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Effect of Fluoride on Acetylcholinesterase Activity and Oxygen Consumption in a Freshwater Field Crab, *Barytelphusa guerini*

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Fluoride is a widespread pollutant released into the environment by a great variety of industrial, agricultural and municipal activities. Fluoride is an element of high biological activity and has a tendency to accumulate in organisms, making adverse effects possible even at very low levels of exposure (Groth 1975). Extensive reviews of research on the biological effects of fluoride are available (Lillie 1970; National Academy of Sciences 1971). Information on potential effects of fluoride toxicity in mammalian models is bountiful (Lee 1983; Shashi **et al.** 1989). A survey of literature reveals that few attempts have been made on the various aspects of fluoride toxicity in aquatic organisms (Pillai and Mane 1984; Reddy **et al.** 1989). However, studies on toxicological effects of fluoride in crustaceans, in particular on freshwater field crabs are virtually non-existent. **Barytelphusa guerini**, is a freshwater field crab, which forms one of the major components of the paddy field ecosystem and has a high edible importance, received little attention to fluoride toxicity. A measure of a metabolism may be a most sensitive parameter such as enzyme activity (Wiseman 1970) or physiological response (Reynolds **et al.** 1978). Acetylcholinesterase (E.C.3.1.1.7) is regarded as biochemical marker to assess the complex effect of a pollutant like fluoride (Krupka 1966). Hence the present study is aimed to evaluate the toxic potentiality of sublethal concentration of sodium fluoride on AchE activity in various tissues and on time-course alterations in whole animal oxygen uptake in a freshwater field crab, **Barytelphusa guerini.** This crab forms a major link in food web of aquatic ecosystem and deserves an understanding of low level toxicological impact of fluoride in biochemical and physiological integrity.

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MATERIALS AND METHODS

Healthy, uniform sized male crabs, **Barytelphusa guerini**, were collected from and around Hyderabad and were acclimatized to the laboratory conditions for a period of 15 days. The animals were fed fishmeat **ad libitum.** To determine the LC_{50} value, the crabs were exposed to six serial concentrations of sodium fluoride. A density of 10 crabs per 8 L of dechlorinated tap water was used with 10 individuals in each tub. The physico-chemical characteristics of water were as follows: pH 7.4; dissolved oxygen 4.6 mg/L; total alkalinity expressed as HCO_3^- 16 ppm; and carbonates (CO_3^-) 4 ppm. Free carbon dioxide was absent. The bioassay experiment of each concentration was repeated six times with parallel controls, and mortality was noted in each concentration at the end of 96 h. No mortality was observed in controls. All the experiments were conducted at 26.5 $\pm$ 0.5°C. The average mortality in each concentration was taken to determine the LC_{50} by plotting a graph, taking log concentration on X-axis and % mortality on Y-axis (Finney 1964). According to graphical plots the 50% mortality corresponds to log concentration 1.95 which is equivalent to 89.13 mg/L of NaF. The crabs were exposed to sublethal concentration (1/3 of LC_{50} i.e., 30 mg/L) as suggested by Konar (1969) for a period of 15 days. The toxicant water and normal water renewed every 24 hours after feeding. The animals were starved a day prior to experimentation to avoid metabolic differences, if any, due to differential feeding and food reserves. Six crabs each from experimental and control tubs were sacrificed on the 4th and the 15th day of exposure. The crabs were held from the lateral side between the thumb and index finger and allowed to adjust for some time and chelate legs were amputated immediately. The carapace was cut open and the tissues, chelate leg muscle, hepatopancreas, heart, gills and thoracic ganglion, were isolated from both control and toxicant-treated animals and were immediately transferred to a deep-freezer for storage prior to analysis of Acetylcholinesterase. A 5% homogenate of the tissues was prepared in ice-cold sucrose (0.25M) solution. Uncentrifuged homogenate was used for initiation of enzyme reaction. AchE was assayed by the method of Metcalf (1951). The incubation mixture contained in 2 ml volume of 100 μ moles of phosphate buffer pH(7.4), 4 μ moles of acetylcholine chloride as substrate and 1 ml of homogenate. The reaction was stopped after allowing an incubation of 30 minutes at 37°C by adding 2 ml of freshly prepared alkaline hydroxylamine hydrochloride (prepared in 1:1 proportion by mixing 2 M hydroxylamine hydrochloride and 3.5 M sodium hydroxide). The tubes were shaken

and 1 ml of 50% HCl was added with further shaking. After filtering the contents, color was extracted with Ferric chloride (0.037 M) solution. The enzyme activity was expressed as μ moles of acetylcholine chloride hydrolysed per mg protein per hour. The protein content of the enzyme source was estimated according to Lowry **et al.** (1951). Oxygen consumption of the crabs was measured by the method of Winkler as described by Welsch and Smith (1953). The crab was weighed and placed in Winkler's chamber and care was taken to make it air tight and free from leakage of water. The crab was allowed to stabilize in the chamber for three minutes. After three minutes water was collected into narrow mouthed bottle and oxygen was estimated by iodometry. After thirty three minutes another sample of water was collected and again oxygen was fixed in similar way. The difference in oxygen tension in initial and final samples gives amount of oxygen consumed by the animal. Oxygen consumption was carried out in six individual animals in both experimental and control groups throughout the exposure span and was expressed as mL of oxygen per gram weight per hour. The results were subjected to statistical treatments. Students 't' test was used to compare the differences between control and experimental groups.

RESULTS AND DISCUSSION

In the present investigation the toxicological effect of sublethal concentration of sodium fluoride on the physiological organisation of crab, as a whole present quite significant changes in both biochemical (AchE) and physiological (O_2 uptake) response patterns indicating time-dependency. Oxygen consumption of crabs measured continuously for a period of 15 days at different intervals of exposure period showed an initial increase up to 2 days, followed by a gradual depression in respiratory rates over a prolonged exposure period. Respiratory activity, apart from reflecting the energy metabolism, is a good indicator of the general condition of an organism. In the present investigation, an initial increase in the oxygen consumption up to 2 days might be the result of fluoride stress, enforcing more intake of fluoride through high rate of gill movements leading ultimately to increased fluoride accumulation (Moore 1971) resulting in depressed O_2 uptake thereafter. Oxygen consumption increased up to 2 days and subsequent decrease as observed in the present study could be due to action of this halogen (F^-) ion upon the cellular energetics. Suketa **et al.** (1976) reported an overall decrease in energy metabolism of rats intoxicated with fluoride. This will lend a support

Table 1. Oxygen consumption of a fresh water field crab, Barytelphusa guerini exposed to sublethal concentration of sodium fluoride.

	Oxygen Consumption Duration of exposure in days								
	0.25	0.50	1	2	4	6	9	12	15
Controls	0.080	0.112	0.086	0.084	0.090	0.092	0.088	0.086	0.084
S.E. ±	0.001	0.002	0.002	0.004	0.004	0.002	0.002	0.001	0.003
Experimental	0.105*	0.164*	0.103*	0.092*	0.066*	0.062*	0.056*	0.050*	0.042*
S.E. ±	0.001	0.004	0.003	0.003	0.001	0.001	0.001	0.001	0.001
% difference	+31.25	+46.43	+19.77	+9.52	-26.67	-32.61	-36.36	-41.86	-56.00

Values expressed as mL of O_2/gramweight/hour; S.E.= Standard Error of Mean; *P<0.001.

Table 2. Acetylcholinesterase activity in the tissues of a fresh water field crab, Barytelphusa guerini, exposed to sublethal concentration of sodium fluoride.

Tissues	Acetylcholinesterase Activity 4 days Control	4 days Experimental	4 days % Difference	15 days Control	15 days Experimental	15 days %Difference
Gills	23.540± 0.686	29.790*±0.763	+26.27	22.411±0.380	17.810±0.361	-20.53
Muscle	13.340±0.343	14.681±0.648	+10.04	12.743±0.328	9.580*±0.322	-24.80
Hepato-pancreas	12.710±0.334	14.671@±0.671	+15.43	11.640±0.321	9.522**±0.419	-18.19
Heart	24.270±0.817	30.850*±0.603	+27.11	23.510±0.192	16.610*±0.232	-29.45
Thoracic ganglion	30.270±0.504	36.390*±0.910	+20.22	29.920±0.390	25.220*±0.280	-15.71

Values expressed as μ moles of Acetylcholine hydrolysed per mg protein per hour; *P<0.001; **P<0.01; @P<0.05.

to the depressed O_2 uptake in later periods of exposure observed in the present study. It is further revealed that fluoride ion has inhibitory effect on respiratory metabolism (Sullivan 1969). Further, a decrease in oxidative potential as indicated by patterns of SDH, LDH activities and alterations in related metabolites of carbohydrate metabolism of present crab model during sublethal fluoride toxicity (Reddy **et al.** 1989), will substantially support the effect of fluoride on the physiological response. Acetylcholinesterase activity reflects the neuronal status of an animal. There was an increase in the enzyme activity on 4th day of exposure in all tissues. This can be attributed to stress caused by fluoride. However, AchE activity was significantly inhibited in all tissues on 15th day of exposure. Inhibition of Acetylcholinesterase by fluoride **in vitro** was reported by Krupka (1966). Interestingly, cholinesterases do not require metal ions for optimum activity (Augustinsson 1950) and hence a possibility for fluoride interaction with co-factors does not arise but a direct interference with enzyme reaction virtually validates the **in vitro** AchE inhibition (Krupka 1966). Acetylcholinesterase activity reflects the neuronal activity which in turn generally reflects in the metabolic rate of an animal (Muralikrishnadass 1967). In the present study, the respiratory rate is not truly reflected in the AchE activity on 4th day of exposure. However, on 15th day of exposure the inhibition in the enzyme activity is reflected in oxygen consumption. The deviation in the response on the initial period needs further investigations on the availability of the ATP reserves as these are essential for maintaining the neuronal activity.

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