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Biochemical Response of Zebra Mussels (*Dreissena polymorpha*) Exposed to Sulfamethazine Antibiotic

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Abstract
Sulfamethazine is an antibiotic that belongs to the pharmaceutically crucial group of heterocyclic sulfonamides. Pharmaceuticals can impact non-target organism in many ways even at very low concentrations and that more studies are needed to highlight and identify potential effects. In this study, it is aimed to assess the biochemical response of *Dreissena polymorpha* exposed to a pharmaceutical pollutant sulfamethazine. *Dreissena polymorpha* individuals were provided with handnets from Keban Dam Lake. During a two-week acclimatization period, mussels were kept in 10 L glass tanks each with approximately hundred individuals at 20 ± 0.5 °C. *Dreissena polymorpha* were exposed to sulfamethazine during 96 h. Five experimental groups were designed as following: control (tap water); A,0.1 g/L; B, 0.25 g/L; C, 1 g/L; 4, g/L sulfamethazine exposure groups.
The samples were weighed first and homogenized using a homogenizer with ice, adding 1/5 w/v of PBS buffer (phosphate buffered saline solution) (pH 7.4). The homogenized samples were centrifuged at 17000 rpm for 15 minutes in a refrigerated centrifuge and the supernatants were stored at -70 °C deep-freezing.
MDA levels in all application groups were increased significantly depending on sulfamethazine exposure ($p<0.05$). GSH levels were increased A and D groups ($p<0.05$) but decreased in B and C groups ($p<0.05$).
MDA and GSH levels in the *Dreissena polymorpha* are sensitive and suitable biochemical biomarkers for evaluating the toxicity of sulfamethazine.
Keywords: *Dreissena polymorpha*, Sulfamethazine, MDA, GSH

INTRODUCTION

Sulfamethazine is an antibiotic that belongs to the pharmaceutically crucial group of heterocyclic sulfonamides. This an- tibiotic is widely used in veterinary and medicine practice as an antibacterial drug in pharmaceutical preparation (Yönten et al., 2017)

The continuous discharge of pharmaceuticals via sewage treatment plants effluents into the environment results in a chronic exposure of aquatic organisms to these substances. Pharmaceuticals are detected in surface waters in levels up to 1 µg/L (Tixier et al., 2003), in sediments (Furlong et al., 2003), and in organisms (Brooks et al., 2005). Due to their chemical and physical properties, many of these compounds end up in the sediment where they accumulate and may induce adverse effects on benthic organisms (Díaz-Cruz et al., 2003).

Since *D. polymorpha* possesses well functioning oxidative defense and has a relatively high resistance to xenobiotics, it is widely used for conducting ecotoxicological experiments (Faria et al., 2009, Pain and Parant, 2003). Its ability to respond to exposure to PhACs has been demonstrated in previous studies (Contardo-Jara et al., 2011).

A limited number of studies so far have tested ecotoxicological effects of human pharmaceuticals on aquatic organisms. Thus, there is evidence that pharmaceuticals can impact non-target organism in many ways even at very low concentrations and that more studies are needed to highlight and identify potential effects. In the present study, the invertebrate species *Dreissena polymorpha* was used as model organism to investigate adverse effects of sulfamethazine on mussels.

MATERIALS AND METHODS

Chemicals

Sulfamethazine was obtained from Sigma–Aldrich (Munich, Germany).

Model organism

D. polymorpha mussels (22–25 mm) were collected in spring after spawning in 1 m depth from Keban Dam Lake, located in a remote area 40 km north of Elazığ. Lake water temperature was 20 °C. During a two-week acclimatization period, mussels were kept in 10 L glass tanks each with approximately hundred individuals at 17 ± 0.5 °C. Photoperiod was set at 12:12 h (light:dark).

Two days prior to exposure mussels were transferred into the flow-through system (9 L tanks (30 cm × 20 cm × 14.5 cm) containing 7 L AFW). Nutrition, water, temperature and light conditions were maintained constant during the time of acclimatization and exposure.

Fig-1 *D. polymorpha*

D. polymorpha Mussels were exposed for 96 to increasing concentrations of sulfamethazine (0,1; 0.25; 1; 4 g L^{-1}). Five tanks of 10 mussels each were used for every exposure concentration and the respective control.

The tissues were divided into two groups to determine GSH and lipid peroxidation levels. In the first group examples, GSH were determined. For this purpose, the samples were weighed first and homogenized using a homogenizer with ice, adding 1/5 w/v of PBS buffer (phosphate buffered saline solution) (pH 7.4). The second group of tissues was used for lipid peroxidation analysis. The tissues were homogenized in 1.15% KCI (potassium chloride) at 1/10 w/v. The homogenized samples were centrifuged at 17000 rpm for 15 minutes in a refrigerated centrifuge and the supernatants were stored at -70 °C deep-freezing. Lipid peroxidation levels (as MDA) in the tissues were measured with the thiobarbituric-acid reaction using methods described by Placer et al. (1966). GSH levels were measured according to Sedlak and Lindsay (1968).

Statistical analysis

Data were analyzed using PASW Statistics 18.0 (SPSS Inc., Chicago, IL, USA). One way ANOVA and the Duncan's multiple range tests were employed to evaluate the statistical differences amoung all groups (A (0,1g/L Group), B (0,25 g/L Group), C (1 g/L Group), D (4 g/L Group) and Control Group) in same exposure time ($^{abc}p< 0.05$).

RESULTS

Table-1. Biochemical parametres of *D. polymorpha* mussels exposed to Different concentration of Sulfametazine.

	MDA	GSH
Control	26,46±6,56^{b}	2,13±0,038^{C}
A	47,71±11,30^{ab}	4,84±0,061^{a}
B	43,61±7,52^{ab}	1,74±0,064^{d}
C	60,54±1,97^{a}	1,83±0,038^{cd}
D	46,28±6,22^{ab}	4,36±0,22^{b}

The letters a, b, c and d in the top icon indicate the difference between the groups.

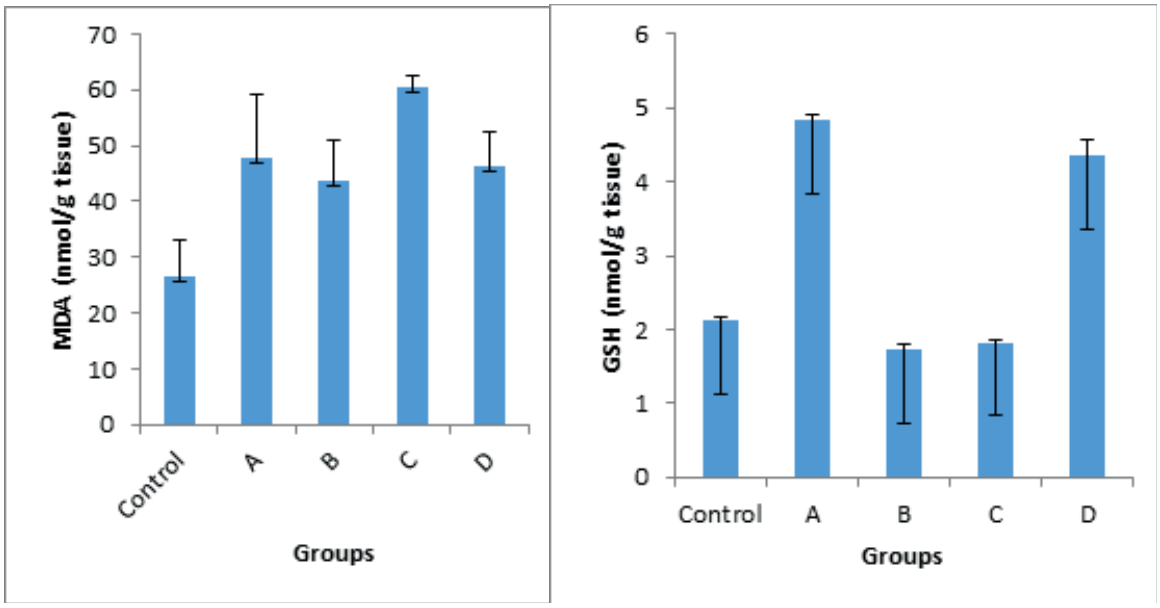

Fig-2 Biochemical parametres of *D. polymorpha* mussels exposed to Different concentration of Sulfamethazine

MDA Levels

MDA levels in all application groups were increased significantly depending on sulfamethazine exposure after 96 h ($p<0.05$)

GSH levels

GSH levels were significantly increased A and D groups ($p<0.05$) but significantly decreased in B and C groups after 96 h ($p<0.05$).

DISCUSSION

Due to anthropogenic activity, large amounts of pollutants such as pesticides, heavy metals and organic contaminants are released into the environment. Pharmaceuticals are an additional group of chemicals, which are discharged into aquatic systems (Kolpin et al., 2002). Although the pharmaceuticals occur in the aquatic environment at low concentrations, in the nanogram per liter or microgram per liter range, they were assessed to pose potential environmental risks (Rohweder, 2003), due to either high persistence or biological activity (Fent et al., 2006). In addition, our knowledge regarding the influence of pharmaceutical mixtures on aquatic organisms remains limited. As the aquatic environment is usually contaminated with a mixture of drugs and not only single compounds, it is important to study possible effects of a single drug. We investigated the effects of sulfamethazine as a single compound on biochemical parametres of *D. polymorpha.*

Due to its abundance in many European and American fluvial habitats, its relative long life span and great ability to bioconcentrate toxic chemicals, the freshwater bivalve zebra mussel (*Dreissena polymorpha*) has been used extensively as sentinel species to monitor persistent organic contaminants and metals (Berny et al., 2002).

Failure of antioxidant defenses to remove exogenous ROS produced by redox cycling chemicals either by being inhibited by those compounds or overwhelmed by an excess ROS, will disrupt the balance between the antioxidant/pro-oxidant system within the organisms leading to oxidative damage (Livingstone, 2003). In this study oxidative tissue damage was evaluated determining lipid peroxidation measured as MDA. Quin et al., (2011) investigated the effects of the pharmaceuticals gemfibrozil and diclofenac

on biomarker expression in the zebra mussel (*Dreissena polymorpha*). Exposure to gemfibrozil and diclofenac at 1 and 1000 µg L^{-1} concentrations significantly increased the level of lipid peroxidation, a biomarker of damage. Sampaio et al., (2016) investigated the effects of Dietary Exposure to Sulfamethazine on the Hematological Parameters and Hepatic Oxidative Stress Biomarkers in Nile Tilapia (*Oreochromis niloticus*). The sulfamethazine-fed group showed the same hepatic lipid peroxidation (LPO) concentration as the control group. Condarto-jara et al., (2011) tested Carbamazepine (CBZ), Ibuprofen (IBU) and Bezafibrate (BEZ) for their potential to bioaccumulate and provoke oxidative stress in the non-target organism *Dreissena polymorpha.* Ramesh et al., (2018) investigated antioxidant status, biochemical, and hematological responses in a cultivable fish *Cirrhinus mrigala* exposed to an aquaculture antibiotic Sulfamethazine. Plasma LPO level was found to be increased in Treatment I, and II throughout the study period (except 14th Treatment II) (The values were ranged from 2.9 to 4.0 n mole MDA/mg protein and a maximum LPO activity was found on 28th day of sulfametazine exposure period. Kliminova et al., (2018) analyzed the indices of oxidative stress activity in freshwater bivalve *Dreissena polymorpha* (Pallas) from areas of the Rybinsk Reservoir with different levels of anthropogenic load. Mussels that were collected in the most polluted part of the reservoir, had a higher activity of catalase, glutathione S-transferase, and glutathione reductase and a higher content of malondialdehyde than zebra mussels taken from the relatively clean Volga stretch. Antre et al., (2017) investigated the cumulative effects of ibuprofen and air emersion in zebra mussels Dreissena polymorpha. They revealed that mussels exposed to ibuprofen had increased the Lipid peroxidation levels.

GSH is an important defence molecule and radical scavenger. The alteration at GSH level is an important indicator of cellular stress. GSH levels decreased in mussels exposed to methyl mercury, were unaffected by inorganic mercury ($HgSO_4$) and increased in individuals exposed to Cd and Aroclor 1260 (Faria., 2008) . Recently, Lehmann et al. (2007) and Osman et al. (2007) also found increased levels of GSH in Asiatic clams (*Corbicula fluminea*) and in zebra mussels exposed to Aroclor 1260 and sediment extracts contaminated with PCBs and polycyclic aromatic hydrocarbons, respectively. *D. polymorpha*, were exposed to extracts of sediments obtained from two sites,a contaminated lake (Ketelmeer, Km) and a relatively clean lake (Drontenmeer, Dm). Osman et al., (2006) After a short (24 h) and a long-term (7 days) exposure, A 4-fold decrease of total glutathione concentration relative to the control, were observed in the gills of mussels exposed to the more contaminated Km extract.

Parolini et al., (2014) investigated the oxidative alterations of the freshwater bivalve *Dreissena polymorpha* induced by a 14-d exposure to an environmentally relevant mixture of the most common illicit drugs found in the aquatic environment, namely cocaine (50 ng L−1), benzoylecgonine (300 ng L−1), amphetamine (300 ng L−1), morphine (100 ng L−1) and 3,4-methylenedioxymethamphetamine (50 ng L−1). They indicated that the illicit drug mixture caused a slight variation in lipid peroxidation.

Paraloni et al.(2011) evaluated the cytogenotoxicity of paracetamol on the zebra mussel (*Dreissena polymorpha*). The biomarker battery demonstrated moderate cyto-genotoxicity in zebra mussel hemocytes since no primary DNA fragmentation was measured by the SCGE assay and only a slight increase in fixed DNA damage was registered by apoptotic and MN frequencies.

CONCLUSIONS

Our results showed that sulfamethazine at realistic environmental concentrations can impair the oxidative status of the zebra mussel, posing a serious hazard to the health status of this bivalve specie.

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