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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 +4ºC. Samples were separated and kept at -20ºC 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.

REFERENCES

1. Park SW, Lee JY, Yang JS, Kim KJ, Baek K: Electrokinetic remediation of contaminated soil with waste-lubricant oils and zinc. *J Hazard Mater*, 169,

1168-1172, 2009.

2. Coruh S, Ergun ON: Use of fly ash, phosphogypsum and red mud as a liner material for the disposal of hazardous zinc leach residue waste. *J Hazard Mater*, 173, 468-473, 2010.

3. Nussey G, Van Vuren JHJ, Du Preez HH: Effect of copper on the haematology and osmoregulation of the mozambique tilapia, *Oreochromis mossambicus* (Cichlidae). *Comp Biochem Physiol C*, 111, 369-380, 1995.

4. Eisler R: Zinc hazards to fish, wildlife, and invertebrates: A synoptic review. *US Fish Wildl Serv Biol Rep*, 10, 106, 1993.

5. Valee BL, Falchuk K: The Biochemical basis of zinc physiology. *Physiol Rev*, 73, 79-118, 1993.

6. Akahori A, Jozwiak Z, Gabryelak T, Gondko R: Effect of zinc on carp (*Cyprinus carpio* L.) erythrocytes. *Comp Biochem Physiol C*, 123, 209-215, 1999.

7. Adhikari S, Ayyappan S: Behavioural role of zinc on primary productivity, plankton and growth of a freshwater teleost, *Labeo rohita* (Hamilton). *Aquaculture*, 231, 327-336, 2004.

8. Razra GC, Mandal B: Desorption of adsorbed zinc in soils in relation to soil properties. *J Indian Chem Soc Soil Sci*, 4, 233-237, 1996.

9. Parvez S, Raisuddin S: Protein carbonyls: novel biomarkers of exposure to oxidative stress-inducing pesticides in freshwater fish *Channa punctata* (Bloch). *Environ Toxicol Phar*, 20, 112-117, 2005.

10. Mendes R, Cardoso C, Pestana C: Measurement of malondialdehyde in fish: A comparison study between HPLC methods and the traditional spectrophotometric test. *Food Chem*, 112, 1038-1045, 2009.

11. Draper HH, Hadley M: Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol*, 186, 421-430, 1990.

12. Rubbo H, Radi R, Trujillo M, Telleri R, Kalyanaraman B, Barnes S, Kırk M, Freeman BA: Nitric oxide regulation of superoxide and peroxynitrite dependent lipid peroxidation. Formation of novel nitrogen containing oxidized lipid derivatives. *Chemistry*, 269, 26066-26075, 1994.

13. Gonenc A, Ozkan, Y, Torun M, Simsek B: Plasma malondialdehyde (MDA) levels in breast and lung cancer patients. *J Clin Pharm Ther*, 26, 141-144, 2001.

14. Wink DA, Hanbauer I, Krishna MC, DeGraff W, Gamson J, Mitchell JB: Nitric oxide protects against cellular damage and cytotoxicity from reactive oxygen species. *P Natl Acad Sci USA*, 90, 9813-9817, 1993.

15. Laspina NV, Groppa MD, Tomaro ML, Benavides MP: Nitric oxide protects sunflower leaves against Cd-induced oxidative stress. *Plant Sci*, 169, 323-330, 2005.

16. Singh HP, Batish DR, Kaur G, Arora K, Kohli RK: Nitric oxide (as sodium nitroprusside) supplementation ameliorates Cd toxicity in hydroponically grown wheat roots. *Environ Exp Bot*, 63, 158-167, 2008.

17. Schauer R: Chemistry, metabolism and biological functions of sialic acids. *Adv Carbohyd Chem Biochem*, 40, 131-234, 1982.

18. Varki NM, Varki A: Diversity in cell surface sialic acid presentations: implications for biology and disease. *Lab Invest*, 87, 851-857, 2007.

19. Schroder PN, Giannis A: From substrate to transition state analogues: The first patent inhibitör of sialytransferases. *Angew Chem Int Edit*, 38, 1379-1380, 1999.

20. Erdil A, Tüzün A, Yönem A, Erbil MK: Serum sialic acid concentrations in patients with non-insulin dependent diabetes mellitus and its relation with retinopathy. *Turk J Med Sci,* 21, 349-354, 2001.

21. Karakuş S, Gey H: A preliminary study of heavy metals in transcaucasian barb (*Capoeta capoeta capoeta* Guldenstaedt, 1772) from the Kars Creek, Turkey. *Rev Med Vet,* 157, 551-556, 2006.

22. Unlu E, Akba O, Sevim S, Gumgum B: Heavy metal levels in mullet, Liza abu (Heckel, 1843) (Mugilidae) from Tigris River, Turkey. *Fresenius Environ Bull*, 5, 107-112, 1996.

23. Hilmy AM, El-Domıaty NA, Daabess A, Abdel Latife HA: Some physiological and biochemical ındices of zinc toxicity in two freshwater fishes, *Clarias lazera* and *Tilapia zilli*. *Comp Biochem Physiol C,* 87, 297-301, 1987.

24. Köck G, Bucher F: Accumulation of zinc in rainbow trout (*Oncorhynchus mykiss*) after waterborne and dietary exposure. *Bull Environ Contam Toxicol,* 58, 305-310, 1997.

25. Cicik B: The effects of copper-zinc interaction on the accumulation of metals in liver, gill and muscle tissues of common carp (*Cyprinus carpio* L.). *Ekoloji*, 12, 32-36, 2003.

26. Murugan SS, Karuppasamy R, Poongodi K, Puvaneswari S: Bio-accumulation pattern of zinc in freshwater fish *Channa punctatus* (Bloch) after chronic exposure. *Turk J Fish Aquat Sci,* 8, 55-59, 2008.

27. Lushchak VI: Environmentally induced oxidative stress in aquatic animals. *Aquat Toxicol,* 101, 13-30, 2011.

28. Miranda M, Espey MG, Wink DA: A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide*, 5, 62-71, 2001.

29. Sydow G: A simplified quick method for determination of sialic acid in serum. *Biomed Biochim Acta,* 44, 1721-1723, 1985.

30. Ergün G, Aktaş S: ANOVA modellerinde kareler toplamı yöntemlerinin karşılaştırılması. *Kafkas Univ Vet Fak Derg,* 15 (3): 481-484, 2009.

31. Glover CN, Bury NR, Hogstrand C: Intestinal zinc uptake in freshwater rainbow trout: evidence for apical pathways associated with potassium efflux and modified by calcium. *Biochim Biophys Acta,* 1663, 214-221, 2004.

32. Bury NR, Walker PA, Glover CN: Nutritive metal uptake in teleost fish. *J Exp Biol* 6, 11-23, 2003.

33. Grosell M, Nielsen C, Bianchini A: Sodium turnover rate determines sensitivity to acute copper and silver exposure in freshwater animals. *Comp Biochem Physiol C,* 133, 287-303, 2002.

34. Garcia-Santos S, Fontainhas-Fernandes A, Wilson JM: Cadmium tolerance in the nile tilapia (*Oreochromis niloticus*) following acute exposure: Assessment of some ionoregulatory parameters. *Environ Toxicol,* 21, 33-46, 2006.

35. Skidmore JF: Toxicity of zinc compounds to aquatic animals, with special reference to fish. *Q Rev Biol,* 39, 227-247, 1964.

36. Sanpera C, Vallribera M, Crespo S: Zn, Cu and Mn levels in the liver dogfish exposed to Zn. *Bull Environ Contam Toxicol,* 31, 415-417,1983.

37. Spry DJ, Hodson PV, Wood CM: Relative contributions of dietary and waterborne zinc in the rainbow trout, *Salmo gairdneri*. *Can J Fish Aquat Sci,* 45, 32-41,1988.

38. Willis JN, Sunda WG: Relative contributions of food and water in the accumulation of zinc by two species of marine fish. *Mar Biol,* 80, 273-279, 1984.

39. Yılmaz M, Ersan Y, Koç E, Özen H, Karaman M: Toxic effects of cadmium sulphate on tissue histopathology and serum protein expression in european chub, *Leuciscus cephalus* (Linnaeus, 1758). *Kafkas Univ Vet Fak Derg,* 17 (Suppl. A): S131-S135, 2011.

40. Mendil D, Demirci Z, Tüzen M: Seasonal investigation of trace element contents in commercially valuable fish species from the Black Sea, Turkey. *Food Chem Toxicol,* 48, 865-870, 2010.

41. Monserrat JM, Marıınez PE, Geracitano LA, Amado LL, Martins CM, Pinho GL, Chaves IS, Ferreira-Cravo M, Ventura-Lima J, Bianchini A: Pollution biomarkers in estuarine animals: Critical review and new perspectives. *Comp Biochem Physiol C,* 146, 221-234, 2007.

42. Malek RL, Sajadi H, Abraham J: The effects of temperature reduction on gene expression and oxidative stress in skeletal muscle from adult zebrafish. *Comp Biochem Physiol C,* 138, 363-373, 2004.

43. Olsvik PA, Kristensen T, Waagbø R,Rosseland BO, Tollefsen KE, Baeverfjord G, Berntssen MHG: mRNA expression of antioxidant enzymes (SOD, CAT and GSH-Px) and lipid peroxidative stress in liver of Atlantic salmon *(Salmo salar)* exposed to hyperoxic water during smoltification. *Comp Biochem Physiol C,* 141, 314-323, 2005.

44. Vidal ML, Bassères A, Narbonne JF: Influence of temperature, pH, oxygenation, water-type and substrate on biomarker responses in the fresh-water clam *Corbicula fluminea* (Müller). *Comp Biochem Physiol C,* 132, 93-104, 2002.

45. Henricks PA, van Erne-van der Tol ME, Verhoef J: Partial removal of sialic acid enhances phagocytosis and the generation of superoxide and chemiluminescence by polymorphonuclear leukocytes. *J Immunol,* 129, 745-750, 1982.

46. Kumagai R, Lu X, Kassab GS, Role of glycocalyx in flow induced production of nitric oxide and reactive oxygen species. *Free Radic Biol Med,* 47, 600-607, 2009.

47. Eguchi H, Ikeda Y, Ookawara T, Koyota S, Fujiwara N, Honke K, Wang PG, Taniguchi N, Suzuki K: Modification of oligosaccharides by reactive oxygen species decreases sialyl lewis x-mediated cell adhesion. *Glycobiology*, 15, 1094-1101, 2005.

48. Karapehlivan M, Uzlu E, Atakişi O, Erdoğan HM, Uzun M, Citil M: Effect of L-carnitine on plasma sialic acid, MDA and blood GSH levels in doxorubicine administered rabbits. *Kafkas Univ Vet Fak Derg,* 13 (2): 155-160, 2007.

49. Smith AR, Hagen TM: Vascular endothelial dysfunction in aging: Loss of Akt-dependent endothelial nitric oxide synthase phosphorylation and partial restoration by (R)-alpha-lipoic acid. *Biochem Soc T,* 31, 1447-1449, 2003.