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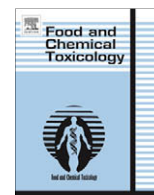

Effect of ferrous sulphate on aspartate and alanine aminotransferases of brain of *Tilapia mossambica*

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ABSTRACT

Iron in the form of ferrous sulphate coming from sources such as mines, writing inks, blue pigments, dyeing industries, photography, medicine, deodorizers, disinfectants, fungicides and molluscides, etc. contributes in elevating ferrous sulphate of water bodies. The present study investigated the action of ferrous sulphate on the local fish *Tilapia mossambica*. Tilapia exposed to 0.001 g/L ferrous sulphate for 30 days showed reduction of cytosolic AST and ALT activities of cerebral cortex by 35.4% and 29.1%, respectively, while exposure to 0.01% ferrous sulphate promoted 49.2% and 38.4% reduction of AST and ALT activities. Similarly mitochondrial AST and ALT activities reduced by 50% and 34.8%, respectively, on exposure to 0.001 g/L ferrous sulphate while 0.01 g/L ferrous sulphate promoted 51% and 47.8% reductions of AST and ALT activities at the end of 30 days, suggesting interference in the glutamate and protein metabolism of Tilapia brain.

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1. Introduction

Ferrous sulphate or ferrous sulphate heptahydrate occurs in nature as the mineral melanterite, either crystalline or fibrous, and appears to have formed by the oxidation of pyrite or marcasite. It is a constituent of mine effluents and acid mine drainage from mines (Maltby et al., 1987; Singh, 1987). When dissolved in water it readily gets degraded to form Fe (III) and sulphuric acid. The iron (III) hydroxide is precipitated out of solution as reddish brown precipitate normally known as ferric hydroxide that increases the turbidity of water (Adams et al., 2004). It is also used in preparations of writing inks, blue pigments, textile dyes, medicines, deodorizers, disinfectants, fungicides and molluscides, reducing agents, feed additives, fertilizer additives and soil acidifiers to prevent ferric chlorosis or iron deficiency (HCN:CUOEL, 2004).

Accidental spillage or discharges of untreated effluents or sludge containing iron into natural water bodies may have harmful effects on the fish population and other forms of aquatic life (Van Anholt et al., 2002; Sotero-Santos et al., 2007). On exposure to water contaminated with iron, the fish tend to accumulate iron in their tissues. Bioaccumulation of iron in fish is well documented (Patin, 1984; Mohamed et al., 1990; Lappivaara et al., 1999; Wepener et al., 2001; Burger et al., 2002). The increase of iron load in animal tissues may impair normal physiological processes of fish leading to serious health problems for fish and consumers at large. Lappivaara and Marttinen (2005) reported that waterborne iron promotes accumulation of iron in liver and gills with decreased haemocrit value, decreased levels of catecoholamine and β-estradiole levels in plasma of white fish. Further, they observed that fish shows lowered responses to the external threat which is likely to promote decline in local fish population. The lowered responses of the fish to external threats indicate neuronal dysfunction. Therefore, it was necessary to investigate the action of ferrous sulphate on the local fish *Tilapia mossambica*, with reference to aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities of brain as these enzymes of brain are involved in glutamate metabolism (Peng et al., 1998) and the impairment of it could lead to neuronal dysfunction.

2. Materials and methods

2.1. Biological material

The fish species, *T. mossambica*, were obtained from Dhaujem farm, Old Goa, India and maintained in glass aquaria of 1000 L capacity containing well-aerated unchlorinated ground water. Fish weighing 11.60 ± 2.18 g were transferred to a 100 L glass aquarium (180 × 90 × 90 cm) for 20 days for conditioning and fed with commercial dry feed pellets (Fish Dry Pellets; Waterbabies Products, Madgaon, Goa, India). The aquarium water was renewed daily, was aerated with an aquarium air pump (Jumbo-Jet, Super-8300, made in India) and the natural photoperiod of

Abbrevations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; AR, analytical reagent; L:D, light:dark; LC, lethal concentration; EC, effective concentration; DNPH, dinitrophenyl hydrazine; SD, standard deviation; NADH, nicotinamide adenine dinucleotide reduced form; ROS, reactive oxygen species.

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13:11 h (L: D) was maintained. The conditions for acclimatization and tests were maintained at: temperature 26 ± 2 °C, pH 7.10 ± 0.05 and dissolved oxygen 8.15 ± 55 mg/L. Following acclimatization to laboratory conditions for 20 days, the healthy male fish were used. Experiments were performed according to the guidelines of committee for the purpose of Control and Supervision of Animals in India and were approved by the Institutional Animal Ethics Committee of Goa University, Goa, India.

2.2. Test substance

Ferrous sulphate heptahydrate (CAS registry number: 7782-63-0, EU number: 231-753-5, AR grade purity 99.5%, common names: Green vitriol, Copperas, Melanterite) was used as a toxicant. LC_{50} for different exposure periods was determined by using probit analysis (Finney, 1971). The EC_{50} value of ferrous sulphate for *T. mossambica* was 0.3 g/L for 96 h. Considering the EC_{50} value, the sublethal doses of 0.001 g/L and 0.01 g/L were chosen for the present study. Similar concentrations of iron were found in a few water bodies in Goa (unpublished data). A set of five fish each for respective doses and exposure periods (15 days and 30 days) was used for both control and experiments. Thirty minutes prior to the termination of exposure period escape test was performed for each fish of every set. Escape test constituted of elucidation of escape response by tactile stimulation with the rod (4 mm diameter) which was presented randomly from both side of the caudal fin of a stationary fish, followed by an attempt to capture by hand trapping. This phenomenon was repeated three times for every fish with a gap of 5 min after each test. Escape response was noted in terms of fish being caught which served as no escape response or fish not being caught which served as successful escape response. At the end of exposure period fish were cold narcotised and dissected open to collect their cerebral cortices for enzyme assays and histological analysis.

2.3. Enzyme assay

For estimation of cytosolic enzymes, the frozen tissue was homogenized at 0 °C in 0.32 M sucrose solution containing 5 mM Hepes buffer (0.1 g/10 ml; pH 7.4). The homogenate was freeze centrifuged (4 °C) for 20 min at 12,000*g*. Undiluted supernatants were used for enzyme assay. Whereas for estimation of mitochondrial enzymes, cerebral cortices were homogenized in nine volumes of 0.32 M sucrose. Homogenate was centrifuged at 1000*g* for 10 min. The pellet thus obtained was washed twice and applied on a discontinuous sucrose gradient consisting of 0.8, 1.0, 1.2 and 1.4 M sucrose. After centrifugation at 75,000*g* for 2 h, the mitochondrial pellet was obtained at the bottom of the tube, which was used as sample source for estimation of mitochondrial enzymes. Aminotransferases were measured according to 2, 4 dinitrophenyl hydrazine (2, 4-DNPH) method. The incubation mixture for ALT contained 500 µl of alanine-α-ketoglutarate, 100 µl of enzyme extract, 500 µl of 2, 4-DNPH and 5 ml of 0.4 N NaOH. The incubation mixture for AST contained 500 µl of aspartate-α-ketoglutarate, 100 µl of enzyme extract, 500 µl of 2, 4-DNPH and 5 ml of 0.4 N NaOH. Optical density of corresponding brown coloured hydrazone formed in alkaline medium was read at 505 nm. Protein was measured by the method of Lowry et al. (1951) using bovine serum albumin as standard.

2.4. Histological investigation

For histopathological evaluation, cerebral cortices were embedded in paraffin and sections were cut on microtome at 8-µm. Sections were stained with hematoxylene and eosin. Histopathological changes were evaluated by light microscopy.

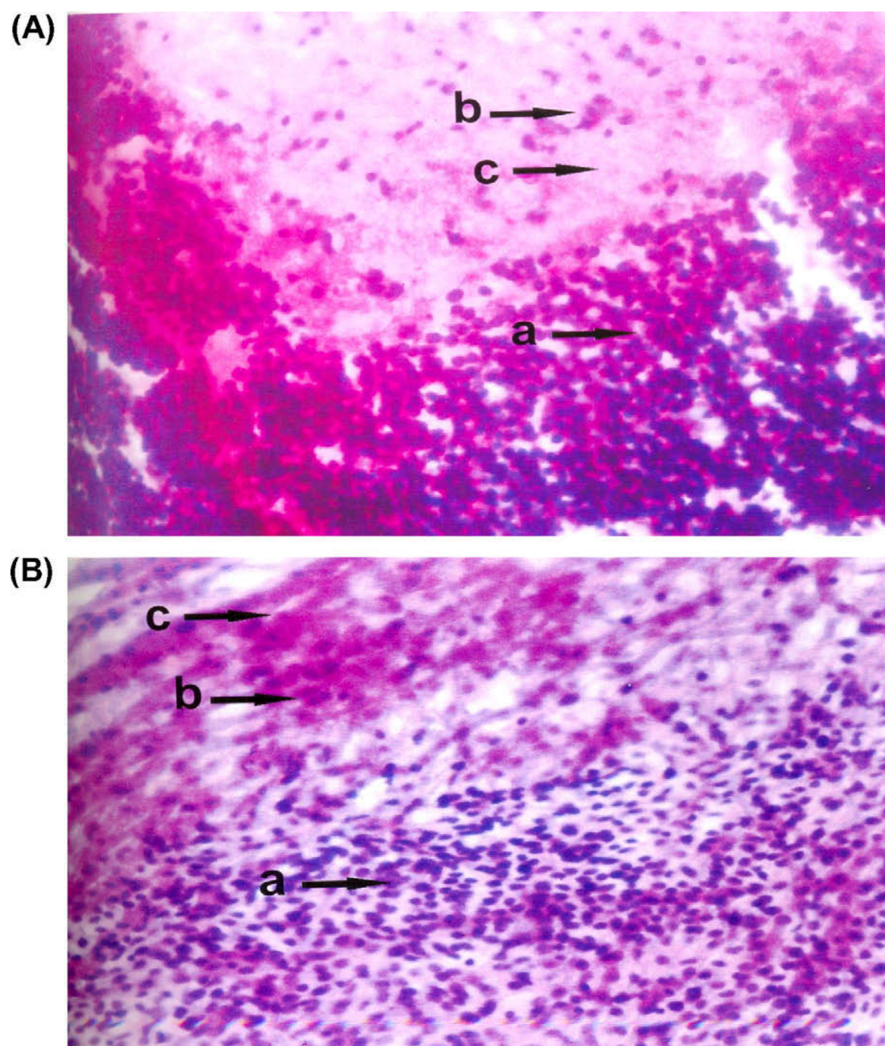

Fig. 1. (40×) (A): Section of cerebral cortex of control fish and (B): section of cerebral cortex of fish exposed to 1 mg/L of ferrous sulphate for 30 days of exposure period (a), granular cells; (b), Purkinje cells and (c), mossy mat of fibers.

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