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Research Article

EFFECTS OF SODIUM FLUORIDE ON ACETYLCHOLINE ESTERASE ACTIVITY IN *OREOCHROMIS MOSSAMBICUS* AND *LABEO ROHITA*

J. Thadani and *S.P. Salunke
Department of Zoology, Faculty of Science, The M.S. University of Baroda, Vadodara, Gujarat. 390002
**Author for Correspondence*

ABSTRACT
Acetylcholinesterase (EC 3.1.1.7) is a serine hydrolase that belongs to the esterase family within higher eukaryotes. This family acts on different types of carboxylic esters. Apart from its catalytic function in hydrolyzing acetylcholine, acetylcholinesterase (ache) affects cell proliferation, differentiation and responses to various insults, including stress. Fluoride is a known enzyme inhibitor and it is conceivable that certain concentrations of this ion in the water could directly affect the metabolism, growth, reproduction, or other physiological processes. In the present study, adult tilapia (*Oreochromis mossambicus)* of 15±0.5cm and fingerlings of *Labeo rohita* were exposed to 20ppm and 30ppm of sodium fluoride (naf) for 28 days (15 fishes/group) after acclimatization at standard laboratory conditions. Fishes were sacrificed on 7^{th}, 14^{th}, 21^{st} and 28^{th} day. Membrane bound fraction and soluble fraction of enzyme was estimated as described by elman *et al.,* (1961). The activity of acetylcholine esterase showed progressive inhibition with increasing concentration of sodium fluoride as well as time of exposure in all the tissues examined.

Keywords: *Acetylcholine Esterase, Sodium Fluoride*

INTRODUCTION
Fluoride (F) occurs naturally in public water systems as a result of runoff from weathering of fluoride-containing rocks and soils and leaching from soil into groundwater. Atmospheric deposition of fluoride-containing emissions from coal-fired power plants and other industrial sources also contributes to amounts found in water, either by direct deposition or by deposition to soil and subsequent runoff into water. Owing to expanded industrial emissions and commercial uses of F compounds, the concentration of F is increasing in both ground water and surface water. Many municipal water supplies are treated with alum and fluoride, and evidences that Fluoride crosses the Blood brain barrier raises the possibility that Fluoride can affect the structure and functions of the central and peripheral nervous system. Yu (1996), Du (1992) and Han (1989) have found that fluoride accumulates in the brain of foetus, causing damage to cells and neurotransmitters. Fluoride is also known to cross the cell membranes and to enter the soft tissues. Impairment of the soft-tissue function has been demonstrated in fluoride-intoxicated animals. Various changes occur after chronic administration of fluoride in the blood (Zen-Zhong *et al.,* 1989), brain, and liver (Singh, 1984) of animals. These include abnormal behaviour patterns, altered neuronal and cerebrovascular integrity, and metabolic lesions. Generation of free radicals, lipid peroxidation, and altered antioxidant defence systems are considered to play an important role in the toxic effects of fluoride (Rzeuski *et al.,* 1998, Sharma and Chinoy, 1998, Shivranjashankara; 2001). The toxic effects of elevated fluoride on various aquatic species are also well documented by Gikanju JK (1992), Dwivedi *et al.,* (1997) and Camargo JA (2003)

Acetylcholinesterase is a serine hydrolase that belongs to the esterase family within higher eukaryotes. This family acts on different types of carboxylic esters. Acetylcholinesterase's biological role is the termination of impulse transmissions at cholinergic synapses within the nervous system by rapid hydrolysis of the neurotransmitter, acetylcholine (Schumacher *et al.,* 1986). The determination of acetylcholinesterase (AChE) activity is important in monitoring and studying of exposure to pesticides and chemical warfare agents; therapeutic monitoring of organophosphate poisoned patients and titrating the anticholinesterase dosage used in Alzheimer’s disease.

Cholinesterase inhibitors act indirectly by preventing the enzyme from hydrolyzing (inactivating) acetylcholine at the receptor site. This inhibition permits the buildup of acetylcholine and results in more intensive and prolonged activation of the receptor site. The effects of cholinergic stimulation include: vasodilattion of blood vessels; slower heart rate; constriction of bronchioles and reduced secretion of mucus in the respiratory tract; intestinal cramps; secretion of salvia; sweat and tears; and constriction of eye pupils.

The present investigation was undertaken to study the effect of two different NaF concentrations on AChE in Brain and Muscles of adult *Oreochromis mossambicus* and fingerling of *Labeo rohita*

MATERIALS AND METHODS

Healthy species of adult *O.mossambicus* of average size 12 ± 2 cm and fingerlings of *L. rohita* of average size 5± 0.5 cm were procured from local supplier and fish breeding centre respectively. They were kept in tanks and glass aquarium respectively under standard laboratory conditions for a period of 15 days for acclimatization before splitting them into Three Groups of 15 each. 45 fishes were randomly selected and were grouped in 3 groups of 15 each. Two groups were treated with 20 ppm and 30 ppm of NaF resptectively for 28 days, while one group was kept as Control. Food was supplied *ad libitum*. Care was taken to maintain normal dark/light cycle and uniform conditions (water temperature 27 ± 2°C) throughout the experiment.

Three fishes from each group were sacrificed by decapitation every 7^{th} day and the sacrificed fingerlings and fishes were dissected to collect the muscle and brain, which were immediately stored at -20 °C and were later used for Biochemical Estimations. Biochemical Estimations by standard methods were conducted for both soluble and membrane bound AChE (E.C.3.1.1.7) in brain, and muscle of Control and Fluoride treated fishes. The protein content were determined by the method of Lowry *et al.*,

Protocol

AChE was estimated using Ellman *et al.,* 1961, briefly tissue was homogenized in chilled mortar pestle in Phosphate buffer pH 8.. Homogenate was divided in two equal parts (for membrane bound and soluble fractions of AChE). One fraction was treated with 0.1 % triton X100 for membrane bound enzyme. Both fractions were centrifuged at 20, 000 rpm for 15 min. the supernatant served as the source of enzyme.

The final assay mixture contained 2.7 ml phosphate buffer (0.1M, pH 8), 0.2ml DTNB (9 5, 5'-dithio-bis (2-nitro benzoic acid) (0.33mM), and 0.02 ml of Acetylthiocholine iodide (1.0mM). The reaction was initiated by adding 0.2ml supernatant (enzyme source) and the increase in absorbance was read at 412nm. Rate of color change was proportional to enzyme activity and was deduced from extinction coefficient

R = ΔA/(1.36*104) x 1/(200/3320)Co = 1.22(10-3) A/Co where

R = rate, in moles substrate hydrolyzed/min. g tissue, ΔA = change in absorbance/min. Co = original concentration of tissue (mg/ml), 200/3320 are volume corrections, 1.36 (104) is the extinction coefficient of the yellow product.

Protein estimation was done according to Lowry *et al.,* (1951)

RESULTS AND DISCUSSION

Fish are extremely sensitive to many water-borne toxicants including NaF, due to their prolonged, constant and direct contact with the aquatic environment where chemical exposure occurs over the entire body surface and are of ecological significance in any natural systems (Little *et al.,* 1993). However a wide range of environmental and genetic factors cause fish to respond differently to given levels of fluoride, but they do display characteristic fluoride intoxication signs.

The result of the present studies showed progressesive decrease in the AChE activity of soluble as well as membrane bound enzyme fractions in all the experimental tissues in time and dose dependant manner.

Chinoy *et al.*,(1993) reported enhanced AChE activity in skeletal muscle of rats treated with NaF. In contrast as shown in Figure 2, AChE activity was significantly inhibited in both fractions of muscle tissue of Tilapia *mossambica* and *L. rohita.* Similar inhibition in enzyme activity was also observed in Brain tissues (Figure 1).

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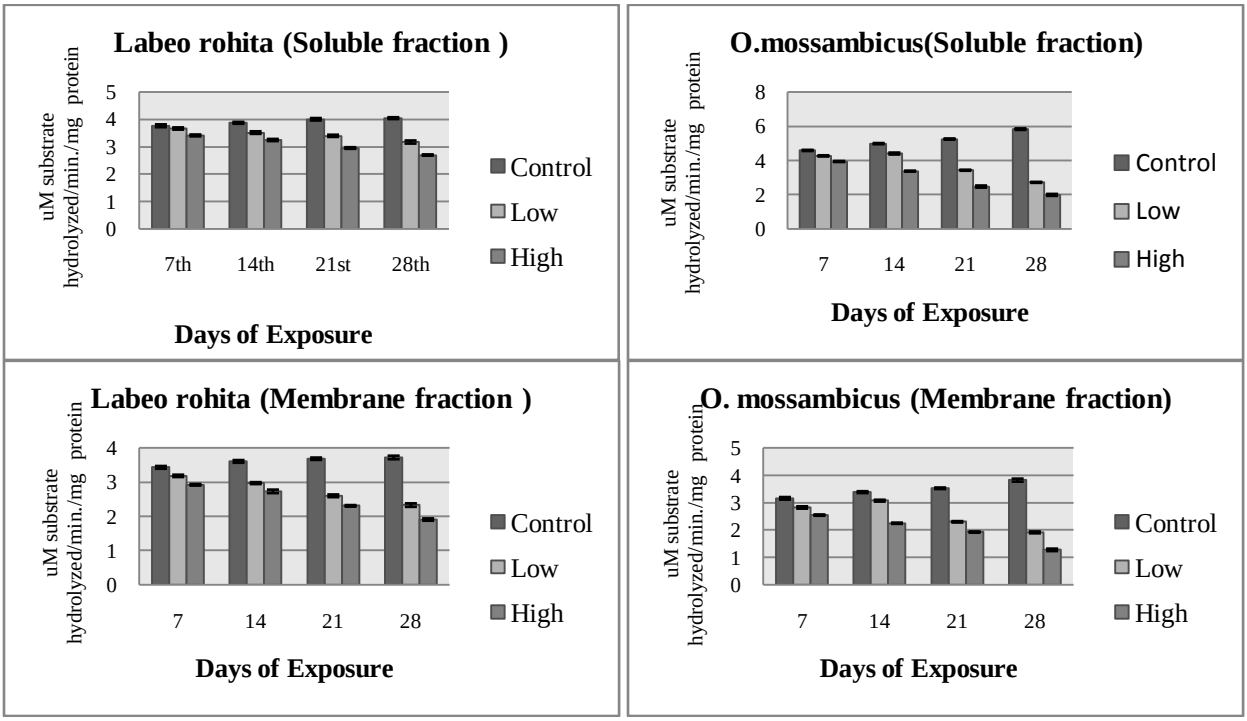

Figure 1: Acetylcholine Esterase activity in Brain

Acetylcholine esterase activity in brain of Tilapia mossambica and L. rohita. Both fractions of enzyme show progressive decrease in its activity at low concentration (20ppm) and high concentration (30ppm) of NaF exposure

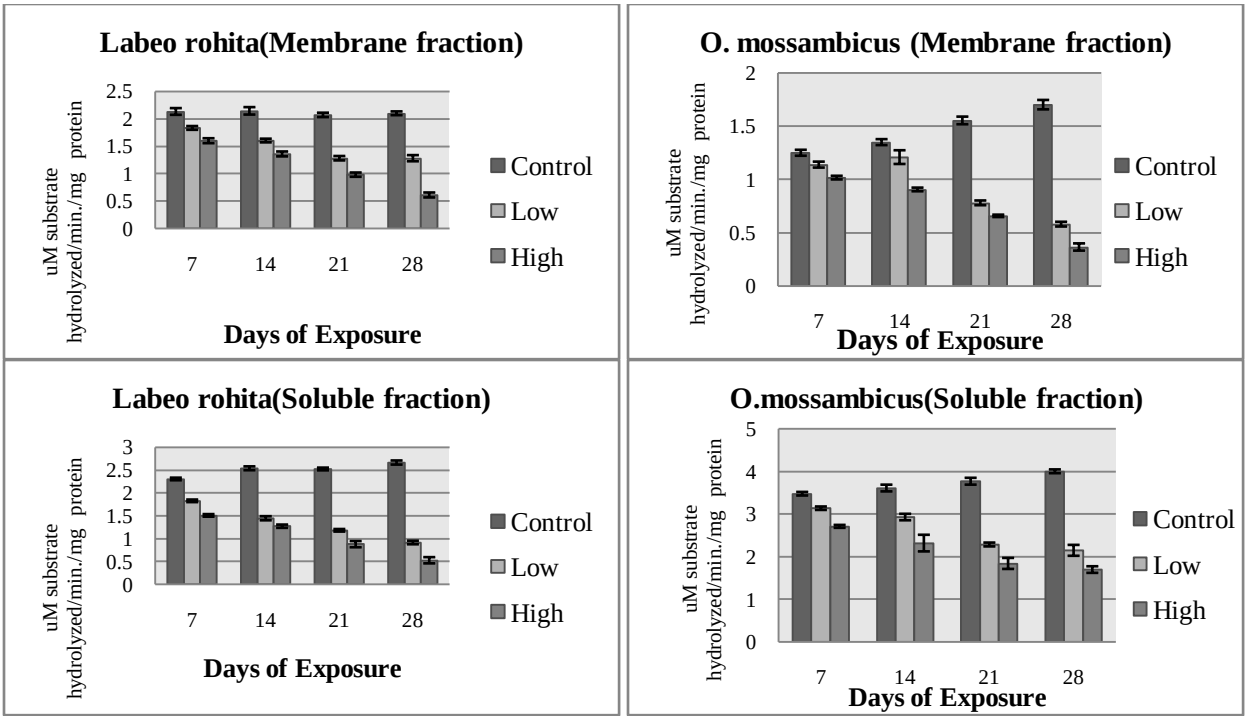

Figure 2: Acetylcholine Esterase activity in Muscle

Acetylcholine esterase activity in brain of Tilapia mossambica and L. rohita. Both fractions of enzyme show progressive decrease in its activity at low concentration (20ppm) and high concentration (30ppm) of NaF exposure

Studies reported by Ekambaram and Paul (2002); Bhatnagar *et al.,* (2006); Vani and Reddy (2000); in rats and mice treated with NaF are in agreement with our studies. Koteeswarai *et al.,* (2003) have also reported significant inhibition of AChE activity in sodium fluoride (NaF) exposed fish whole brain homogenates.

The role of AChE in cholinergic transmission is to regulate nervous transmission by reducing the concentration of acetylcholine (ACh) in the junction through AChE-catalyzed hydrolysis of ACh. When AChE is inactivated, the concentration of ACh in the junction remains high in comparison with unaffected organisms (Bocquen´e *et al.,* 1990; Galgani & Bocquen´e 1990, 2000; Galgani *et al.,* 1992; Escartin & Porte, 1997; Narbonne *et al.,* 1999). The decrease in AChE activity after Fluoride exposure could be due to loss of neuronal cell bodies in hippocampus as reported by Mullenix (1995), Lu *et al.,* (2000), Bhatnagar *et al.,* (2002) and Ge Yu *et al.,* (2005); loss of synaptic structures (Chlubeck *et al.,* 1998; Zhang *et al.,* 2001) or inhibition of enzyme activity (Zhao and Wa, 1998; Zhai *et al.,* 2003). A similar decrease in the activity of the cholinesterases in liver and muscles could be due to inhibition of the enzyme activity or loss of synaptic structures.

In conclusion, we have shown that sub-lethal exposure of the *Tilapia mossambica* and *L. rohita* to excess F causes significant inhibition of AChE activity in brain and muscle tissues of these fish.

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