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Effect of Mercury Chloride on Both Nitrosative and Oxidative Stress in the Gill Tissue of Rainbow Trout (*Oncorhynchus Mykiss*).

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ABSTRACT

The aim of the study was to determine the toxic effect and the dynamics of oxidative stress caused by mercury chloride ($HgCl_2$) in rainbow trout (*Oncorhynchus mykiss*) weighing 59.43±7.21 g.For this purpose, a total of 40 fish in 4 groups, 10 fish in each group (n=10), were exposed to 25% and 50% (137.75 µg L and 275 µg L) of the LD_{50} for 2 and 7 days. To determine the oxidative/nitrosative stress, Peroxynitrite ($ONOO^-$ mmol L), Total Oxidant Level (TOS mmol H_2O_2 Eq L), Total Antioxidant Level (TAS mmol Trolox Eq L), OSI (TOS/TASx10) and Malondialdehyde (MDA) level were determined. While the difference between the groups in terms of TAS, OSI and MDA levels in gill tissues was statistically significant ($P<0.05$), this difference was statistically insignificant ($P>0.05$) in terms of $ONOO^-$ and TOS values. As a result, $HgCl_2$ was found to cause stress and toxic to fish as it increases the levels of $ONOO^-$, OSI and MDA, which are indicators of stress in gill tissue.

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Gökkuşağı Alabalığı (*Oncorhynchus Mykiss*) Solungaç Dokusunda Civa Klorür'ün Hem Nitrozatif Hem de Oksidatif Stres Üzerindeki Etkisi

ÖZET

Çalışmanın amacı, civa klorürün ($HgCl_2$) 59,43±7,21 g ağırlığındaki gökkuşağı alabalıklarında (*Oncorhynchus mykiss*) toksik etkisinin ve neden olduğu oksidatif stresin dinamiklerini belirlemektir. Bu amaçla, her grupta 10 balık (n=10) olacak şekilde 4 grupta toplam 40 balık, LD_{50}'nin %25 ve %50'sine (137.75 µg L ve 275 µg L) 2 ve 7 gün süreyle maruz bırakıldı. Oksidatif/nitrozatif stresi belirlemek için, Peroksinitrit ($ONOO^-$ mmol L), Toplam Oksidan Seviyesi (TOS mmol H_2O_2 Eq L), Toplam Antioksidan Seviyesi (TAS mmol Trolox Eq L), OSI (TOS/TASx10) ve Malondialdehit (MDA) seviyesi belirlendi. Solungaç dokularında TAS, OSI ve MDA düzeyleri açısından gruplar arasındaki fark istatistiksel olarak anlamlı iken ($P<0.05$), bu fark $ONOO^-$ ve TOS değerleri açısından istatistiksel olarak önemsiz çıktı ($P>0.05$). Sonuç olarak, $HgCl_2$ solungaç dokusunda stres göstergeleri olan $ONOO^-$, OSI ve MDA seviyelerini arttırdığı için strese neden olduğu ve balıklar için toksik olduğu görüldü.

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INTRODUCTION

Mercury (Hg) is found in nature in the form of an elemental form, inorganic (iHg) and organic Hg (MeHg). It is released into the environment from natural and anthropogenic sources (Clarkson and Magos, 2006). iHg is transmitted to the air from mineral deposits, burning coal and wastes or production facilities. It passes to the water or soil

through natural deposits, waste disposal and the use of Hg containing fungicides. In the aquatic environment, Hg uptake depends on many environmental parameters, including its bioavailability and toxicity, hydrophobicity, pH, salinity, hardness, and the interaction of metals with biotic and abiotic ligands (Erickson et al., 2008).

In teleost fish species, the bioavailability of Hg is not only the total chemical condition in the environment, but also how far the fish operate these different Hg forms on the gill, skin and digestion, although the toxic mechanisms of Hg still need to be clarified. In recent field and laboratory studies, Hg toxicity in fish has a key role in initiating oxidative stress. The gills, digestive system and skin are the main part of metal intake in fish (Erickson et al., 2008). The photos study is used by the antioxidant system of Hg associated with the production of reactive oxygen species (ROS) in the oxidative stress response (Mieiro et al., 2010; Monteiro et al., 2013a; Brandao et al., 2015; Guardiola et al., 2016).

Fish gills are considered to be the main way to absorb pollutants in the environment due to their anatomical locations, large surfaces and direct and continuous contact with the external environment (Mieiro et al., 2010; De Domenico et al., 2011). Therefore, fish gills have been recognized as appropriate indicators of water pollution and are often used in environmental monitoring studies to assess the impact of water pollution (Mauceri et al., 2005; Fasulo et al., 2010; Matos et al., 2010; De Domenico et al., 2011). The combination of TAS, TOS, OSI, MDA, $ONOO^-$ related processes with Hg-induced oxidative and nitrosative stress biomarkers in fish can help to explain the mechanisms underlying oxidative damage. For this reason, in this study, it was aimed to determine the changes of nitrosative and oxidative stress ($ONOO^-$, TOS, TAS, OSI and MDA) parameters that occur in rainbow trout liver tissue with $HgCl_2$.

Rainbow trout was chosen as the study material because it does not die under the effect of heavy metals (in low concentrations), has the ability to accumulate heavy metals in its body, is easily available, can survive in laboratory conditions, and has enough tissue or organs to be analyzed.

MATERIAL and METHOD

The experimental model of inorganic mercury toxicity was carried out in Bingöl University Faculty of Agriculture, Department of Aquaculture. The fish used in the trial (59,43±3,73 g and 17,24±1,64 cm) were obtained from a commercial fish farm located in the Keban district of Elazig province. In this study, the acute toxicities of $HgCl_2$ on rainbow trout (LD_{50}) were determined by the use of logit analysis of Statistics 20 SPSS IBM. LD_{50} value was calculated as 551 µg L after 96 hours of $HgCl_2$ application to fish. After calculating the LD_{50} value, two subletal doses (25% LD_{50}= 138 µg L and 50% LD_{50}= 276 µg L) were determined, and the fish were exposed to $HgCl_2$ for 2 and 7 days. After euthanasia of fish, necessary autopsy was performed and -80 °C was maintained until gill tissues were used. Frozen gill tissue samples are individually 1:10 (w v) (10 mM Tris-buffer (pH=7.4), 0,1 mM NaCl, 1% TritonX-100, 0,2% SDS, 2.5 mM was homogenized in ethylenediaminetetraacetic acid.

Determination of $ONOO^-$ Value

Evaluation of nitrosative stress status in gill tissue is obtained by determining $ONOO^-$ value. $ONOO^-$ value was measured by phenol nitration (Vanuffelen et al., 1998; Al-Nimer et al., 2012; Ahlatci et al., 2014). To obtain a final volume of 2 ml, 10 µl of sample was added to 5 mM phenol in 50 mM sodium phosphate buffer (pH 7.4). After 2 hours of incubation in a dark place at 37 °C, 15 µl of 0.1 M NaOH was added and the absorbance of the samples at 412 nm wavelength was recorded. Nitrophenol yield was calculated from ε= 4400/M/cm. Results were expressed as µmol/g wet tissue. Biochemical measurements were made using a spectrophotometer (Shimadu U 1601, Japan).

Determination of TAS, TOS and OSI Values

TAS and TOS values of gill tissues, Rel Assay brand commercial kits (Rel Assay Kit Diagnostics, Turkey) was measured. Trolox, a water-soluble analog of vitamin E, was used as calibrator for TAS tests. Results are expressed as mmol Trolox equiv L (Erel, 2004). Hydrogen peroxide was used as calibrator for TOS tests. Results are expressed as µmol H_2O_2 equiv. L. While calculating OSI, which is expressed as the percentage of the ratio of TOS levels to TAS levels, the mmol value in the unit of the TAS test was converted to µmol as in the TOS test (Erel, 2005). The results were calculated according to the formula below.

$$OSİ = \frac{TOS,\mu mol\ H2O2\ equiv./L}{TAS,mmol\ Trolox\ equiv./Lx10}$$

MDA Measurements

MDA determination of tissue samples Ohkawa et al. (Ohkawa et al., 1979) according to the method. 200 µl of each group was taken and 200 µl of 8.1% SDS was added. Then it was kept in a boiling water bath at 95 °C for one hour and then cooled and vortexed by adding a mixture of 1 ml distilled water and 5 ml of n-butanolpyridine in a ratio of 15:1 (v v). After centrifuging at 4000 rpm for 15 minutes, the top organic layer was taken and measured spectrophotometric at 532 nm wavelength, and the results were recorded in nmol ml.

Statistical Analysis

SPSS 20.0 package program was used to calculate the statistical analysis of the data obtained. One-way

analysis of variance (oneway ANOVA) was used to determine the differences between the groups and Duncan Test was used to compare the groups.

RESULTS

TAS, TOS, OSI, ONOO- and MDA measurements

Upon completion of the study, the averages of the measured parameters were tabulated on the basis of groups. The mean values of TAS, TOS, OSI, $ONOO^-$ and MDA of the control and experimental groups were statistically interpreted. While the difference between the groups in terms of TAS, OSI and MDA levels was statistically significant ($P<0.05$), this difference was statistically insignificant ($P>0.05$) in terms of $ONOO^-$ and TOS values (Table 1).

Table 1. The values of the gill samples TAS (mmol L), TOS (µmol L), OSI, $ONOO^-$ (mmol L) and MDA (nmol mg pro)

Çizelge 1. Solungaç örneklerinin TAS (mmol L), TOS (µmol L), OSI, $ONOO^-$ (mmol L) and MDA (nmol mg pro) değerleri

	*Trial Groups ($\bar{x}$±SD)**				
	1	2	3	4	5
TAS	0.30±0.04^{a}	0.28±0.03^{a}	0.31±0.05^{a}	0.33±0.04^{a}	0.45±0.14^{b}
TOS	4.82±2.26^{b}	2.95±0.99^{a}	3.26±1.76^{ab}	2.56±0.69^{a}	3.21±0.90^{ab}
OSI	155.46±56.12^{b}	111.70±43.03^{ab}	107.41±63.13^{ab}	80.70±29.56^{a}	71.91±10.01^{a}
ONOO-	58.21±25.23^{a}	63.21±23.08^{a}	70.00±48.84^{a}	78.92±35.55^{a}	49.28±5.90^{a}
MDA	106.12±24.29^{b}	96.77±4.78^{ab}	99.69±18.98^{b}	97.08±13.40^{ab}	78.95±17.58^{a}

* The difference between average values carrying different letters in the same line is statistically significant ($P<0.05$).TAS= Total Antioxidant Level, TOS= Total Oxidant Level, OSI= Oxidative Stress Index, $ONOO^-$= Peroxynitrite, MDA= Malondialdehyde 1= 25% LC50 2 Days, 2= 50% LC50 2 Days, 3= 25% LC50 7 Days, 4= 50% LC50 7 Days, 5= Control group

DISCUSSION

Due to the high use of mercury in agricultural, mining or industrial areas, water enters ecosystems from natural sources due to anthropogenic or volcanic activities in land and/or oceans and their concentrations increase day by day. In water environments, mercury is available in elemental form, inorganic or organic compounds (Dean et al., 2007). Mercury level in non-polluted waters does not exceed 0.1 µg L (Devlin, 2006). However, in the waters close to the mercury industry, mercury levels can reach levels that threaten both aquatic life and human health fed by aquatic organisms. For example, in the samples of chlorine-alkali, cellulose, paper and ceramic industry, the level of mercury was found between 0.0005-0.23 mg L (Bollen et al., 2008).

In the mining activity region, mercury levels in the water can rise to very high levels such as 0.0001-19.82 mg/L (Gammons et al., 2006). In the aquatic ecosystems contaminated with mercury, high levels of mercury accumulation were found in the tissues of the fish. It has been stated by the World Health Organization that the levels of mercury in fish tissues should not exceed 0.5 µg g for food safety and human consumption (Lima et al., 2005).

Mercury is known to be a metal that causes mutagenic, teratogenic and carcinogenic effects. All mercury compounds can interfere with thiol metabolism, thereby causing inactivation or inhibition of proteins containing thiol ligands, thereby preventing the normal function of important biochemical parameters. Mercury can also suppress important defense mechanisms of cells by causing the formation of free oxygen species and oxidative stress and show effects that may cause lipid peroxidation (Berntssen et al., 2003). Mercury has a high affinity for the -SH groups of cellular biomolecules. For this reason, it can be attached to low molecular weight thiols such as mercury, cysteine and glutathione and proteins containing thiol after being taken into the body, and can remain in tissues and organs for a long time, causing free radicals that cause lipid, protein and DNA oxidation (Perottoni et al., 2004). Mercury is known to have a high affinity for sulfhydryl groups in the structure of biomolecules. Although mercury is a redox-inactive metal and does not participate directly in the redox cycle, it interacts with important antioxidants of cells such as antioxidants and enzymes such as thiol-containing glutathione and can cause indirect production of ROTs by stopping the activity of these molecules (Bagchi et al., 1995). ROTs cause oxidative stress and cause damage such as membrane lipids, proteins, enzymes, and loss of activity and cellular elements such as nucleic acids. However, the cells have the ability to neutralize this oxidative stress toxicity with enzymatic and non-enzymatic antioxidant defense systems such as SOD, CAT, which are the most important protective mechanisms against ROTs and their harmful effects. Since it is well known that pollutants entering the aquatic environment cause serious damage to aquatic ecosystems and living organisms, it is important to study the oxidative stress responses that occur in aquatic organisms by toxicants (Soares et al., 2008). For this reason, in recent years, especially in scientific researches in the field of aquatic toxicology, oxidative toxicity studies induced by various toxicants in aquatic organisms are included

(Echeverria-Saenz et al., 2018; Jiang et al., 2018; Lungu-Mitea et al., 2018; McRae et al., 2018; Rather et al., 2018; Vicari et al., 2018; Zhang et al., 2018).

Appropriate mechanisms such as large surface area, direct contact with water and opposite flow principle (opposite the flow direction of blood and blood) of fish gills to absorb less water in the air also facilitates the entry of pollutants dissolved in water into high levels. Therefore, gills are expressed as the first target tissues of metal toxicity (Kirici et al., 2016). Studies (Jagoe et al., 1996; Oliveira Ribeiro et al., 2000) have shown that exposure to dissolved Hg impairs gill epithelium and potentially affects gas exchange and permeability of cell membranes to cations. In this study, the increase in the indicators of oxidative and nitrosative stress as a result of the application of $HgCl_2$ to rainbow trout gills shows that $HgCl_2$ has a toxic effect for the gills. Cappello et al. (Cappello et al., 2016b) reported mercury toxicity in the gills of the golden mullet fish (*Liza aurata*) in a study they conducted using 1H NMR based metabolomics and oxidative stress biomarkers. In a study with mosquito fish (*Gambusia holbrooki*) that added $HgCl_2$ to dietary sources (Hopkins et al., 2003), the results of stopping gill damage and metabolic rate increase due to Hg accumulation through intestinal absorption also show that mercury is toxic to fish gills, which results from the results of this study. It is parallel. In a study conducted by exposing European sea bass (*Dicentrarchus labrax*) fish to Hg (Giari et al., 2008), it was reported that chlorine cells increased in the gills of European sea bass. It was stated that after $HgCl_2$ administration in *Hoplias malabaricus*, CAT activity in the gills did not show any change, while there was a significant decrease in GPx and GR activities (Monteiro et al., 2013b). In this study, MDA activity increased compared to the control group without $HgCl_2$. In a study on capillary mullet (*Liza aurata*) caught from regions contaminated with Hg (Cappello et al., 2016a), GST and CAT activities showed a great increase while gill GPx and SOD activities were exhausted.

Peroxynitrite is a biologically important molecule produced as a result of the $NO^{\cdot}$ and $O_2^{\cdot -}$ reaction, and the reaction rate is four times faster than the conversion of superoxide dismutase (SOD) catalyzed $O_2^{\cdot -}$ to H_2O_2. Under regular conditions, $ONOO^-$ formation is very low. However, when NOOO and $O_2^{\cdot -}$ concentrations increase and/or pathological conditions where SOD activity decreases, $ONOO^-$ pato formation will increase significantly. In addition to initiating radical reactions, $ONOO^-$ induces nitration of biomolecules. In addition, $ONOO^-$ activates a nuclear enzyme known as poly (ADP-ribose) synthetase (PARS). The PARS enzyme uses excessive amounts of NAD as the substrate, which can lead to depletion of adenosine 5p-triphosphate (ATP) and cell apoptosis. $ONOO^-$ is a powerful initiator of DNA single strand breakage, a mandatory stimulus for activation of PARS. PARS activation triggered by DNA single-strand breakage occurs after exposure to various environmental stimuli and oxidants, especially hydroxyl radical and $ONOO^-$ (Szabo, 1998; Crocker et al., 2005; Ertosun et al., 2020).

In this study, $ONOO^-$ induction can be attributed to a local inflammatory reaction or stimulation of the stress response, which is similar to the study conducted by Smith et al. (Smith et al., 2000) As a result, oxidant stress detected in fish treated with $HgCl_2$ causes oxidative stress and many biochemical damages in organisms. More research should be done to better understand the specific oxidative stress and toxic response of aquatic organisms.

Contribution of the Authors as Summary

Authors declares the contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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