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INTERACTION OF SODIUM FLUORIDE ON ALUMINIUM CHLORIDE INDUCED EFFECTS ON CHOLINESTERASE ACTIVITY IN TISSUES OF THE FISH *OREOCHROMIS MOSSAMBICUS*

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ABSTRACT

The combined action of sodium fluoride and aluminum chloride on Cholinesterase activity was studied by exposing fresh water fish *Oreochromis mossambicus* to varying concentration at different time intervals. In cerebellum, Cholinesterase activity decreased at 150ppm and 337.5ppm of aluminum chloride. In 225ppm of aluminum chloride no significant inhibition was found. In the brain tissue of medulla oblongata, Cholinesterase activity was significantly increased at ($P<0.05$) at 48 hrs and 96 hrs for 150ppm for aluminium chloride. The Cholinesterase activity significantly decreased ($P<0.05$) for 225ppm and 337.5ppm of aluminium chloride exposure the same was observed for all duration of exposures. Cholinesterase activity was significantly high at all duration of exposure. In the liver tissue the acetyl chloine activity decreased at all low concentration of exposure with respect to aluminium chloride. Whereas higher concentration (337.5ppm) significant ($p<0.05$) enzyme inhibition was decreased at 96hrs and 21 day of exposure. Sodium fluoride significantly ($P<0.001$) increased the enzyme activity at 24hrs and 96hrs of exposure alone. But the effect was counteracted when same animal was exposed to aluminium chloride for all duration of exposure periods.

Keywords: Induced effects, sodium fluoride, aluminum, cholinesterase, *Oreochromis mossambicus.*

INTRODUCTION

Aluminum is one of the most prevalent metals on the earth's crust. However in biological system, aluminium is present only in trace amounts and has no accepted role in normal physiological process. In addition there are several circumstances in which aluminium accumulation occurs within the biological systems that have proven to be harmful (Giteman, 1989). In addition to the low bioavailability of aluminum, the presence of physiological barriers also effectively hurdles the toxicological manifestation of aluminium within the biological systems.

Due to low bioavailability, not much attention has been paid on the effect of aluminum aquatic organisms. Consequently, the available information on the impact of aluminum and its interaction with other substances on aquatic biota is very limited (Driscoll and Schecher, 1989).

On the other hand Fluorides are protoplasmic poisons, changing the permeability of the cell membrane by inhibiting certain enzymes. Some of the sources of fluoride intoxication are from the fluorides used in the smelting of metals, such as steel and aluminum.

Aluminum has been found to decrease fluoride absorption (Spencer *et al.*, 1977) and has been used in the treatment for fluorosis (Navia, 1970). This has been related to the fact that aluminium forms insoluble compounds with fluoride preventing its absorption. In contrast the effect of fluoride on aluminium absorption has not been directly studied. It has been shown that aluminium inhibits acetyl cholinesterase induced concentration of rodent and gastric smooth muscle and free aluminium in the stomach reduces gastric emptying (Alfery, 1984). Aluminium has major effects on gastro intestinal tract. The absorption of aluminium is altered by a number of inorganic elements including iron.

MATERIALS AND METHODS

Maintenance of the Animals

Fresh water fish *Oreochromis Mossambicus* were supplied by the commercial animal supplier and maintained in laboratory condition under room temperature. Fish feed was provided every day. Aged tap water was used and oxygenated using an aerator. Animals were acclimated for a week before experimental analysis.

Fish taken for the experiments was size ranging about 5 ± 1gms. 10 animals were taken in 5 groups. One group being control and four groups were exposed to different concentration of Aluminium chloride. Similarly another set of 5 groups, each containing 10 fish were taken and four groups were exposed to varying concentration of sodium fluoride. These experiments were conducted to find the median lethal concentration of aluminimum

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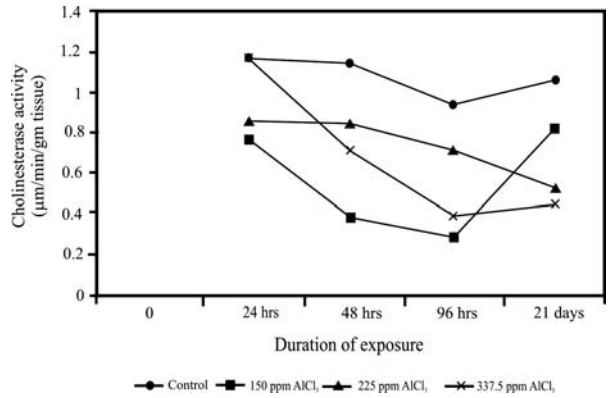

Fig. 1. *Orechromis mossambicus*-Effect of $AlCl_3$ on Cholinesterase activity in Cerebrum

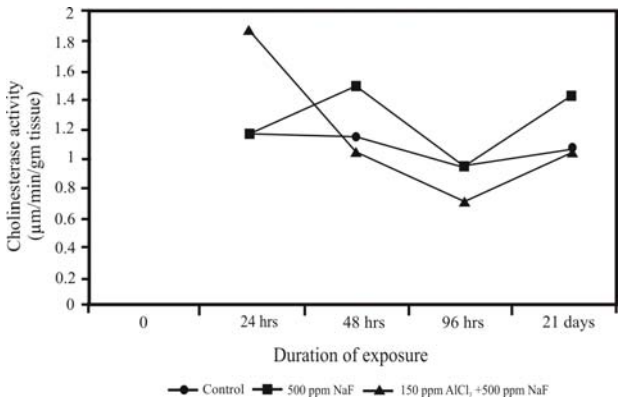

Fig. 2. *Orechromis mossambicus*-Effect of $AlCl_3$ and NaF on Cholinesterase activity in Cerebrum

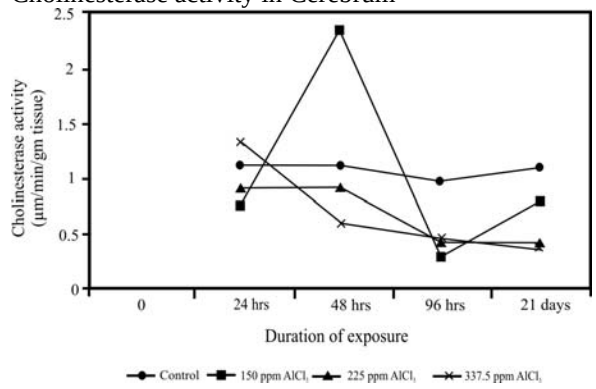

Fig. 3. *Orechromis mossambicus*-Effect of $AlCl_3$ on Cholinesterase activity in Mesen/diencephalon

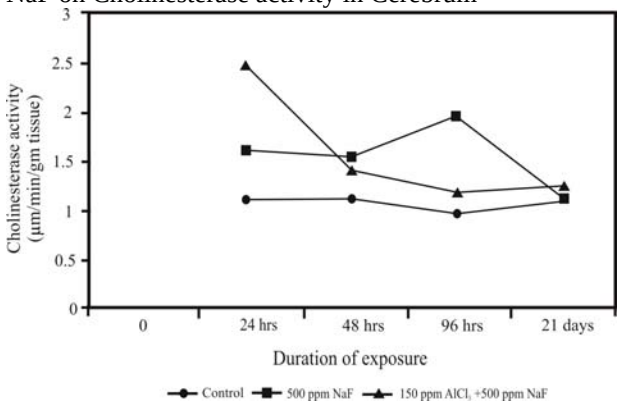

Fig. 4. *Orechromis mossambicus*-Effect of $AlCl_3$ and NaF on Cholinesterase activity in Mesen/diencephalon

chloride were chosen and fish were exposed to various duration of 24hrs, 48hrs, 96hrs, and 21 days in different concentration.

Aluminium Chloride

Five groups containing 10 fishes were subjected to various concentrations of aluminium chloride ranging from 150ppm, 225ppm and 337.5 for different time intervals such as 24hrs, 48 hrs, 96hrs and 21 days. Always one group was maintained for control.

Sodium Fluoride

Five groups of fish were exposed to 500ppm of sodium fluoride. One group was maintained as control. Whereas other group were sacrificed at different time interavals 24hrs, 48hrs, 96 hrs and 21days.

Sodium Fluoride and Aluminium Chloride

Least concentration of 150ppm of aluminium chloride and 500ppm of sodium fluoride were prepared and fishes were subjected to the chemicals in combination (Aluminium chloride and Sodium fluoride) and sacrificed at various time intervals of 24hrs, 48hrs, 96 hrs, and 21 days.

In order to study the effects of aluminium and its interaction with fluoride fishes were exposed to 150 ppm of aluminium chloride and 500 ppm of sodium fluoride individually. The effect of aluminium and its interaction with fluoride in the brain tissue was determined by the assay of acetyl cholinesterase in various regions of the brain tissue. For this, animals were sacrificed at 24 hrs, 48 hrs, 96 hrs and 21st day to the study of acetyl cholinesterase.

Cholinesterase activity was assayed following the procedure of Ellman *et al.* (1961). One way analysis of variance (ANOVA) was performed on the data to analyse to the significant chemical treatment and different periods of exposure.

RESULTS

Enzyme activity in the brain tissue of cerebrum with respect to Aluminium Chloride ($AlCl_3$) was found to significantly decrease ($P<0.001$) at all duration (24 hrs, 48hrs, 96hrs and 21 day) and concentrations 150 ppm, 225 ppm, 500 ppm, 337.5 ppm of exposure. Sodium fluoride was not found to significantly alter the enzyme activity in cerebrum at all duration of exposure. In contrast continued exposure of aluminium and sodium fluoride did not alter enzyme activity.

In mesen/diencephalon aluminium chloride was found to increase the cholinesterase enzyme activity significantly ($P<0.05$) at 48 hrs duration of exposure at 150 ppm of

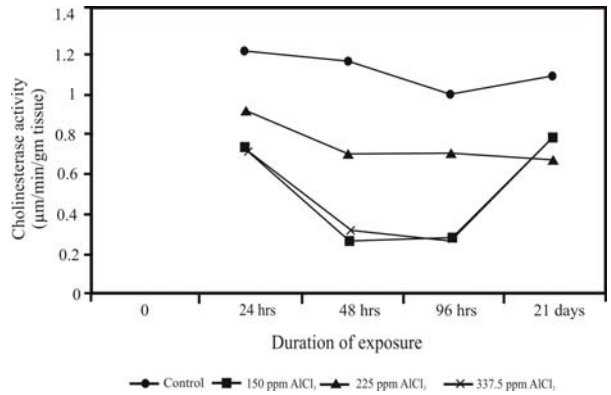

Fig. 5. *Orechromis mossambicus*-Effect of $AlCl_3$ on Cholinesterase activity in Cerebellum

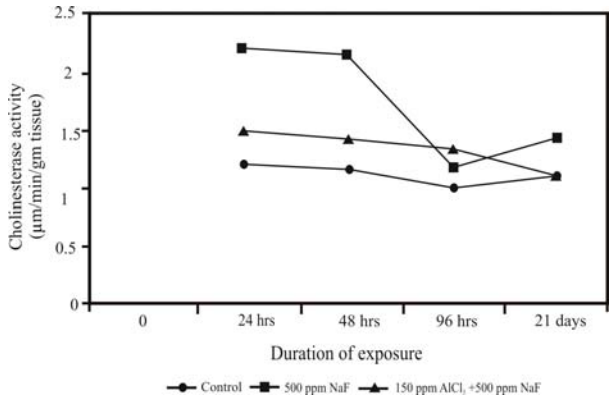

Fig. 6. *Orechromis mossambicus*-Effect of $AlCl_3$ and NaF on Cholinesterase activity in Cerebellum

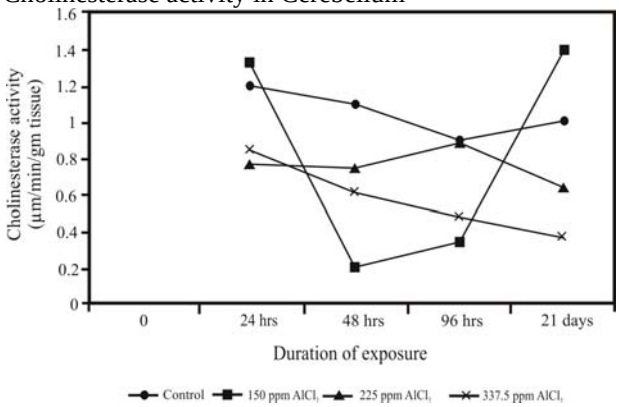

Fig. 7. *Orechromis mossambicus*-Effect of $AlCl_3$ on Cholinesterase activity in Medulla oblongata

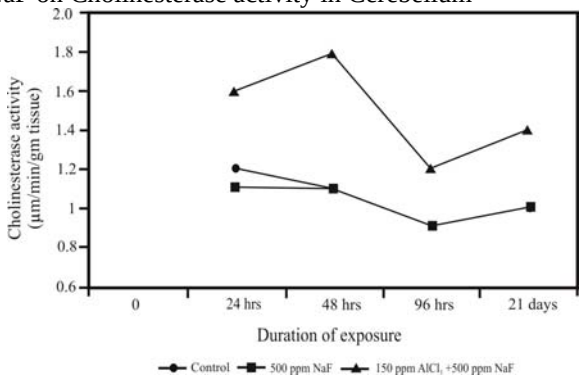

Fig. 8. *Orechromis mossambicus*-Effect of $AlCl_3$ and NaF on Cholinesterase activity in Medulla oblongata

aluminum chloride. On the other hand, there was no effect on the cholinesterase activity for the other treatment groups, at different periods of exposure.

In the cerebellum, aluminium chloride was found to significantly decrease ($P<0.001$) the cholinesterase activity at 150 ppm and 337.5 ppm at all exposure periods. No significant inhibition was found at 225 ppm concentration of exposure.

In the medulla oblongata aluminium chloride was found to significantly alter the enzyme activity significantly ($P<0.05$) at 48 hrs and 96 hrs for 150 ppm. The cholinesterase activity was significantly decreased ($P<0.05$) for 225 ppm and 337.5 ppm of aluminium chloride exposure and a decrease in the enzyme activity for all durations of exposures. Whereas sodium fluoride was found to significantly enhance the cholinesterase activity irrespective of the exposure periods. It was also found to be highly significant ($P<0.00l$) at 24 hrs, 48 hrs, and significant ($P<0.005$) at 96 hrs and at the 21st day of exposure.

In liver tissue aluminium chloride did not affect enzyme activity at lower concentration for all periods of exposure. However, at a higher concentration (337.5 ppm), significant ($P<0.05$) enzyme inhibition was observed only at 96 hrs and 21 days of exposure. Individual exposure to sodium fluoride significantly ($P<0.00I$) increased the enzyme activity at 24 hrs and 96 hrs. However this effect was suppressed when the same individuals were exposed to aluminium chloride for all the exposure periods.

Aluminium chloride at all concentration and duration of exposure seem to have little effect on the cholinesterase activity in kidney for all exposure periods. Sodium fluoride was found to significantly ($P<0.05$) alter the cholinesterase activity at 24 hrs and 48 hrs of exposure periods. However a combined exposure to sodium fluoride and aluminium chloride showed little changes during all exposure periods.

DISCUSSION

Aluminum chloride is a strong inhibitor of the fish cholinesterase activity. The cerebrum cholinesterase activity was found to be inhibited around 70% at a concentration of 337.5 ppm of aluminium chloride (Fig. 1 and 2). Cerebral cholinesterase activity was found to be highly susceptible to aluminium chloride at all the concentration and durations of exposure. Inhibition of cholinesterase activity in the medulla oblongata and cerebellum to aluminium chloride at all the concentration and duration of exposure was evidenced. Cholinesterase

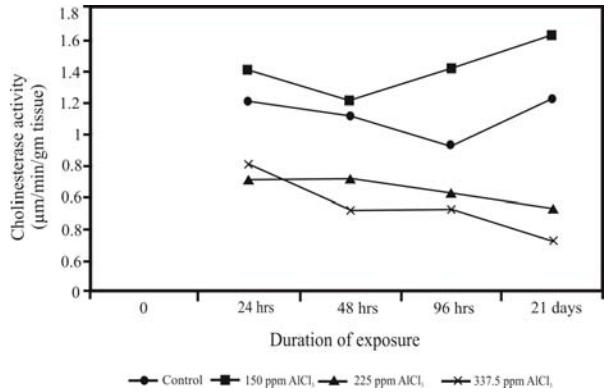

Fig. 9. *Orechromis mossambicus*-Effect of $AlCl_3$ on Cholinesterase activity in Liver

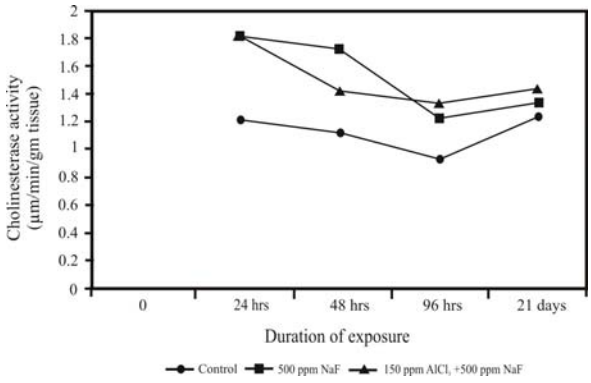

Fig. 10. *Orechromis mossambicus*-Effect of $AlCl_3$ and NaF on Cholinesterase activity in Liver

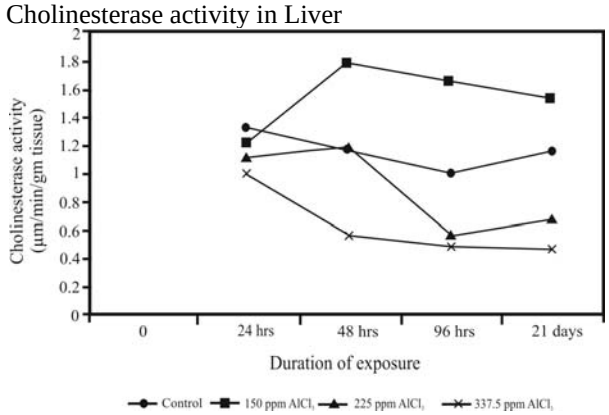

Fig. 11. *Orechromis mossambicus*-Effect of $AlCl_3$ on Cholinesterase activity in Kidney

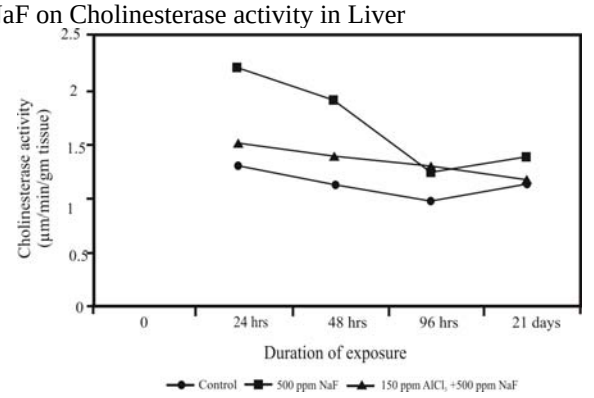

Fig. 12. *Orechromis mossambicus*-Effect of $AlCl_3$ and NaF on Cholinesterase activity in Kidney

activity in mesen / diencephalon was found to be the least affected at different exposure of aluminium chloride, even when the exposure period was extended (Fig. 3 and 4). The differential toxic effect of aluminium chloride on fish brain regions indicates a varying mechanism of action and metabolism. Aluminium chloride exerts a direct effect on cholinesterase activity or altered enzyme activity due to an indirect effect by causing hypoxia which in turn affects the cholinesterase activity. According to Handy (1993) oral dose of aluminium (10g/kg dry wt) increased toxicant accumulation in the muscle, gill, liver, kidney and mucus of trout. In the present study though aluminium chloride seems to decrease the cholinesterase activity in liver and kidney was also not significant. Insignificant inhibition of cholinesterase in liver is due to the clearance through the blood by gills, skin and bile as they are the major routes of excretion and also due to the fast metabolism by the hepatic tissues. For fish possible routes excretion includes the gills, bile kidney and skin (Romanenko *et al*., 1986; Heath, 1987). Possible routes of metal excretion via the urine of teleost fish were generally through kidney for certain metals (Rogers and Beamish, 1982). The kidney also accumulates metals to rather high concentrations by mere relocation for sequestering them and this does not necessarily mean excretion of these metals. Baker and Schofield (1982) have attributed the mechanism of aluminium toxicity in fish to the effects of aluminium on osmoregulatory balance and respiratory problems associated with the coagulation of mucus on the gills. have reported losses of Na^+ and Cl^- from the blood of brown trout when exposed to solution containing 7µmol/L aluminium at pH 5.0. Apart from blocking of Na^+ and Cl^- ions aluminium is found to block neural transmitters of the central nervous system which results in reduction in coordination and thereby swimming efficiency in fishes (Heath, 1987).

In the medulla oblongata, sodium fluoride at 500 ppm was found to significantly increase the cholinesterase activity for all exposure periods (Fig. 7 and 8), whereas in the cerebellum the increase in cholinesterase activity was found only at 24hrs and 48 hrs (Fig. 5 and 6). However, there was no significant change in cholinesterase activity in mesen / diencephalon (Fig. 3 and 4) and cerebrum (Fig. 1 and 2) due to sodium fluoride for different periods of exposure. In contrast to the brain tissue, liver (Fig. 9 and 10) and kidney (Fig. 11 and 12) exhibited a decrease in cholinesterase activity for shorter duration of 24 and 48 hrs suggesting an acute toxicity symptom and a regulatory mechanism for longer periods of exposure. A combined exposure to aluminum chloride (150ppm) and sodium fluoride (500ppm) had reduced the effects as observed when exposed to the sodium fluoride alone. The observations of the present study indicate that in the brain

tissue itself cerebrum, (Fig. 1 and 2) medulla oblongata (Fig. 7 and 8) and cerebellum (Fig. 5 and 6) were more vulnerable to Aluminium chloride at both lower and higher concentrations. Activity in liver, (Fig. 9 and 10) and kidney (Fig. 11 and 12) and mesen / diencephalon (Fig. 3 and 4) are least affected at all concentrations exposed. Though, sodium fluoride without Aluminum chloride affects the cholinesterase activity at shorter duration in cerebellum, (Fig. 5 and 6) medulla oblongata, (Fig. 7 and 8) liver (Fig. 9 and 10) and kidney, (Fig. 11 and 12) these effects were not observed with combined exposure Sodium fluoride-Aluminium chloride treatment, thus suggesting that sodium fluoride reduces the aluminum chloride induced stress in fish. Influence of Aluminium chloride and sodium fluoride observed in the present study indicates the possibility of functional changes in nervous system. Alteration in function of the nervous system leads to indirect effects such as motivational disturbances. Hamilton and Haines (1995) have studied the influence of fluoride on aluminium toxicity to Atlantic salmon and reported that low fluoride concentrations reduces gill morphological damage in fish exposed to aluminium in acidic waters, where as high fluoride concentrations (>100µg/L) did not reduce aluminium inducted effects as observed in the present study.

The enzyme activity with aluminium chloride was found to significantly decrease in cerebrum, (Fig. 1 and 2) at all duration (24 hrs, 48 hrs, 96hrs, 21 days) of exposure and concentration (150 ppm, 225 ppm, 337.5 ppm and 500 ppm). Aluminium chloride at 150 ppm and 337.5 ppm was found to significantly decrease the cholinesterase activity in cerebrum at all periods of exposure. In mensen / diencephalon (Fig. 3 and 4) at 150 ppm concentration of aluminium chloride there was increased activity of cholinesterase enzyme. In medulla oblongata (Fig. 7 and 8) there was significant decrease in enzyme activity at all duration of exposure with aluminium chloride. Aluminium chloride at lower concentration did not affect enzyme activity in liver (Fig. 9 and 10) and kidney (Fig. 11 and 12) at all duration of exposure. The effect of sodium fluoride alone was significant in medulla oblongata, liver (Fig. 9 and 10) and kidney (Fig. 11 and 12). However sodium fluoride and aluminium chloride continued exposure seems to have least effect at all duration of exposure. The results from the experiment suggest that in sodium fluoride and aluminium chloride treatment, sodium fluoride reduce the aluminum induced stress in fish tissue. The tissue content of aluminium chloride and sodium fluoride may be analysed to substantiate the changes in cholinesterase activity.

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