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Acute and sublethal effects in an Indian major carp *Cirrhinus mrigala* exposed to silver nitrate: Gill Na^+/K^+-ATPase, plasma electrolytes and biochemical alterations

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ABSTRACT

Due to prolonged use of silver in many applications, it enters into the freshwater and affects the aquatic organisms. Fingerlings of *Cirrhinus mrigala* were exposed to acute and sublethal concentrations of silver nitrate and the alterations of gill Na^+/K^+-ATPase, plasma electrolytes and biochemical parameters were assessed. The median lethal concentration of silver nitrate to the fish *C. mrigala* for 96 h was found to be 0.107 mg/l (with 95% confidence limits). 1/10th of LC 50 96 h value (0.0107 mg/l) was selected for sublethal study. During acute treatment branchial Na^+/K^+-ATPase activity was inhibited approximately 44.34% after 96 h of exposure. In sublethal treatment, silver nitrate could not produce a significant change in the activity of the enzyme at the end of 7th day. However, after 14th day, significant ($p < 0.05$) decrease was noted showing 22.52%–49.11% in rest of the study period. Silver intoxication resulted hyponatremia, hypokalemia, hypochloremia, and hypoproteinemia in both the treatments. Despite the decrease in these parameters, plasma glucose level was found to be increased in both the treatments to endure the silver toxicity. We suggest that the alterations in branchial Na^+/K^+-ATPase activity, plasma electrolytes, and biochemical parameters of fish may be useful in environmental biomonitoring and to assess the health of fish in freshwater habitat contaminated with silver.

1. Introduction

Heavy metals and their components from natural and anthropogenic activities contaminate the aquatic environment all over the world [1,2]. These heavy metals are considered as a core group of aquatic pollutants due to their toxicity and accumulative behavior [3]. Silver is discharged into the aquatic environment through its various industrial applications such as mining, photographic processing, manufacture of silverware and jewellery, effluents from sewage treatment plants and medical wastes as nanoparticles (AgNPs) [4–8]. In the aquatic environment silver may exist in a variety of chemical forms and has received much attention due to its toxicity to aquatic organisms at low concentrations [9]. Silver in its ionic form (Ag^+) is highly toxic to freshwater fish, due to its accumulation in gills and causing an inhibition of carbonic anhydrase and branchial Na^+/K^+ ATPase activity [10–13]. The inhibition of branchial Na^+/K^+ ATPase activity due to silver toxicity leads to ionoregulatory disturbances, circulatory collapse and finally death [10,14,15]. The Na^+/K^+ ATPase molecules which are located at the basolateral membrane of the gill epithelium is highly sensitive to silver and may be the key target for silver ion [6,12].

Due to extensive use of silver in many industrial applications several studies have been conducted to assess the silver toxicity in aquatic environments [15–19]. However, most investigations have been limited acute silver toxicity in aquatic environments [20,21]. Fish is one of the chief sources of food all over the world due to its nutritional values like essential protein, polyunsaturated fatty acids and liposoluble vitamins [22]. Moreover, fish are highly sensitive to environmental changes and thus the health condition of the fish may reflect the status of the aquatic ecosystem [23,24]. An understanding of the impact of metal toxicity both at short and long term effects on fish play an important role in establishment of water quality criteria and environmental health risk assessment [25–27]. Moreover, use of biomarkers for monitoring both environmental quality and the health of organisms are widely used as early diagnostic tools [26,28,29]. The biomarkers such as serum levels of metabolites, ionoregulatory disturbances, hormonal changes and biochemical alterations are widely used for monitoring of water pollution [30–32]. Likewise serum parameters, such as glucose, protein, and cholesterol, are widely used as stress indicators in aquatic environment [32].

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The contamination of aquatic environment by industrial and agricultural chemicals may cause significant tissue damage in fish and ultimately affect the activity of enzymes. Alterations in the activity of enzymes are routinely used as an index reflecting metal toxicity or in biomonitoring of chemical pollutants. Na^+/K^+-ATPase is a membrane bound enzyme and used as a sensitive indicator of environmental contaminants [33]. Gill Na^+/K^+-ATPase is involved in osmoregulation of fish particularly for ion homeostasis at the cellular and organism levels and can used as a sensitive biomarker for evaluation of the membrane fragility of the gills [34,35]. The gill is considered the proximate site of silver uptake in freshwater fish and the binding of silver with gill may inhibit the enzyme Na^+/K^+-ATPase leading to a net loss of Na^+ and Cl^- ions [10,11,36].

In aquatic organisms, maintenance of constant internal ion concentration is necessary for active regulation of water influx and ion efflux [37]. Inorganic ions such as sodium, potassium and calcium are important for the normal metabolic/physiological functioning of an organism. Na^+ and Cl^- are the major cation and anion of the extra cellular fluid, respectively whereas; K^+ is the major cation of the intracellular fluid [38]. These ions are very sensitive to environment stressors and their measurement in blood of aquatic organisms can be used to monitor the polluted water bodies [39,40]. Any imbalance in the levels of these ions in fish may leads to impairment of various physiological activities. Hence, the measurement of ion levels in blood of fish provides an appropriate biomarker to environmental stressors.

Measurement of biochemical parameters is a commonly used diagnostic tool in aquatic toxicology particularly in fish exposed to sublethal toxicity of different toxic substances [41]. The alterations of biochemical blood profile also used in the detection and diagnosis of metabolic disturbances and disease processes [42]. Among the biochemical parameters, plasma glucose and protein are widely used to assess the toxic stress [43]. Glucose is an important source of energy in fish and the alterations of blood glucose can be employed as general indicators of stress in teleosts. Blood glucose can be utilized as a parameter of stress response, as it is rapid, practicable and quantitative [44]. Similarly, proteins performing different biological functions are greatly influenced by environmental stressors. Measurement of proteins in blood can be used to determine the physiological phases of organism and mostly used as an indicator for general state of health [45,46].

Among the different forms of silver, silver nitrate is used in various industrial applications, for instance in electronics, photographic industry, ink manufacturing, coloring porcelain etc. The literature on the toxicity and effects of silver nitrate on gill Na^+/K^+ ATPase, plasma electrolytes and biochemical parameters of Indian major carps are scanty. Hence, the present investigation is aimed to evaluate the acute and sublethal effects of silver nitrate in the fingerlings of an Indian major carp, *Cirrhinus mrigala*. This carp is endemic to Indo-Gangetic riverine systems and cultured mainly as a component of carp polyculture systems in the ponds and also an edible fish in India. About 900,000–1,000,000 ha of water bodies in India are ponds and tanks and used for carp culture. It grows very quickly and hence the fish is cultivated in fish farms on large scale [47]. Moreover, the selected biomarkers endpoints could be used to determine the adverse effects of silver on *C. mrigala* and also developing monitoring strategies.

2. Materials and methods

The Department of Zoology, School of Life Sciences, BharathiarUniversity, Coimbatore-641046 has been registered with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The experiments and handling of organisms were carried out as per the guidelines of CPCSEA.

2.1. Fish specimen and maintenance in the laboratory

Specimens of *C. mrigala* were selected as an experimental animal model. Fish with an average weight of 8.0 ± 0.4 g and length of 6.5 ± 0.1 cm were purchased from Tamilnadu Fisheries Development Corporation Limited, Aliyar Fish Farm, Aliyar, Tamilnadu and India. They were safely brought to the laboratory in well packed polythene bags containing aerated water. Care was taken to minimize physical stress during transportation. After arrival, fish were stocked in a large cement tank (1000 L capacity) for a period of 20 days. During stocking period, fish were fed *ad libitum* with rice bran and ground nut oil cake in the form of dough once in daily. Feeding was given at least 1 h prior to replacement of water. The water (three forth of the water) was changed daily along with the waste feed and fecal material. Dechlorinated tap water with physico-chemical features such as; temperature 24.7 ± 1.2 °C, pH 7.2 ± 0.09, salinity 0.29 ± 0.1 ppt, dissolved oxygen 6.4 ± 0.04 mg/L and total hardness 17.5 ± 0.5 mg/L were used throughout the experimental period. After acclimatization, healthy fish were transferred to clean glass aquarium tanks (200 L capacity) which were continuously aerated. These fish served as the stock for experimental schedule.

2.2. Toxicity assessment and determination of 96 h LC50 value

Preliminary toxicity tests were carried out to find the median lethal tolerance limit of fish *C. mrigala* to silver nitrate for 96 h. Separate circular plastic tubs of 50 L of water capacity were taken and different concentrations of silver nitrate (0.05, 0.1, 0.15, 0.2 mg/L) were added. Then, 10 healthy fish were introduced into each tub, which were starved 48 h prior to the commencement of the experiment. 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 in control and silver nitrate tubs were recorded after 96 h. The concentration at which 50% mortality of fish observed after 96 h was considered as median lethal concentration (LC50) of silver nitrate, which was 0.107 mg/L. The LC50 concentration for 96 h was calculated by the probit analysis method [48]. Homogenicity of the population used in the present investigation was tested using chi-square test. The dead fish were removed immediately from the tank.

2.3. Acute toxicity studies

For acute toxicity study of $AgNO_3$, five tubs (50 L capacity) were taken, LC50 96 h concentration of the $AgNO_3$ (0.107 mg/L) was added to each tub and 15 fish from the stock were introduced. A common control was also maintained with similar setup. At the end of 96 h fish were randomly collected from control and $AgNO_3$ groups and blood was withdrawn for the estimation of plasma electrolytes (Na^+, K^+ and Cl^-), plasma glucose and protein. Concurrently, gills were removed for the estimation of Na^+/K^+-ATPase activity.

2.4. Sublethal toxicity studies

For sublethal studies, 200 healthy fish were selected from the stock and divided into two groups and then introduced into two separate aquarium tanks. 1/10th value of LC50 concentration for 96 h of silver nitrate (0.0107 mg/L) was taken as the sublethal concentration and added to each tank. Three similar replicates