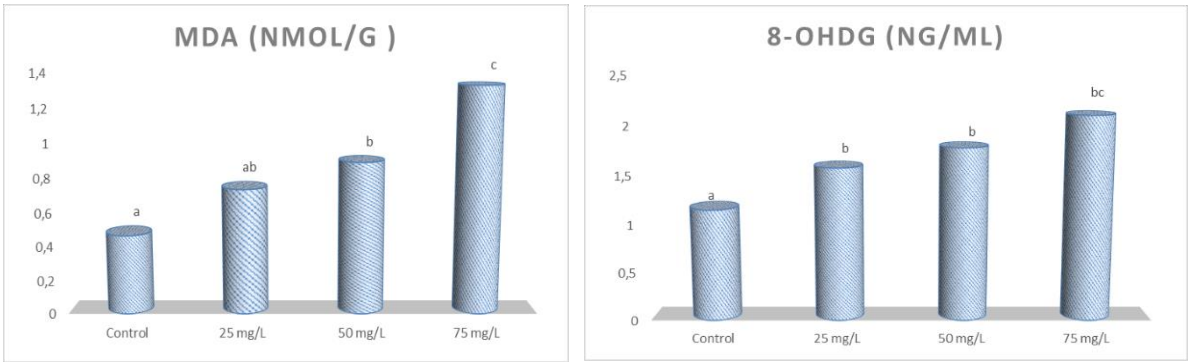

Figure 2. Changes in MDA and 8-OHdG levels in rainbow trout muscle tissue.

DISCUSSION

In our study, significant changes in the levels of antioxidant enzymes and some parameters (MDA, CAT, SOD, GSH-Px and 8-OHdG) in the muscle tissues of juvenile trout (*Oncorhynchus mykiss*) treated with arsenic were examined and the findings were investigated. The data obtained after the study were evaluated.

It is known that reactive oxygen species are formed as a result of heavy metal exposure in aquatic creatures, and oxidative stress occurs within the cell as a result of the formation of reactive oxygen species in the organism. It has been determined that the effectiveness of some antioxidant enzymes increases or, on the contrary, decreases, in order to completely eliminate or reduce ROS-induced oxidative stress occurring within the cell [13].

Min Li et al. In Channa argus fish exposed to lead (Pb), SOD, CAT and GSH-Px levels were examined in the liver, kidney and gill tissues of the fish as a result of lead exposure. As a result of exposure, a significant decrease was observed in SOD, CAT and GSH-Px levels in the tissues. It has been observed that intracellular oxidative stress increases with the accumulation of lead in tissues [14]. In our arsenic study, a decrease was observed in the levels of SOD, CAT and GSH-Px in the muscle tissue of juvenile trout. This decrease is parallel to the results of the above study.

In a study conducted by Abdal-Gawad et al. on Nile Tilapia, the oxidative status of the fish after exposure to copper (Cu) and Zinc (Zn) was examined, and after the examination, it was observed that there was a decrease in the levels of SOD, CAT, GST and GSH-Px in the liver tissues of the fish exposed to the metal [15]. The changes in the parameters in our study were found to be statistically significant compared to this study.

Wagh et al. In [16], zebra fish were used and the fish were exposed to certain levels of Lead (Pb), Nickel (Ni) and Cadmium (Cd). After the application, the gill and liver tissues of the fish were examined and after the examination, a significant decrease in SOD, CAT and GSH-Px levels and a significant increase in MDA levels were observed in the tissues. The changes in trout muscle tissue parameters in our study are similar to the results in this study.

In a study by Wang et al., the liver tissues of Nile Tilapia fish exposed to copper (Cu) and zinc (Zn) were examined after the application. As a result of examination of the tissues, it was observed that there was a significant decrease in SOD, CAT and GSH-Px levels and a noticeable increase in MDA levels (17). The results of this study are similar to the results of the study in which we applied arsenic. In a study conducted by Iyorah et al., kidney and gill tissues of African Catfish living in the Warri River exposed to heavy metal pollution (Pb, Cd, Mn, Ni, Cu) were examined and MDA levels were examined. It was observed that there was a significantly higher increase in the specified tissues of fish exposed to heavy metal pollution compared to the control group [18]. In our application, a similar increase was observed in MDA levels in the muscle tissues of trout.

In a study conducted in rainbow trout in 2023, the oxidative stress status and lipid peroxidation of fish exposed to heavy metals were examined, and it was found that MDA levels in samples taken from the gonads of trout exposed to heavy metals increased significantly compared to the control groups [19]. In our study, the increase in MDA levels in the muscle tissues of juvenile trout was found to be statistically significant.

In a study, 4 fish species (Argus fish, Striped Eel, Giant Catfish and Mullet) were taken from Pattani Bay in Thailand, which is known to have heavy metal pollution, and the muscle and liver tissues of these fish were examined. After the examination, it was observed

that the MDA rates in the specified tissues were at a significantly higher level than in the fish in the control group [20]. In our study where we applied arsenic, MDA levels in the muscle tissues of fish are parallel to the results of this study.

In a study by Ifenkwe et al., albino rats were used and these rats were divided into two groups for application. While the first group of rats were fed normally, certain amounts of manganese (Mn), Lead (Pb) and Cadmium (Cd) were added to the feed of the second group of rats. Blood samples were taken from the second group of rats and examined, and it was seen that the 8-OHdG levels in the blood were higher than the control group [21]. In a study conducted with goldfish in 2023, the fish were exposed to certain concentrations of Lithium (Li) and the muscle and gill tissues of the fish were examined after exposure. After the examination, it was observed that MDA and 8-OHdG levels increased in the tissues [22]. In our study of arsenic application, a similar increase in muscle tissues was observed after application. It gave similar results to the study above. In a study conducted on zebrafish, certain concentrations of antimony (Sb) were applied to the fish every two days, and the liver of zebrafish was examined after the application. After examination, it was observed that 8-OHdG levels increased in the liver tissues of zebrafish. In our study, 8-OHdG levels increased similarly in the muscle tissue of fish. This increase was found to be statistically significant.

CONCLUSION

When we look at the results of our arsenic application study; It has been observed that due to the presence of a heavy metal such as arsenic in high concentrations in the regions where aquatic creatures live, and especially as a result of long-term exposure of aquatic creatures to this heavy metal, excessive arsenic accumulates in the tissues of the living creature and oxidative stress occurs in the cells of the creature due to the effect of reactive oxygen species formed as a result of arsenic accumulation. Free radicals, which are shown to be the main cause of oxidative stress occurring in the cells of the muscle tissue of young trout, accumulate in large amounts in the tissues of fish, and in order to reduce or completely eliminate the oxidative stress occurring within the cells, the intracellular levels of some of the antioxidant enzymes, cytokines and antioxidant markers increase, resulting in lipid peroxidation. It was understood that DNA damage occurred in the tissues as a result.

The presence of heavy metals in large amounts in the living spaces of aquatic creatures negatively affects the life stage and developmental stages of the living creatures, and the presence of these creatures in the food chain and the use of these creatures as food by other species can cause significant toxicity in living creatures.

In order to eliminate the situation caused by the presence of a heavy metal such as arsenic in aquatic organisms and to prevent heavy metals from entering the food chain, all fish farming enterprises must periodically check water quality and water health and take precautions. and the amount of heavy metals in water must be eliminated in a controlled manner or harmful heavy metals in water must be completely eliminated.

This study can be used as a good resource in other scientific studies on the toxicity of a heavy metal such as arsenic in aquatic organisms and nature.

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