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RESEARCH ARTICLE

Effects of Zinc Sulphate on Transcaucasian Barb, (*Capoeta capoeta* [Guldenstaedt, 1773]) Plasma Nitric Oxide, Malondialdehyde and Total Sialic Acid Levels

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Summary

The aim of this study was to investigate plasma total sialic acid (TSA), nitric oxide (NO) and malondialdehyde (MDA) levels in response to different doses of zinc sulphate ($ZnSO_4$) in *Capoeta capoeta*. The fishes were kept in tanks for 15 days for adaptation. Three groups of fish (control, 1st, and 2nd), each containing 9 fishes, were placed in separate tanks containing no, 5 and 10 mg/L $ZnSO_4$, respectively for 10 days. At the end of the study, blood samples were taken, and plasma TSA, NO and MDA levels were analyzed. An increase in plasma TSA and MDA levels were found along with increasing $ZnSO_4$ amounts, and a decrease in NO levels was observed. In conclusion, it was determined that levels of TSA, MDA and NO were altered depending on $ZnSO_4$ doses applied in *C. capoeta*.

Keywords: *Freshwater fish, Capoeta capoeta, Zinc, Total sialic acid, Malondialdehyde, Nitric oxide*

Siraz Balığı (*Capoeta capoeta* [Guldenstaedt, 1773]) Plazma Nitrik Oksit, Malondialdehit ve Total Sialik Asit Düzeyleri Üzerinde Çinko Sülfatın Etkileri

Özet

Bu çalışmada farklı dozlarda çinko sülfat uygulanan *Capoeta capoeta*'larda plazma total sialik asit (TSA), nitrik oksit (NO) ve malondialdehit (MDA) düzeylerinin belirlenmesi amaçlandı. Balıkların 15 gün süre ile laboratuvar ortamına adaptasyonları sağlandıktan sonra 9'ar adet üç grup oluşturuldu. Grup I'de bulunan balıklar normal su ortamında, grup II ve III ise sırasıyla 5 ve 10 mg/L $ZnSO_4$ eklenen tanklarda 10 gün süre ile bekletildi. Balıklardan kan örnekleri alındıktan sonra plazma TSA, NO ve MDA seviyeleri analiz edildi. Artan $ZnSO_4$ miktarlarına göre plazma TSA ve MDA seviyelerinde artış olduğu belirlenirken, NO seviyelerinde düşüş olduğu gözlendi. Sonuç olarak, $ZnSO_4$ uygulanan *C. capoeta*'da plazma TSA, MDA ve NO seviyelerinde doza bağlı değişiklikler tespit edildi.

Anahtar sözcükler: *Tatlısu balığı, Capoeta capoeta, Çinko, Total sialik asit, Malondialdehit, Nitrik oksit*

INTRODUCTION

Increasing household, industrial and agricultural wastes in association with increasing human population extremely rise heavy metal levels in aquatic media [1,2]. Heavy metals normally join to aquatic systems by natural ways, and their levels in water generally are measured as nanogram and microgram in litter [3]. It has been stated that zinc is an essential microelement which has potential toxicity in aquatic environment [4]. Also, it has been reported that zinc plays an important role as a heavy metal in the structure and function of about 300 proteins and is needed for many physiological events such as growing up and cell dividing, immunity, durability of cell, taste and eyesight function [5,6]. The importance of zinc for living organisms originates from being an integral part of metalloenzymes and the cofactor for regulation of zinc dependent enzyme activities such as carbonic anhydrase, alkaline phosphatase and alcohol dehydrogenase [7]. Therefore, fertilizers with high zinc have been used in soils which are insufficient for zinc [7,8].

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Reactive oxygen species (ROS) could be originated from environmental polluters exhibit harmful effects on lipid, protein, carbohydrate and nucleic acids because of its high reactive features [9]. Lipid oxidation may be increase during fish tissue and cells' processing with cause of having high amount polyunsaturated fatty acid (PUFA) in fishes [10]. One of the most important products of oxidation is malondialdehyde (MDA) which has carcinogenic and mutagenic features; the level of this products has been used as an indicator of lipid peroxidation [10,11]. Peroxides has been formed as a result of lipid peroxidation, also constitute peroxinitrite radical by composing reaction with nitric oxide (NO) affecting organic matters [12,13]. NO molecules show a protective effect on tissue cells by decreasing tissue damage occurred through ROS [14]. Besides NO's harmful effects as constituting peroxinitrite, DNA damage and causing being spoiled of enzyme functions, as an antioxidant, it has protective and arrangement effects to decrease oxidative damage [15,16].

Sialic acids, (N-and-O-acyl species) as different kind of N-acetyl neuraminic acid (NANA), are constituents of macromolecules and receptors as glycoprotein, glycolipid and terminal carbohydrate ruins of glycosaminoglycans oligosaccharides' side chain [17,18]. Sialic acids (SA) have ionization features because of carboxyl groups in its structure and apply negative polarity to the cell surface. Because of negative polarity, they are held accountable for electrostatic pushing seen in trombosite, erythrocyte and cancer cells [17]. SAs is used to follow of metabolic activities in living organisms due to a lot of important physiological and pathological roles [17-19]. Serum total sialic acid (TSA) levels may stated as both free and related to SA levels' total [20].

The main spreading zone of *Capoeta capoeta* including cyprinidae family is in North-East Turkey Anatolian Region Kura and Aras Rivers and source of branches in these rivers' borders. This subspecies has economical and ecological importances in terms of both nourishment and used as bioindicator for degree of the aquatic environment damage [21,22]. The effects of zinc have been extensively studied on physiology and biochemistry of variety freshwater organisms especially for fishes [23-27]. However, it can not be met by searching on studied effects of zinc on freshwater fish *Capoeta capoeta*'s biochemical parameters. So, in this study, it was aimed to determine plasma NO, MDA and TSA levels in *Capoeta capoeta capoeta* applied $ZnSO_4$.

MATERIAL and METHODS

In this study, 27 *Capoeta capoeta* weighing 200 to 250 gram were used. Fishes caught from Kars creek in autumn were taken to 500 L tanks in laboratory environment. After adaptation of fishes to environmental conditions during 15 days, 3 groups including 9 fishes in each group were constituted. First group of fishes was placed in normal water environment; second and third group fishes were placed in water environment having 5 and 10 mg/L $ZnSO_4$ and exposed for 10 days. At the end of the study, blood samples were taken into the tube with EDTA and centrifuged at 3.000 rpm for 10 min in +4°C. Samples were separated and kept at -20°C until analysed.

Nitric oxide levels were colorimetrically determined according to the method Miranda et al.[28]. Therefore, NO levels were determined with absorbance levels read in 540 nm as spectrophotometric about nitrate and nitric values. TSA analyses were done according to the spectrophotometric method determined by Sydow [29]. MDA values were determined using spectrophotometric measure declared by Draper and Hadley [11].

Statistic analyses were done for statistically evaluation of data. Results were expressed as median (X)±standard declination (SD). Importance level of difference between group averages was determined by using SPSS 15.0 for Windows statistic packet program with variance analyze and Duncan poly comparison test [30].

RESULTS

Biochemical changes constituted in blood taken after 10 days from control group fishes not included $ZnSO_4$ with fishes added 5 and 10 mg/L $ZnSO_4$ to life environments were shown in *Table 1*. It was obtained that in plasma TSA and MDA levels ($P<0.001$) a statistically meaningful increase occurred in group II and group III when comparing to control group. It was determined that plasma NO levels were statistically ($P<0.05$) low level comparing to the control group.

DISCUSSION

Relationship between oxidative stress and sialic acid levels with zinc is important in terms of being lightened of sensitive balance between decreasing to the least level

Table 1. *Plasma NO, TSA and MDA levels in Capoeta capoeta capoeta applied $ZnSO_4$ and control group*
Tablo 1. *$ZnSO_4$ uygulanan ve kontrol grubu Capoeta capoeta capoeta'da plazma NO, TSA ve MDA seviyeleri*

Parameters (n=9)	Control	$ZnSO_4$ (5 mg/L)	$ZnSO_4$ (10 mg/L)	P
NO µmol/L	39.47 ± 2.43^{a}	30.61 ± 3.16^{b}	36.75 ± 1.36^{ab}	P<0.05
TSA mg/dl	68.72 ± 3.36^{a}	78.97 ± 2.42^{b}	89.85 ± 4.31^{c}	P<0.001
MDA µmol/L	11.84 ± 0.54^{a}	17.25 ± 1.22^{b}	20.35 ± 1.84^{b}	P<0.001

Values with different letters within the same row indicates significant differences (P<0.05 and P<0.001) of environmental zinc pollution with sufficient zinc uptake. In aquatic environment, essential metals in fishes are absorbed through digestive systems and gills, and it has been stated that zinc may be taken from gills through apical calcium in ion transporter chloride cells that are rich in terms of mitochondria [31,32]. It is indicated that variety in terms of sensitivity against metals in living species refers to changes in antioxidant defense system's cell mechanism with variety met in metals' dispersion and accumulation in tissues [33,34]. In a study, it has been stated that *Cyprinus carpio*'s protein levels in the mixture added zinc decrease in muscle tissue although it increased in gills and liver [25]. According to this, it has been claimed that proteins needed for carrying of the zinc in blood may have been provided through muscle tissue. In a study which has been carried out by adding zinc to *Tilapia zilli* and *Clarias lazera*'s living environment, it has been claimed that glycogen amount decreased and this might be related to increasing of lactic acid levels in muscle and liver [23]. It has been stated that acute effects appeared with high zinc concentration in fishes also caused hypoxia related to structural defect in gills and finally an increase in mortality [35]. A study was carried out with *Chana punctatus* by Murugan et al.[26] by fixing the relationship of zinc in different tissues in decreasing measurement sequentially with liver, kidney, intestine, gill and muscle. According to these results, it has been expressed that liver and kidney are target tissues in terms of zinc toxicity. In another study related to *Scyliorhinus canicula*, it has been recorded that metal accumulation is at most in liver and least in muscle tissue in the environment, including sublethal zinc concentrations [36]. In other studies, it has been stated that in fact zinc is absorbed by fishes through nourishments by digestive means [24,37].

In a study carried out with rainbow trout (*Oncorhynchus mykiss*) by Köck and Bucher [24], it has been stated that in experimental aquatic environment zinc concentration is 1600 µg/L, in the beginning, it has been met with fish deaths and zinc taking's basic source has been constituted by gills. In case of exceeding zinc concentration of aquatic environment to 400-500 µg/L in terms of taking the zinc to the body in fishes, the idea that gills will be the most urgent thing is supported in different studies as well [24,37,38]. A study with rainbow trout by Glover et al. [31] has been stated that zinc requirements of fishes in aquatic environment is 15-30 mg/kg, also zinc concentration reaching to the intestine lumen through nourishments 1-100 µM will be suitable. In this study, it was determined that the application of 5 and 10 mg/L $ZnSO_4$ caused statistically important toxic effects in plasma.

Heavy metal toxicity in fishes shows variation not only species but also environment conditions [39,40]. Obtained informations from some studies are important for relation among zinc concentration and MDA used as an indicator of lipid peroxidation. In a study by Mendes et al.[10], it has been stated that MDA levels are about 29 µmol/kg in hake and 700 µmol/kg in sardine. Chaijan et al.[41] claims that this level may be exceed to 8.000 µmol/kg. In studies carried out with different fish species, it has been reported that zinc toxicity shows variation related to physical and chemical features of water as water hardness, heat, pH and dissolved oxygen concentration [39]. Although the life of fishes in environment has generally low heat, in some conditions, it has been claimed that determined decrease in environment heat may cause oxidative stress in fishes, as well [27,42]. This situation may be related to decreasing ROS elimination and disabilty to protect the balance between productions. It has been stated that oxidative stress increases in different fish species and aquatic environments with high oxygen content (*Salma salar, Carassius auratus* etc.) [27,43]. In another study on freshwater oysters, it has been stated that catalase and glutathione peroxidase enzyme activities known as anti oxidative enzymes in sufficient aquatic environment in terms of oxygen increase [44]. In this study, significantly higher MDA levels of fish caught before the winter season can be associated with these conditions. The present study also reports a statistically important increase in TSA levels with MDA in *Capoeta capoeta* exposed to high zinc concentration. Therefore, it may be concluded as good adaptation ability against low biochemical heats in this species against oxidative stress.

It has been claimed that SAs have an antioxidative role which are responsible for taking O_2 away from vein system basically [45,46]. In a study by Henricks et al.[45], it has been stated that O_2 production in vein system increases in case of taking SAs away in neutrophils. It has been stated that oxidative stress may start to let SA loose from oligosaccharides in cell surface without sialidase activation or induction [47,48]. Also, it is reported that NO is susceptible to interact with heavy metals and effective to decrease oxidative damage because of heavy metal pollution [15,16]. In vascular system, especially as a result of function loss met to endothelium cells because of NO is not able to continue to function in metabolic reactions, thus, it has been fixed that there is an increase in O_2 production and accumulation [46,49]. In our study, this situation can be associated with significantly decreased NO levels in group II and increased TSA levels in application groups. In the present study, in NO and MDA levels with TSA levels including both free and bound SA levels were observed statistically important changes.

In conclusion, it was inferred that environment conditions have high Zn concentrations caused changes on MDA and NO levels related to oxidative stress and TSA levels in *Capoeta capoeta*. In addition, the relationship of sialic acids with numerous functional molecules in the body is important to decrease the heavy metal toxicity in fishes. Therefore, further detailed studies should be carried out on sialic acid and oxidative stress related to Zn toxicity.

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