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Research Article

Cadmium Toxicity in Rainbow Trout (*Oncorhynchus Mykiss*): A Study on Heart and Muscle Tissue

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Abstract

This study aims to determine and compare oxidative stress responses in muscle and heart tissues of rainbow trout (Oncorhynchus mykiss) resulting from exposure (24, 48 and 96 hours) to sub-lethal concentrations of cadmium (1, 3 and 5 mg/L). Malondialdehyde (MDA) and Peroxynitrite ($ONOO^-$) parameters were studied to determine oxidative/nitrosative stress, respectively. The MDA and ONOO− values of heart and muscle tissues showed statistically significant ($P<0.05$) differences between each other in terms of both hours and doses. The difference in muscle tissue ONOO− and MDA values between control and other groups was found to be statistically significant ($P<0.05$). Similarly difference in heart tissue ONOO− and MDA values between control and other groups was found to be statistically significant ($P<0.05$). For both parameters as the amount of the administered dose increased, the average of the ONOO− and MDA values obtained increased significantly. As a result, it was determined that cadmium increases ONOO− and MDA levels in heart and muscle tissues, causes stress and is toxic to fish even in small doses.

Key words: Cadmium toxicity, oxidative, peroxynitrite, muscle, heart

Gökkuşağı Alabalıklarında (*Oncorhynchus Mykiss*) Kadmiyum Toksisitesi: Kalp ve Kas Dokusu Üzerine Bir Araştırma

Öz

Bu çalışma, gökkuşağı alabalığının (Oncorhynchus mykiss) kas ve kalp dokularındaki ölümcül olmayan kadmiyum konsantrasyonlarına (1, 3 ve 5 mg / L) maruz kalmadan (24, 48 ve 96 saat) kaynaklanan oksidatif stres tepkilerini belirlemeyi ve karşılaştırmayı amaçlamaktadır. Malondialdehit (MDA) ve Peroksinitrit ($ONOO^-$) parametreleri sırasıyla oksidatif / nitrosatif stresi belirlemek için çalışıldı. Kalp ve kas dokularının MDA ve ONOO− değerleri, hem saatler hem de dozlar açısından birbirleri arasında istatistiksel olarak anlamlı ($P<0.05$) farklılıklar gösterdi. Kontrol ve diğer gruplar arasında kas dokusu ONOO− ve MDA değerlerindeki fark istatistiksel olarak anlamlı bulundu ($P<0.05$). Kontrol grubu ile diğer gruplar arasında benzer şekilde kalp dokusu ONOO− ve MDA değerlerinde de fark istatistiksel olarak anlamlı bulundu ($P <0.05$). Her iki parametre için de uygulanan doz miktarı arttıkça, elde edilen ONOO− ve MDA değerlerinin ortalaması önemli ölçüde artmıştır. Sonuç olarak kadmiyumun kalp ve kas dokularında ONOO− ve MDA düzeylerini artırdığı, strese neden olduğu ve küçük dozlarda bile balıklar için toksik olduğu tespit edildi.

Anahtar kelimeler: Kadmiyum toksisitesi, oksidatif, peroksinitrit, kas, kalp

Introduction

The release of heavy metals into the aquatic environment is known to cause harm to the environment and living organisms, and this generates considerable interest in the study of oxidative/nitrosative stress responses in aquatic organisms excited by toxic metals. Cadmium (Cd) is a metal that has no beneficial properties for life, there is no biological evidence that it is necessary or beneficial. Even low concentrations are toxic to plants, fish, birds, mammals, including humans and all microorganisms (Nordberg et al. 2007; Kirici et al. 2017). In a study where comparative acute toxicity tests of 63 heavy metals were performed, Cd was found to be the most toxic metal (Borgmann et al. 2005). Cd is inseparable in its basic form and can turn into different compounds. These compounds can also bond tightly to the soil, depending on the acidity of the water. Coal and other fossil fuels can be shown as sources of Cd in general. In clean waters, the natural Cd content is often less than 1 µg/L (Nordberg et al. 2007).

Cd and other metals emitted from mining sites pollute drinking and other water resources. Some biological studies have linked metals such as lead, cadmium, copper, and zinc with their induction to produce reactive oxygen species (ROS), leading to lipid peroxidation and changes in antioxidant enzymes that lead to oxidative stress (Hu 2000). Studies have shown that the heart and muscle tissue exhibit great vulnerability to metal toxicity (Tort and Madsen 1991; Sarkar et al. 1995; Rodrigues et al. 1998; Wang et al. 1999; Aureliano et al. 2002; Soares et al. 2006; Soares et al. 2007). Fish muscle is a rich source of polyunsaturated fatty acids (PUFA) (Ren et al. 2012). Because of this, the high PUFA level in fish muscle makes it susceptible to oxidation (Kenari et al. 2009). Several studies of Cd-induced oxidative stress have focused on fish's gills, liver, intestines or cells (Gomes et al. 2016; Luczynska et al. 2019).

Since the potential effects of cadmium toxicity on possible oxidative / nitrosative stress on fish heart and muscle tissues remain unclear, in this study, we aimed to investigate the changes in MDA, which are important parameters of oxidative stress, and ONOO−, one of the important parameters of nitrosative stress, in order to determine the possible damage to these tissues from cadmium exposure.

Materials and Methods

The application part of the study was carried out in Bingöl University Faculty of Agriculture Aquaculture Laboratory, while the laboratory part was carried out in Gaziantep University Medical Biochemistry Laboratory. Rainbow trout (Oncorhynchus mykiss) (75.43 ± 5.73 g and 27.24 ± 2.14 cm) was purchased from a commercial trout facility in Elazig and brought to the laboratory in a healthy way. Fish brought to the laboratory were placed in 600 liter tanks and adapted for 21 days. During the study, water temperature was 14±3 °C, dissolved oxygen level was 8.24±0.5 mg/L, alkalinity 128±11 mg/L, and total hardness was measured as 132±29 mg/L and pH 7.3±0.2 as CaCO3. The fish were fed twice a day with a commercial fish feed of 2% of the fish weight. In the study, fish were exposed to 1, 3 and 5 mg/L doses of Cd for 24, 48 and 96 hours (Hisar et al. 2009). Fish were placed in 50 L aquariums, with 20 fish in each group. Circulation was provided in the aquariums with a flow rate of 1.5 liters per minute. The study was carried out in duplicate (80 fish for each iteration, total of 160 fish were used, 70 females and 90 males.). 5 fish were sampled randomly from the aquariums at 24, 48 and 96 hours. The tissues from the fish samples were taken and stored at -20 ºC until they were used.

To determine the ONOO− value 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 (Ahlatci et al. 2014; Al-Nimer et al. 2012; Vanuffelen et al. 1998). Biochemical measurements were made using a spectrophotometer (Shimadu U 1601, Japan).

MDA determination of tissue samples was made 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 spectrophotometrically at 532 nm wavelength, and the results were recorded in nmol/ml.

Statistical analysis of the data obtained SPSS 20.0 package program was used to calculate. Duncan Test was used to determine the differences between groups.

Results

ONOO− values of heart and muscle tissues showed statistically significant ($P<0.05$) differences between each other in terms of both hours and

doses. The difference between the control group and the other groups between the muscle tissue ONOO− values was found to be statistically significant (P<0.05). As the amount of administered dose increased, the average of the absorbance values obtained increased significantly. The difference between the ONOO− values between 24 and 48 hours was not statistically significant in terms of applied hours (P>0.05), while the 96-hour application showed a statistically significant difference (P<0.05) compared to the other 24 and 48 hours (Table 1).

Table 1. $ONOO^-$ values (mmol/L)

Tissue	Groups (mg/L)	Times (hours)		
		24	48	96
Muscle	Control	7,91±5,34[a]	12,91±5,10[a]	6,66±5,62[a]
	1	14,16±5,16[b]	23,75±2,62[b]	22,91±5,34[b]
	3	28,75±4,67[c]	31,25±5,41[c]	35,41±3,67[c]
	5	43,75±2,62[d]	37,08±4,30[d]	51,66±5,40[d]
Heart	Control	46,66±6,45[a]	58,33±7,01[a]	53,75±7,20[a]
	1	81,25±5,64[b]	81,66±4,65[b]	72,08±5,79[b]
	3	106,25±4,67[c]	107,91±6,20[c]	96,25±11,03[c]
	5	125,41±8,86[d]	123,33±5,40[d]	128,75±6,66[d]

*The difference between average values carrying different letters in the same cloumn is statistically significant (p<0.05). Values are given in x̄±SD. $ONOO^-$: Peroxynitrite.

The difference between cardiac and muscle tissue MDA values between control and other groups was statistically significant (P<0.05). As the amount of administered dose increased, the average of the absorbance values obtained increased significantly. MDA values in terms of hours applied showed a statistically significant difference (P<0.05) from each other (Table 2).

Table 2. MDA values (nm/mg protein)

Tissue	Groups (mg/L)	Times (hours)		
		24	48	96
Muscle	Control	1,99±0,18[a]	2,06±0,37[a]	1,03±0,17[a]
	1	4,86±0,26[b]	5,24±0,34[b]	2,15±0,52[b]
	3	5,06±0,41[c]	6,95±1,43[c]	4,75±0,61[c]
	5	9,02±0,97[d]	9,55±0,90[d]	10,74±0,83[d]
Heart	Control	5,07±,0,27[a]	4,12±0,61[a]	1,66±0,25[a]
	1	5,86±0,90[b]	5,08±0,37[b]	2,29±0,25[b]
	3	7,87±0,44[c]	9,13±0,49[c]	10,34±0,51[c]
	5	9,57±,0,44[d]	12,77±0,73[d]	11,89±0,31[d]

*The difference between average values carrying different letters in the same cloumn is statistically significant (p<0.05). Values are given in x̄±SD. MDA: Malondialdehyde.

Discussion

Cadmium is a toxic metal widely used in industries. It is causes oxidative/nitrosative stress and then causes serious pathological conditions due to long-term retention in some tissues (Bagchi et al. 2000). MDA and ONOO− levels were investigated as Cd-induced oxidative/nitrosative stress markesrs in trout muscle and heart tissues. The results show that the MDA and ONOO− levels are statistically significantly increased in these tissues. In this study, due to the Cd toxicity, the most pronounced effect was observed in heart tissue. This has shown us that heart tissue is more sensitive to Cd toxicity than muscle tissue. Nitric oxide (NO) is a highly reactive endogenous radical overproduced by cells under nitrosative stress conditions and functions as a mediator expressing cytotoxic activity (Hibbs et al. 1988). Heart and muscle cells produce superoxide anion (O2−) and NO radicals that cause the formation of peroxynitrite anion (ONOO−). Therefore, induction of O2− production in heart and muscle tissue may contribute to the cytotoxicity of Cd. MDA is a marker of lipid peroxidation and a strong indicator of oxidative damage in tissues (Del Rio et al. 2005).
A large increase in MDA levels in both tissues compared to the control group indicates extensive tissue lipid peroxidation. It has been reported in many studies that cadmium accumulates in muscle tissue and this metal causes oxidative damage in

muscle tissues (Yano and Marcondes 2005; Gonzalez et al. 2006; Manna et al. 2008). In this study, changes in oxidative stress parameters in the heart and skeletal muscle of fish exposed to cadmium showed that cadmium has a toxic effect on fish. The increase in the level of oxidative/nitrosative stress in muscle and heart tissues appears to be consistent with a mechanism involving increases in reactive oxygen and nitrogen species production following exposure to Cd. However, the underlying mechanisms causing such effects are still not clearly understood. Based on the results of this study, it can be concluded that Cd may cause depletion of the antioxidant-enzymatic system and lipid peroxidation in trout heart and skeletal muscle. However, further molecular studies are needed on the subject.

Conflict of Interest Statement: The manuscript's authors declare that, they do not have any conflict of interest.

Researchers' Contribution Rate Statement Summary: The authors declare that, they have contributed equally to the manuscript.

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