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Investigation of oxidative stress, lipid peroxidation and DNA damage in the muscle tissue of young trout treated with arsenic

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ABSTRACT

Arsenic, a metalloid and metal compound commonly found in the earth's crust, dissolves in underground waters and can cause toxic effects by spreading through industrial processes or the environment. In this study, the changes in levels of antioxidant enzymes and several parameters (GSH-Px, CAT, MDA, SOD, and 8-OHdG) in the muscle tissue of juvenile trout (Oncorhynchus mykiss) after arsenic exposure were investigated, and the data obtained were analysed.

In our study, juvenile trout were divided into three groups, and arsenic was administered at saturations of 25, 50, and 75 mg/L. 96 hours after application, the muscle tissue from young trout was removed and homogenised. The 8-OHdG content in the muscle tissue was determined using an ELISA kit. The levels of SOD, CAT, GSH-Px and MDA were determined using spectrophotometric methods.

In young trout with arsenic accumulation in muscle tissue, DNA damage was observed, and the 8-OHdG content increased accordingly. There was deterioration in the cell membrane structure and lipid peroxidation. When the muscle tissue of young trout treated with arsenic was examined, a significant decrease in the concentrations of CAT, GSH-Px and SOD was observed. It is assumed that this is due to oxidative stress caused by the accumulation of free radicals in the muscle tissue of the fish. These changes occurred after arsenic was applied. The presence of heavy metals in large quantities in the habitats of aquatic organisms negatively affects their life and developmental stages, and their presence in the food chain and use as food by other species can cause significant toxicity.

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

Introduction

Arsenic, a metalloid and metal-based compound commonly found in the earth's crust, dissolves in underground waters and can cause toxic effects when it enters industrial processes or the environment (Hughes, 2002; Cilingir Yeltekin, 2018). Although it is used as a raw material in agriculture, pharmaceuticals and other industries, it has toxic effects on various organisms, including humans. Arsenic is found in water, especially after groundwater dissolves certain compounds and minerals (e.g., after arsenic has passed through soil and rock) (Baskan & Pala, 2009).

The toxicity of heavy metals to living organisms is generally associated with the formation of reactive oxygen species (ROS). Upon exposure to a toxicant, ROS levels increase, leading to oxidative stress. Oxidative stress alters the electron transport system and causes cellular damage in tissues and organs (Méndez-Armenta et al., 2011). Hydroxyl radicals and hydrogen peroxide are among the free oxygen radicals with the highest activity in ROS. To minimise the destructive effects of ROS arising from various influences, living organisms have developed an antioxidant self-defence system with two classes. These systems are enzymatic antioxidant systems containing enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione reductase (GR), and catalase (CAT), as well as non-enzymatic antioxidants such as glutathione and thioredoxin (Özdek et al., 2020). CAT, SOD and GSH-Px are the most important antioxidant defence systems in living cells. Moreover, these enzymes are always fighting against free radicals and oxidative stress (Hematyar et al., 2019).

Low levels of ROS do not cause destruction, but high levels of ROS have adverse effects on intracellular components such as proteins, lipids and DNA. In particular, ROS can trigger lipid peroxidation (LPO) of polyunsaturated fatty acids, leading to cell membrane disruption and changes in membrane structure and permeability (Doğan et al., 2022). In addition to the excessive production of reactive oxygen species, lipid peroxidation is also observed in aquatic organisms exposed to heavy metals. An increase in malondialdehyde (MDA), a degradation product of polyunsaturated fatty acids, is an indicator of oxidative stress. One of the most important indicators of lipid peroxidation is MDA. As a result of these effects, free radicals cause lipid peroxidation and severe membrane damage through an autocatalytic effect (Atmaca & Aksoy, 2009).

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 studied base. An increase in the concentration of 8-hydroxy-2'-deoxyguanosine is an indicator of DNA damage (Özcan et al., 2015). It is known that intracellular oxidative stress disrupts base and sugar modifications on DNA through various mechanisms, induces single- and double-strand breaks, creates abasic sites and DNA-protein crosslinks, and causes other forms of damage. DNA damage, which occurs in the cell for various reasons, is one of the most common destructive events in the life of a cell. DNA damage in living organisms can lead to mutations, cancer, ageing and, as a result, cell death. In the course of its life, DNA is exposed to numerous changes caused by cellular metabolites (ROS) and exogenous substances. These changes in DNA can lead to cell death in unicellular organisms. They can also lead to wear and tear and ageing in multicellular organisms (Sancar et al., 2004).

Studies show that the amount of arsenic in water is increasing daily. This rising arsenic level poses a threat to all living things (Berg et al., 2001; Fernández et al., 2012). This rising arsenic level threatens all living things. The doses for our study were determined based on the arsenic concentrations found in these studies. The aim of this study is therefore to investigate the effects of increasing arsenic concentrations in water on the metabolism of living organisms.

Materials and Methods

Fish

Juvenile trout (*Oncorhynchus mykiss*) were purchased from trout farms in the Van-Çatak district. 60 juvenile trout (80-100 g) from this facility were distributed among 4 fibreglass aquaria (300 L), each containing the same number of fish. The fish were maintained for one week to acclimatise to the environment. The young trout were fed twice a day, morning and evening, with commercial food. The fish were kept in tanks at 18.0±1°C, which were aerated with an air stone.

The fish in one of the tanks served as the control group, and the fish in the other three tanks served as the groups treated with the As concentration. Arsenic-containing $NaAsO_2$ (Sigma-Aldrich, purity 90-95%, CAS number: 7784-46-5, Germany) was added to the aquaria to be treated with arsenic at concentrations of 25, 50, and 75 mg/L, respectively, and the concentrations were maintained for 96 hours. Determination of the lethal dose according to the work of Buhl and Hamilton (Yao et al., 2023). 96 hours after arsenic application, the fish were anaesthetised with MS222 (0.1 g/L) and their muscle tissue was detached. The detached muscle tissue was homogenised and frozen at -83 °C for analysis.

Preparation of Tissue Homogenate

The fish muscle tissue is weighed, a homogenization buffer containing EDTA and KH2PO4 is added, and the mixture is homogenised using a homogeniser. The resulting mixture was centrifuged, transferred to Eppendorf tubes, and used for the analyses.

Determination of the Concentrations of the Mentioned Antioxidant Enzymes and Apoptosis Markers

The SOD and GSH-Px content in homogenised muscle tissue of young trout was determined using a UV kit, and the CAT and MDA content was determined using spectrophotometric methods. The Randox-Ransel enzyme kit (SD 125, LOT 343177, United Kingdom BT294QY, RS 505, LOT 430430, United Kingdom BT294QY) was used to detect SOD and GSH-Px, and the absorbance values were measured using the spectrophotometer. Xanthine and xanthine oxidase were used together to determine the SOD enzyme activity, and the solution took on a red appearance, forming the superoxide radical 2-(-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) 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 enzyme GSH-Px, the reagents were pipetted into the cuvette, and the absorbance of the samples was measured at 340 nm on a spectrophotometer using the Randox-Ransel enzyme kit (Yeltekin, 2024).

The 8-OHdG content was measured using an ELISA kit specifically manufactured for fish (BT LAB, Cat. No. E0084FI, LOT 202403002, China). The solutions and standard solutions required for the analysis were added to each well of the ELISA kit plates, and the numerical data obtained were read and calculated in a microplate reader at 450 nm (Yeltekin, 2022).

Statistical Analyses

The SPSS v25 package was used to precisely interpret the results. The significance levels were determined at the .05 level. Duncan's multiple-comparison test was applied to data that appeared to be significantly skewed.

Results and Discussion

The study of the data obtained shows that the levels of antioxidant enzymes in the muscle tissue of young rainbow trout treated with a specific arsenic concentration change significantly. Statistically, a significant decrease in the levels of SOD, CAT and GSH-Px was observed in the muscle tissue of juvenile trout. There was a statistically significant increase in MDA and 8-OHdG levels due to oxidative stress in the muscle tissue of the arsenic-treated fish (Figure 1-2).

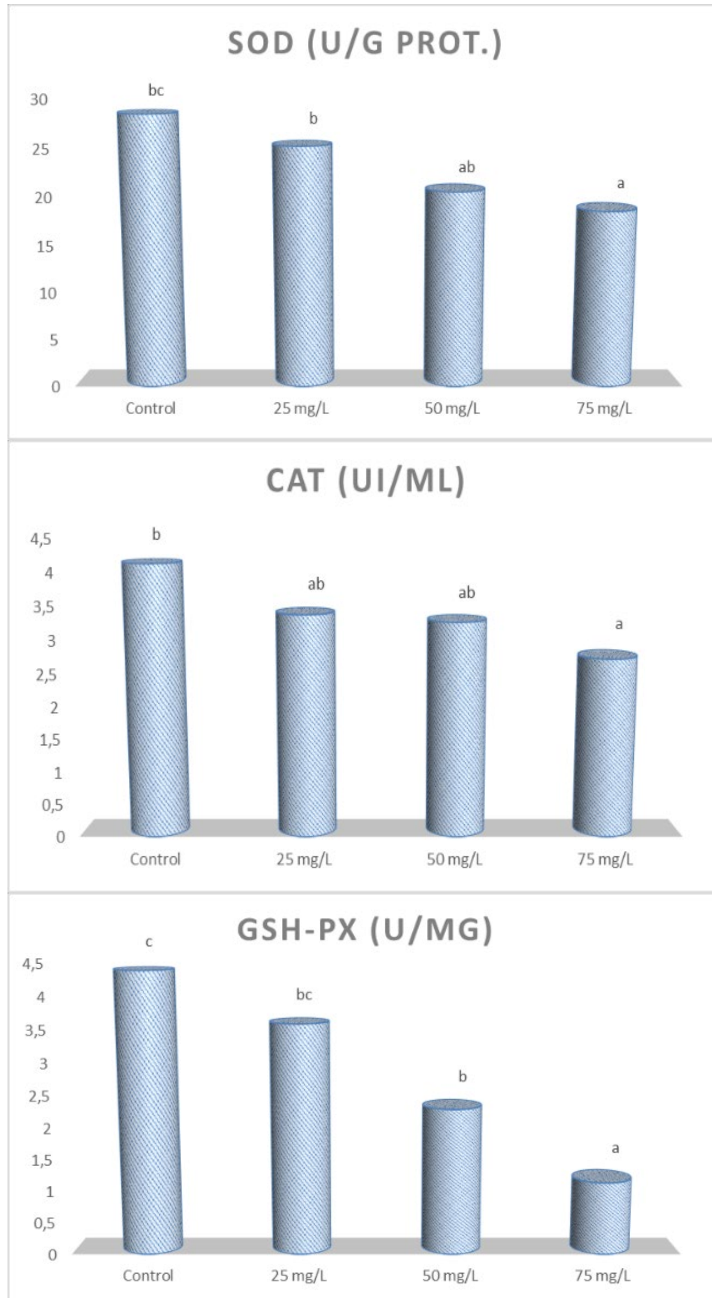

Figure 1. Changes in CAT, SOD and GSH-Px activity levels in rainbow trout muscle tissue. The letters a, b, c, and d indicate a significant difference between the groups ($p<0.05$)

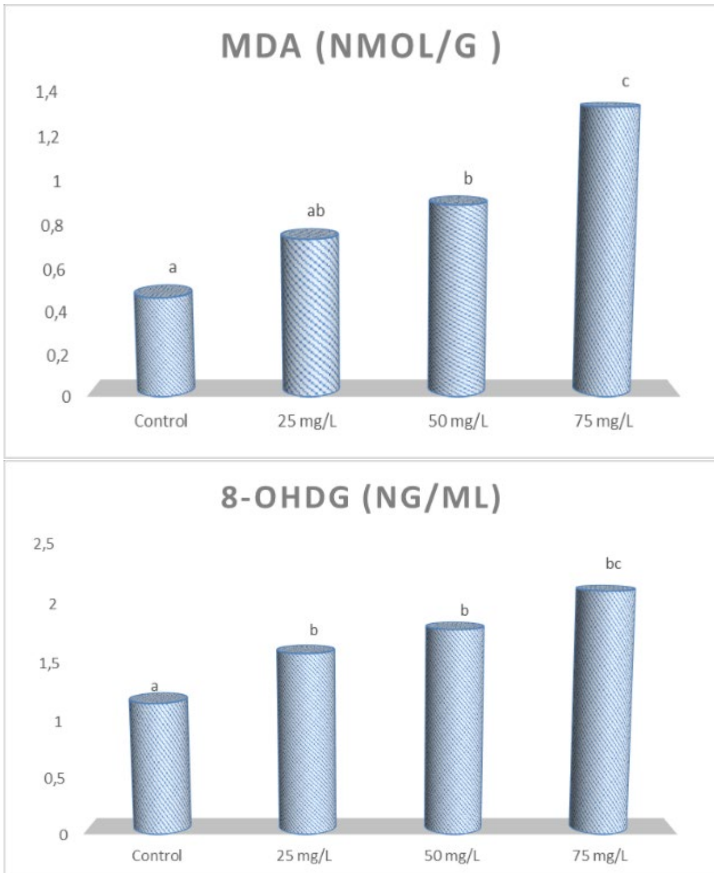

Figure 2. Changes in MDA and 8-OHdG levels in rainbow trout muscle tissue. The letters a, b, c, and d indicate a significant difference between the groups ($p<0.05$)

In our study, significant changes in the levels of antioxidant enzymes and several parameters (MDA, CAT, SOD, GSH-Px, and 8-OHdG) were examined in the muscle tissue of arsenic-treated juvenile trout (Oncorhynchus mykiss), and the results were verified. The data obtained after the study were analysed.

It is known that reactive oxygen species are produced in aquatic organisms following heavy metal exposure and that oxidative stress occurs within cells as a result. It has been found that the effectiveness of some antioxidant enzymes increases or decreases, depending on whether they eliminate or reduce ROS-induced oxidative stress in the cell (Lee et al., 2023). An arsenic study was conducted on carp (Cyprinus carpio). The study found that these fish had significantly increased levels of oxidative stress, which disturbs the charge balance in the electron transport system and causes disorders (Lu et al., 2024; Saç, Yeltekin, 2023). The results of the present study support this finding.

Li et al. In *Channa argus* fish exposed to lead (Pb), the SOD, CAT and GSH-Px levels in the liver, kidney and gill tissues of the fish were examined as a result of lead exposure. Following exposure, significant decreases in SOD, CAT, and GSH-Px levels were observed in the tissues. Intracellular oxidative stress increased with lead accumulation in tissues (Li et al., 2021). In our arsenic study, a decrease in SOD, CAT and GSH-Px concentrations was observed in the muscle tissue of the young trout. This decrease parallels the results of the study mentioned above.

In a study conducted by Abdal-Gawad et al. on Nile tilapia, the oxidative status of the fish was examined after exposure to copper (Cu) and zinc (Zn). After the study, a decrease in the levels of SOD, CAT, GST and GSH-Px was observed in the liver tissue of the fish exposed to the metal (Abdel-Gawad et al., 2020). The changes in the parameters in our study were found to be statistically significant compared with those in this study. In addition, an arsenic study conducted in zebrafish showed that arsenic exposure had significant adverse effects on fish development. Arsenic toxicity during development was even found to affect the sex of the fish (Rachamalla et al., 2024).

Wagh et al. (2023). In zebrafish, the fish were exposed to specific concentrations of lead (Pb), nickel (Ni) and cadmium (Cd). After the application, the gill and liver tissues of the fish were examined, and significant decreases in SOD, CAT, and GSH-Px levels and significant increases in MDA levels were observed. The changes in trout muscle tissue parameters in our study are comparable to those reported in this study.

In a study by Wang et al., liver tissue from Nile tilapia exposed to copper (Cu) and zinc (Zn) was examined after application. Examination of the tissue revealed significant decreases in SOD, CAT, and GSH-Px levels and a marked increase in MDA levels (Wang et al., 2020). The results of this study are similar to the results of the study in which we administered arsenic. In a study conducted by Iyorah et al., kidney and gill tissues of African catfish living in the Warri River and exposed to heavy metal pollution (Pb, Cd, Mn, Ni, Cu) were examined, and MDA levels were measured. It was found that the tissues of fish exposed to heavy metal pollution showed a significantly greater increase than those of the control group (Iyorah et al., 2023). In our application, a similar increase in MDA levels was observed in trout muscle tissue.

In a 2023 study on rainbow trout, oxidative stress and lipid peroxidation were investigated in fish exposed to heavy metals, and MDA levels increased significantly in gonadal samples from trout exposed to heavy metals compared with control groups (Bhat et al., 2023). In our study, the increase in

MDA levels was statistically significant in the muscle tissue of juvenile trout.

In one study, four fish species (argus fish, striped eel, giant catfish, and mullet) were sampled from Pattani Bay in Thailand, which is known to be contaminated with heavy metals, and their muscle and liver tissue were analysed. After examination, it was found that the MDA levels in these tissues were significantly higher than those in the control group (Aggarwal, 2004). In our study, in which we administered arsenic, the MDA levels in the fish's muscle tissues were parallel to the results of this study. In another study involving about 1600 fish, it was found that arsenic concentration increased steadily and had a severe adverse effect on the organisms (Lau et al., 2025).

In a 2023 study with goldfish, the fish were exposed to various concentrations of lithium (Li), and the muscle and gill tissue were examined after exposure. After the study, it was found that MDA and 8-OHdG levels increased in the tissues (El-Tekreti & Yeltekin, 2023). In another arsenic study, oxidative stress was found to increase at very high levels (Saç & Yeltekin, 2023). In our study on arsenic application, a similar increase in muscle tissue was observed after application. It showed results similar to those of the above study. In a study conducted on zebrafish, specific concentrations of antimony (Sb) were administered to the fish every two days and the livers of the zebrafish were examined after application. After the examination, it was found that the 8-OHdG content in the zebrafish liver tissue increased. In our study, 8-OHdG levels increased similarly in the muscle tissue of the fish. This increase was found to be statistically significant.

Conclusion

If we look at the results of our study on arsenic application: It has been observed that due to the presence of a heavy metal such as arsenic in high concentrations in the regions where aquatic organisms live, and especially as a result of long-term exposure of aquatic organisms to this heavy metal, excessive arsenic accumulates in the tissues of the organism and oxidative stress occurs in the cells of the organism due to the action of reactive oxygen species formed as a result of arsenic accumulation. Free radicals, which are the leading cause of oxidative stress in the cells of muscle tissue of young trout, accumulate in large quantities in the tissues of the fish, and in order to reduce or eliminate oxidative stress in the cells, the intracellular levels of some antioxidant enzymes, cytokines and antioxidant markers increase, leading to lipid peroxidation. This has been hypothesised to lead to DNA damage in tissues. The presence of heavy metals in large quantities in the habitats of aquatic organisms negatively affects their life and developmental stages, and their accumulation in the food chain and use as food by other species can cause significant toxicity.

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

This study can serve as a valuable resource for other scientific studies on the toxicity of heavy metals, such as arsenic, in aquatic organisms and the environment.

Compliance with Ethical Standards

Conflict of interest: The author declares no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: The study was approved in accordance with the decision of the Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee dated 29/04/2021, numbered 2021/04-19.

Data availability: The data will be made available upon request from the author.

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Disclosure: -

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