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Effects of Iron Chloride/Zeolite on GST of Rainbow Trout (Oncorhynchus mykiss)'s Kidney Tissue

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Abstract

Aquatic ecosystems have been negatively affected by the contamination of ground and surface waters as a result of various activities. Due to the ferrous chloride ($FeCl_2$), which is used as the reducing agent for the organic synthesis reactions in the contamination of water column and sediment, iron salts may be very toxic for some aquatic organisms. In order to minimize these effects, natural products such as zeolite have been widely used in recently years. For this reason, rainbow trout were exposed to $FeCl_2$ and/or zeolite (($FeCl_2$ (0.002 mg/l)(A), $FeCl_2$+zeolite (0.002 mg/l+1 gr/l) (B), zeolite (1 gr/l) (C) and control (without $FeCl_2$ and/or zeolite (D)). At the end of the exposure time, 28 days and their oxidative stress responses were investigated. At the end of the treatment period, glutathione-S-transferase (GST) activity was determined in the samples taken from kidney. GST values for kidney tissues were found statistically important in the control and treatment groups ($p<0.01$).

Key words

Ferrouschloride, Zeolite, Fish, Toxicity, Oxidativestress, Enzyme, Detoxificant

1. INTRODUCTION

Pollutant aspects which accumulate in seafood and which are hazardous to health and which also destroy the ecological balance can be identified as pesticides, some organic materials, industrial wastes and artificial agricultural fertilizers [1]. These chemicals, either natural or synthetic, are known to have toxic effects on both humans and other organisms [2]. Contamination from xenobiotics, a threat to the aquatic ecosystems, affecting nontarget inhabitants or causing ecological imbalances [3]- [5]. Aquatic organisms especially fish can also serve as a sensitive bioindicator of environmental contaminants, including the presence of xenobiotics, toxins, and other alterations in natural water quality parameters [2]. Because they can be found everywhere in the aquatic environment and they play a major role in the aquatic ecosystem. [6].

Living things are equipped with an antioxidant defense system (ADS) in order to be protected against oxidative stress. Oxygen is a very reactive molecule due to its electron affinity, and more reactive intermediate compounds are formed during the reduction of O2 into H2O. Antioxidant enzymes are components which are induced by oxidative stress, and consist of endogenous enzymes (Superoxide dismutase (SOD), Glutathione peroxidase (GSH-Px), Glutathione-S-Transferase (GST), Catalase(CAT), Mitochondrial cytochrome oxidase system, hydroperoxidase) and exogenous enzymes (Vitamin E and C, some drugs) [7].

Ferrous molecule (Fe^{+2}), which has important roles in the living organism system, is found in different forms in the nature and mostly taken in through drinking water and food. It is known that the increase of Fe molecule in the body increases the radical production and also has a role in the increment of the hydroxyl radical, which is effective particularly in the lipid peroxidation of $FeCl_2$, and hydroxyl- like radicals. Interest for the natural products has increased in order to prevent the negative effects of materials such as iron and aluminum in the regulation of water quality parameters, and studies on the utilization of alternative products have intensified. Among these products zeolites, which are found in large reserves in

nature, are widely used for the elimination of heavy materials in water thanks to their sodium aluminoslicade and clay minerals, their ability of ion exchange and detraction of cations and their low cost [8] -[11].

The objective of the current study was to investigate the chronic sub lethal effects of iron chloride/zeolite, using oxidative stress response as an ecologically relevant endpoint. This study is a contribution for aquatic toxicological risk assessments study.

2. MATERIALS AND METHODS

2.1. Fish maintenance and experimental design

Fish were obtained from Ataturk University,Faculty of Fisheries, Inland Water Fish Applicationand Research Center and the study was conducted atFisheries Application and Research Center'sToxicology Experiment Unit during 28 days.Fiberglas tanks of 1 m diameter and 1 m depth withinclined tube drainage system and 40 rainbow trout(*Oncorhynchusmykiss*) of two years old and 165±25g weight were utilized in the research.Filtered water was distributed to the tanks as noless than 0.5 l/min for kg fish. During the research,water temperature was measured as 11.5±2.5 ^{o}C, pHwas 7.4 and dissolved oxygen was 9.1 mg/l. Fishwere randomly distributed to 8 tanks with 5 fish pertank. Two of the tanks were determined as controland the other 6 tanks were the treatment groups.

$FeCl_2$ application dose $LC50_{96}$ value was utilized and ½ (0.002 mg/lt) of this dose was applied to tanks[12]. Stock solutions of$FeCl_2$, obtained from a company (Sigma), wereprepared with ultra distilled water and were appliedto the tanks with determined water volume accordingto the experiment procedure of renewed environmentin the concentration to form this dose once every 12hours. Zeolite was determined as 1 g/lt covering thetank floor [8]. Control and treatmentgroups were designed as ($FeCl_2$ (0.002 mg/lt)(A),$FeCl_2$+zeolite (0.002 mg/lt+1 gr/lt) (B), zeolite (1gr/lt) (C) and control (without $FeCl_2$ and/or zeolite) (D). Enzyme activity of GST was determined in kidney tissues for all groups.

2.2. Enzyme Analyses

At the end of the trial, treatment and controlgroup fish were euthanized by cervical section, theirkidney tissues were taken and freezed inliquid nitrogen and then tissue samples were waited at-86 °C. These tissue sampleswere storaged in ice for 5-15 min at room temperatureto thaw, afterwards, weighted on a precision scalebetween the ranges of 0.5-1 g and completely washedin 0.9% NaCl solution. KH_2PO_4 buffer solution ofthree times weight of the sample was added on thetissue samples splintered into small pieces. Sampleswere homogenised and centrifuged at 13000 rpm foran hour at 4^{o}C. Supernatants were taken and theirenzyme activities were measured [13].

GST activity was assayed by the method of Habig et al. [14]. Using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate; the final reaction mixture contained 1 mM CDNB and 1 mM reduced glutathione (GSH) as determined at 340 nm. Malondialdehydeseconder product is formed as a result ofmalondialdehyde (MDA) lipid peroxidation. Themeasurement is based upon the absorbancemeasurement of the pink complex at 532 nm, formedas a result of the incubation of MDA withthiobarbituric acid (TBA) at 95 ^{o}C [15]. According toBradford [16], protein levels of each sample weredetermined spectrophotometrically at 595 nmwavelength were recorded by determining the bovineserum albumin (BSA) as the standard.

2.3. Statistical Analyses

The obtained data were expressed as mean±SEM.Statistical analysis of data was done using Duncantest and analyzed using SPSS version 10.0 software.A value of $p < 0.01$was considered statisticallysignificant.Lowercase superscripts (a, b) indicate significant differences among experimental treatment group, Each value is the mean±SEM of five individual observations. Enzyme is EU mg protein^{-1}, MDA (nmol/mg prot.).

3. RESULTS AND DISCUSSION

The results of this study indicate that treatment of $FeCl_2$ and/or zeolite in *O. mykiss* did cause a significant alteration on GST enzyme activities in the treatment groups (A, B, C), compared to the non-treatment group (D: control) at the end of the 28 days. At the end of the chronic exposure, the activity of GST enzyme activities in the kidney tissues were increasedin $FeCl_2$+zeolite group (Fig 1). In contrast, MDA content in the tissue decreased due to $FeCl_2$+zeolite (Fig 2).

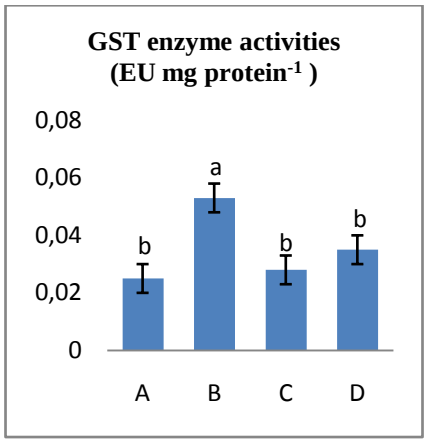

Figure 1. GST activity in kidney

Inthisstudy, the GST activity of rainbowtrout, whichwasexposedto $FeCl_2$orzeolite in differentoncentrations, decreased in smallconcentrationswhiletherewasnodifference in groups.

Manyorganisms have unique systems for protecting themselves against the damaging effects of activated ROSs[17]. SOD, CAT and GSH cycles (glutathioneperoxidase, glutathionereductase, Glutathion S- transferase) convert the well-recognized reactive oxygen radicals into less toxic products. Inourstudy, emphasiswas put on the GST enzymeactivity and MDA levels of the liver. Glutathioneconjugate GST, which is considered as thefirst step in thedetoxification of contaminants, is produced in theliver of someanimals. The most important characteristic of ADS is that all components of the system function mutually against (ROS). Therefore, antioxidant enzymes play a vital role in there gulation of cellular balance and their induction is the result of a reaction against, while antioxidant enzyme activities and lipidperoxidation are importantindicators in analysingcellulardamage in toxicologicalstudies[7].

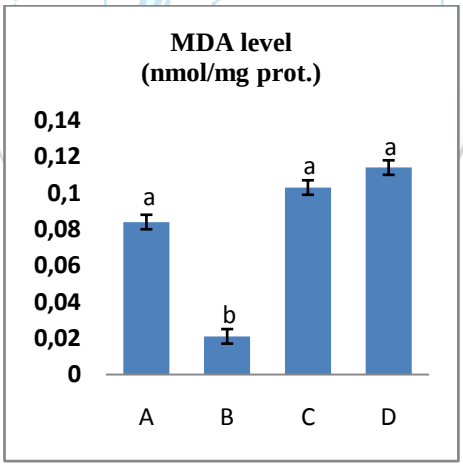

Figure 2. MDA level in kidney

In the present study, exposure to $FeCl_2$+zeolite decreased the MDA levels in the kidney tissues of rainbow trout, which suggests that $FeCl_2$+zeolite, in applied concentrations, reduce peroxidative tissue damage. Decreasing of MDA suggests that ROS inhibited damage may be one of the main detoxic effect of $FeCl_2$+zeolite. The lipid hydro-peroxides formed as a result of lipid peroxidation are broken down to form aldehydes which are mostly biologically active materials. These compounds are either metabolized at the cellular level or they will diffuse from their initial effective region to spread the damage to the other sections of the cell. In determination of these damages, malondialdehyde (MDA) is used, which is a substance that can be frequently measured with thiobarbituric acid. MDA is not a specific or a quantitative indicator of the oil acid oxidation, but it shows a good correlation with lipid peroxidation. The highest MDA values for kidney tissues of rainbow trout have been received in the $FeCl_2$ or zeolite treatment groups and the differences between the $FeCl_2$+zeolite groups have been found to be statistically meaningful.

4. CONCLUSION

The data of this study will help in understanding the biochemical basis of responce of rainbow trout treatment $FeCl_2$or/and zeolite detoxic mechanism. More investigations must be performed to better understand the specific oxidative stress and toxic response of aquatic organism.

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