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Hematological, biochemical and enzymological responses in an Indian major carp *Labeo rohita* induced by sublethal concentration of waterborne selenite exposure

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ABSTRACT

Selenium (Se) pollution in aquatic ecosystem is an environmental issue throughout the world. Elevated concentrations of inorganic Se from agricultural and industrial processes may cause adverse biological effects in aquatic organisms such as fish. In the present study, *Labeo rohita* an Indian major carp were exposed to sublethal concentration of Se (sodium selenite) for 35 days and certain hematological, biochemical and enzymological parameters were estimated. The median lethal concentration of waterborne sodium selenite (Na_2SeO_3) to *L. rohita* was found to be 23.89 mg L^{-1} for 96 h. During sublethal (2.38 mg L^{-1}) treatment, hematological and biochemical parameters such as hemoglobin (Hb) (except 14th day), hematocrit (Hct), erythrocyte (RBC) count and protein levels were found to be decreased in Se treated fish whereas leucocyte (WBC) count and glucose level increased in Se treated fish throughout the study period. The enzymatic parameters such as glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and lactate dehydrogenase (LDH) activities were found to be increased in liver of Se treated fish *L. rohita*. A biphasic response was observed in the value of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). The alterations of these parameters can be used as suitable biomarkers in monitoring of selenium in the aquatic environment and to protect aquatic life.

1. Introduction

Metals and their components are considered to be a major ecological health concerns worldwide [1–3]. Selenium (Se) is a naturally occurring non metallic element and exists in the environment in different forms; they are selenide (Se [II]), elemental selenite (Se [IV] or SeO_3^{2-}) and selenate (Se [VI] or SeO_4^{2-}) [4–6]. The inorganic and/or organic selenium compounds are essential for the development of the acquired immune system in many organisms [7]. Moreover, Se is an important element of many protein molecules with diverse physiological functions [8,9]. However, anthropogenic activities such as metal mining and smelting, coal combustion, and agriculture may leads to large quantities of Se in aquatic environment [10,11]. High concentrations of Se were detected in surface water, sediments, and aquatic organisms in different parts of the world and are an environmental issue throughout the world [9]. In the dynamic aquatic ecosystem selenium can be cycled back into the biota and continue to high levels for many years [12]. Furthermore, Se can be ingested by organisms or binds with particulate matter, or be free without binding to any substance [13]. The accumulation of selenium in aquatic organisms may leads to hematological alterations, tissue pathology, reproductive failure, teratogenic deformities and cytotoxicity [11,14,15].

In aquatic ecosystem, Se contamination is more challenging than any other chemical contamination [16]. Moreover, all chemical form of Se has different toxicological and biological properties [17,18]. Generally, fish model is widely used to study Se toxicity due to their higher nutritional requirement of selenium than mammals [8]. Moreover, the biochemical pathways involved in selenium metabolism in fish are almost unknown. In toxicological assay, hematological and biochemical parameters are widely used as health indicators, because they react before the toxicant enters into the body of the organism [19,20]. Hematological parameters such as hematocrit (Hct), hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs), are used to assess the functional status of the oxygen carrying capacity and also indicate secondary responses of an organism to irritants such as metals [21,22]. The erythrocyte blood indices such as MCV, MCH and MCHC are widely used to diagnose anemia in animals under stress conditions [23].

Likewise, biochemical parameters such as protein and glucose are highly sensitive to stress conditions and often used in detection of status of stress condition [24]. Furthermore, enzymological parameters are frequently used to assess the impact of metals in

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aquatic organisms. Enzymes such as, carboxyl esterase (CE), lactate dehydrogenase (LDH), alkaline and acid phosphates (ALP, ACP), transaminases (ASAT or GOT and ALAT or GPT) are considered to be useful biomarkers in biomonitoring of chemical pollutants in aquatic organisms [25,26]. The alterations of transaminases activity can be taken as a measure of compensatory mechanism to impaired metabolism [27]. Likewise, LDH activity is used as a biomarker in the assessment of stress condition in various organisms [28,29] and serves as a good diagnostic tool in toxicology.

In India during recent years aquaculture receives much attention than the agriculture activity. The Indian major carps, such as catla (*Catla catla* Ham.), rohu (*Labeo rohita* Ham.) and mrigal (*Cirrihinus mrigala* Ham.) are cultivated almost in all Indian fresh water aquaculture farms [30]. In many feed additives, selenium is added as a vital element to preserve health and to maximize animal productivity [31,32]. To our knowledge the impact of selenium toxicity on Indian major carps are very limited. Hence, the present study is aimed to evaluate the sublethal concentration of sodium selenite on hematological, plasma biochemical and enzymological activities of an Indian major carp, *L. rohita*. The fish *L. rohita* is a widespread species and cultured throughout India. They are widely used in carp polyculture systems and have a higher consumer preference and market demand. Moreover, the selected biomarkers endpoints can be used in environmental monitoring of selenium contamination in aquatic ecosystem.

2. Materials and methods

The Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore 46, Tamil Nadu, India, has been registered with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The experiments and the handling of the organisms were carried out as per the guidelines of CPCSEA.

2.1. Collection of fish and maintenance

Specimens of *L. rohita* (length 8.0 ± 0.6 cm and weight 11.0 ± 1.5 g) were obtained from Tamil Nadu Fisheries Development Corporation Limited, Aliyar Fish Farm, Tamil Nadu, India and safely brought to the laboratory. The fish were acclimatized to laboratory conditions for couple of weeks in a large tank (1000 L capacity) and fed *ad libitum* with rice bran and groundnut oil cake in the form of dough once in daily. Water was renewed (one – third of the water) daily to avoid accumulation and contamination of excretory materials. Feeding was withheld for 24 h before to the commencement of the experiment to keep the fingerlings more or less in the same metabolic state. Fish showing any abnormal behavior was removed from water tank as soon as possible. Tap water free from chlorine was used with the following physicochemical characteristics [33]; temperature (27.3 ± 1.2 °C), pH (7.1 ± 0.05), dissolved oxygen (6.3 ± 0.02 mg L^{-1}), total hardness (18.9 ± 0.3 mg L^{-1}) and salinity (0.3 ± 0.05 ppt). Prior to the commencement of experiment, healthy fingerlings were collected randomly and transferred into two glass aquaria (200 L capacity) which were continuously aerated. The study was conducted in 12:12 light–dark cycle.

2.2. Toxicant

Sodium selenite (anhydrous, Na_2SeO_3) was obtained from M/S Loba chemic Pvt. Mumbai, India and used without further purification for the experiment.

2.3. Toxicity assessment and determination of 96 h LC50 of sodium selenite

Preliminary toxicity tests were conducted for the determination of 96 h median lethal concentration of Na_2SeO_3 to *L. rohita*. Separate glass tanks (50 L capacity) were taken and different concentrations of Na_2SeO_3 (5, 10, 15, 20 and 25 mg L^{-1}) were added. Then, to each tank, 10 healthy fish randomly collected from the stock were introduced. To each concentration three replicates were maintained. Control groups (toxicant free) were also maintained simultaneously with three replicates for each concentration. The mortality/survival of fish was recorded at the end of 96 h and the concentration at which 50% mortality of fish occurred was taken as the median lethal concentration (LC50), which was 23.89 mg L^{-1}. The LC 50 concentration was calculated by probit analysis method of Finney [34].

2.4. Sublethal toxicity studies (1/10th of 96h LC 50 of Na_2SeO_3)

To assess the sublethal toxicity of Na_2SeO_3, 300 healthy fish were selected from the stock and divided into three groups (one control and two experiments) and then introduced into three separate aquarium tanks (100 fish in each tank). 1/10th of 96 h LC 50 value of Na_2SeO_3 (2.38 mg L^{-1}) was added directly into two experimental aquarium tanks after removal of the same volume of water. Experiment was conducted for a period of 35 days. The concentration of Na_2SeO_3 (2.38 mg L^{-1}) in experimental tanks were renewed daily in order to maintain constant concentration of the Na_2SeO_3 after removal of the same volume of water. Fish were fed *ad libitum* every day. At the end of 7th, 14th, 21st, 28th, and 35th day of exposure fish were randomly collected from control and experiment aquarium for the study of hematological, biochemical and enzymological assay. No mortality was observed during the exposure period.

2.5. Preparation of samples and analytical procedures

2.5.1. Blood samples

Cardiac blood were collected in plastic disposable syringes fitted with 26 gauge needle which was pre-chilled and coated with heparin and expelled into separate heparinised plastic vials and kept immediately on ice. The whole blood was used for the analysis of hematological parameters (Hb, Hct, RBCs, WBCs,) and the remaining of the blood samples were centrifuged at 93.9*g*, at 4 °C for 20 min to separate the plasma, which was used for the estimation of biochemical parameters (glucose and protein).

2.5.2. Organ samples

After drawing the blood, fish were washed thoroughly in double distilled water and blotted dry with absorbent paper. 100 mg of liver tissues were separated from the control and Na_2SeO_3 exposed fish and homogenized with 1.0 ml of 0.1 M Tris–HCl buffer (pH 7.5) using a Teflon homogenizer in ice cold condition, and then centrifuged at 93.9*g* at 4 °C for 15 min. The supernatant was used for the enzymological assay (GOT, GPT and LDH).

2.5.3. Hematological analysis

Hemoglobin content of the blood was estimated by cyanmethemoglobin method [35]. Hematocrit was estimated by microhematocrit (capillary) method [36]. RBC and WBC were counted by hemocytometer method of Rusia and Sood [37]. Erythrocyte indices like MCV, MCH and MCHC were also calculated according to standard formulas.

$$\mathrm{MCV(cupic\ micra)} = \frac{\mathrm{Hct}(\%)}{\mathrm{RBC(millions} \times \mathrm{cu\ mm} \times 10^6)} \times 100 \qquad (1)$$