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ALTERATION IN TRACE MINERAL CONTENT IN MUSCLE TISSUE OF GUPPY, *POECILIA RETICULATA* PETERS ON CHRONIC EXPOSURE TO SODIUM FLUORIDE

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ABSTRACT:

Fluoride the most electronegative element is of global concern due to its long standing adverse effects on human health & animals, commonly termed as 'Fluorosis'. Fluoride is known to diffuse through cell membrane causing accumulation in soft tissues resulting in impairment in the cellular activities. Present investigation demonstrates significant alteration in the level of few essential trace elements (Iron, Zinc & Copper) in Guppy (*Poecilia reticulata* Peters) chronically treated with 5.75 ppm, 7.18 ppm & 9.58 ppm of Sodium fluoride for the period of 30 & 60 days respectively. Chronic intoxication of fluoride resulted in significant decrease in the concentration of Iron & Copper while the concentration of Zinc increased in the muscle tissue of the test fish. The changes observed were dose dependent & extensively altered the normal level of trace elements either by excess removal or accumulation. Interaction of fluoride with various trace elements & their metabolism is very crucial because chronic ingestion of fluoride influence systemic, biochemical & homeostatic mechanism of the test organism. Role of fluoride in disturbing trace element concentration in Guppy could be due to its binding capabilities with tissue protein & enzymes; forming complexes.

KEY WORDS: *Iron, Zinc, Copper, Poecilia reticulata Peters, Sodium fluoride.*

INTRODUCTION:

Chemicals can play a major role in a living system. Some of them are called as "bulk elements" while others are "trace elements". Certain minerals are integral components of biologically important compounds such as haemoglobin (Fe), thyroxin (I), insulin (Zn) and vitamin B_{12} (Co). Several minerals participate as co-factors for enzymes in metabolism for e.g. Magnesium, Manganese, Copper, Zinc, Potassium, etc. Every element has a whole spectrum of possible effects on a particular animal at a particular dose and concentration. Fluoride is one such element, which plays important role in teeth and bone formation within the maximum permissible limit. It has a very significant role in living systems as less than 0.6 mg/litre prevents dental caries and concentration above the permissible limit (1.5 mg/L, WHO 1996) is responsible for its perilous effects on health termed 'Fluorosis'. Nearly 66 million people in 20 Indian states are at risk due to excessive fluoride in their waters. According to Krasowaska & Wlostowaski (1992) due to high fluoride intoxication, deficiency or excess storage of trace elements occur infrequently in animals & humans, but the evidence of close links between disturbance in trace element concentration & various biological activities & related disorder are scarcely available in literature. Interaction of fluoride with various trace elements and their metabolism is interesting as it has been shown that chronic ingestion of fluoridated water does influence systemic biochemical homeostasis in experimental animals as well as in humans (Bhatnagar et. al. 2003). Trace elements such as Fe, Cu, Mn, I, F, etc. though occur in low concentration in the body serve some useful functions and their imbalance may affect important biological functions in both animals as well humans, including bone and teeth development, immunity, reproduction, etc. (Vohra, 1982). Pillai and Mane (1984) studied the effect of fluoride effluent on some metabolites and minerals in fry of *Catla catla*. Fluorides increase the retention of toxic metals in the body and lower the retention of beneficial trace metals, a death knell to the essential enzyme processes (Jim Phelps 2005). The mechanism for the toxic metal rise in the body by fluorides could be due to the damage to selenium based glutathione enzyme that clears toxic metals from the body. A rising fluoride level in the body shut down the enzyme glutathione and similar others causing rise in toxic metals in the body.

MATERIALS AND METHODS:

The test fish *Poecilia reticulata* Peters measuring 3.5 $\pm$ 0.1 cm & average weight of 0.52 $\pm$ 0.002 g were acclimated in the laboratory for two weeks. Twenty-five acclimated healthy fish were exposed to three sub-lethal concentrations of Sodium fluoride viz. lowest (5.75 ppm), lower intermediate (7.18 ppm) and higher intermediate (9.58 ppm). Control set with same number of test fish but without any toxicant was also maintained simultaneously. The tests were carried out in glass aquaria measuring 60x30x30 cm^3 in size. The amount of water in each tank was 2.0 litres/g body weight of the test fish. Entire water from each tank was replaced every alternate day to avoid any accumulation of metabolic wastes and to keep the level of toxicants in the respective tanks constant. At the end of experimental period of 30 days & 60 days respectively, five fish from control tank and five fish from each of the three sub-lethal concentrations of Sodium fluoride treated tanks were sacrificed by decapitation. The acid wet digestion method as adopted by Chernoff (1975) was used for digestion of the muscle tissue of the test fish. Suitable aliquots of digested tissue were used for the estimation of trace elements like Iron, Zinc and Copper using atomic absorption spectrophotometer (Model No. AA – 20 BQ). The samples for AAS analysis were aspirated at the following wavelengths: Iron - 248.3nm, Zinc - 213.8nm and Copper - 324.8nm. Statistical analysis of the mean of control & treated groups were done using Student's t-test.

RESULTS & DISCUSSION:

Data related to the concentration of trace element in control & Sodium fluoride treated groups of the test fish *Poecilia reticulata* Peters is summarized in Table No.1 & Figure No.1, 2 & 3 for Iron, Zinc & Copper respectively.

Iron

Iron is a very important trace element & haeme is the most predominant iron-containing substance. It is a constituent of several haemoproteins viz. haemoglobin, myoglobin, cytochromes, xanthine oxidase, catalase, tryptophan pyrrolase, peroxidase, etc. Certain other non-haeme iron e.g. transferrin, ferritin, haemosiderin etc. Iron mainly exerts its function through the compounds in which it is present. Haemoglobin and myoglobin are required for the transport of oxygen and carbon dioxide. Cytochromes and certain non-haeme proteins are necessary for electron transport chain and oxidative phosphorylation. Peroxidase is required for phagocytosis. Iron is also associated with effective immunocompetence of the body. The decrease in iron content in the present study signifies an altered state of iron metabolism, which might have been triggered by fluoride ions. Benard et. al. (1958) explained anaemia in fluorosed rabbits due to inhibition of ^{59}Fe and glycine incorporation into protoporphyrin, a precursor of haemoglobin and not due to want of iron. Fluoride is reported to increase the membrane permeability of cells because of its inhibition of pyrophosphate activity (Daniel, 1963). According to Wegner et. al. (1976) fluoride enhances absorption of iron in animals. In experimentally fluorosed rats, Kahl et. al. (1973) observed a decrease in incorporation of ^{59}Fe in blood with concomitant ^{59}Fe uptake by bone marrow and liver. Singh and Kanwar (1981) noted the storage of iron in the bone and liver in mice following fluoride administration implying that iron uptake is in excess of its utilization. Bhatnagar et. al. (2003) studied trace element concentration in various tissues of female mice like brain, liver, kidney and muscles following fluoride administration. They reported significant depletion in concentration of iron in kidney and muscle, but brain and liver showed significant increase of iron. Saralkumari and Ramakrishna Rao (2004) studied the haematological parameters and iron status in human beings afflicted with skeletal fluorosis and observed no significant variation in haemoglobin content, serum iron and iron-binding capacity, indicating existence of hypochromic microcytic anaemia. Decrease in the muscle iron content as observed in the present study could be correlated to the concomitant iron uptake & deposition in tissues else where in the body of the test fish.

Zinc

Zinc is mainly an intracellular element and forms an essential component of several enzymes viz. carbonic anhydrase, alcohol dehydrogenase, alkaline phosphatase, carboxy peptidase, cytosolic superoxide dismutase, etc. The storage and secretion of insulin from β-cells of pancreas requires zinc. It also maintains normal level of vitamin A. Deficiency in zinc results into skin lesions, poorly mineralized bone, impaired reproduction in both sexes & reduced synthesis of proteins & nucleic acid (Osaki et. al. 1971). The steady increase in concentration of zinc in experimentally fluorosed fish in the present investigation reveals poor absorption and transportation of zinc to target organs for various metabolic functions. Excess of zinc is reported to cause electrolyte imbalance, lethargy, muscular incoordination, reduced copper absorption and renal failure (Osaki et. al. 1971). Kanwar & Singh (1981) studied trace element concentration in various tissues following fluoride administration and reported altered levels of zinc, copper and manganese. Significantly reduced concentration of copper, manganese and zinc in kidney and liver and level of zinc in hair has been reported by Singh (1984) and Li et. al. (1994). Bhatnagar et. al. (2003) reported significant decrease in level of zinc in brain, liver and muscles but increase in kidney in mice treated with Sodium fluoride.

Copper

Copper is an essential constituent of several enzymes viz. cytochrome oxidase, catalase, tyrosinase, superoxide dimutase, monoamine oxidase, ascorbic acid oxidase, ALA synthetase, phenol oxidase and uricase. Copper is necessary for synthesis of haemoglobin, ceruloplasmin serve as ferroxidase and involved in the conversion of iron from Fe^{+2} to Fe^{+3}. Severe deficiency of copper causes demineralization of bones, demyelination of neural tissues, anaemia and fragility of arteries. Copper deposited in abnormal amount in liver and lenticular nucleus of brain may lead to hepatic cirrhosis and brain necrosis respectively. The fluoride might interact synergistically with other environmental pollutants to produce greater effects than either pollutant could cause were they interacting alone. This synergistic boost has been demonstrated between fluoride and copper and between airborne hydrogen fluoride and sulphur dioxide. The changes observed in copper level in tissues of the test fish intoxicated with Sodium fluoride are consistent with earlier findings of Kang et. al. (1977); Kanwar & Singh (1981); Singh (1984). Bhatnagar et. al. (2003) reported significant decline in copper content in brain, liver and kidney of fluorosed female mice. However they noted concomitant increase in muscle copper content during fluoride intoxication.

The finding of the present investigation clearly demonstrates a close link between chronic fluoride intake and its possible consequence causing imbalance in levels of the physiologically important elements of the test fish. Disturbance in trace elements observed is very significant as fluoride, a trace element, when taken in excess causes, bone and dental fluorosis as well as non-skeletal fluorosis. Role of fluoride in fluctuating the concentration of these elements could be due to its binding capabilities with tissue proteins. Fluoride is highly electronegative and thus forms complexes with proteins. Alternatively it might be binding with copper, iron and zinc to form complexes resulting in altered level of respective elements. Though Guppy, *Poecilia reticulata* Peters is not an edible variety of fish, its utility in biological control to prevent the menace of mosquito cannot be ruled out especially in the present scenario when malaria, encephalitis & dengue epidemics are on the rise.

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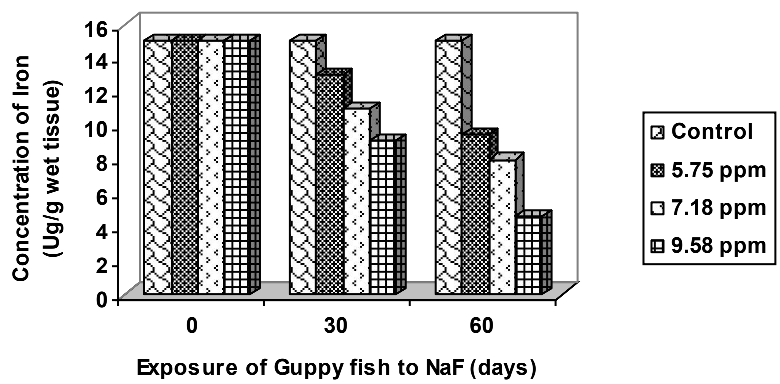
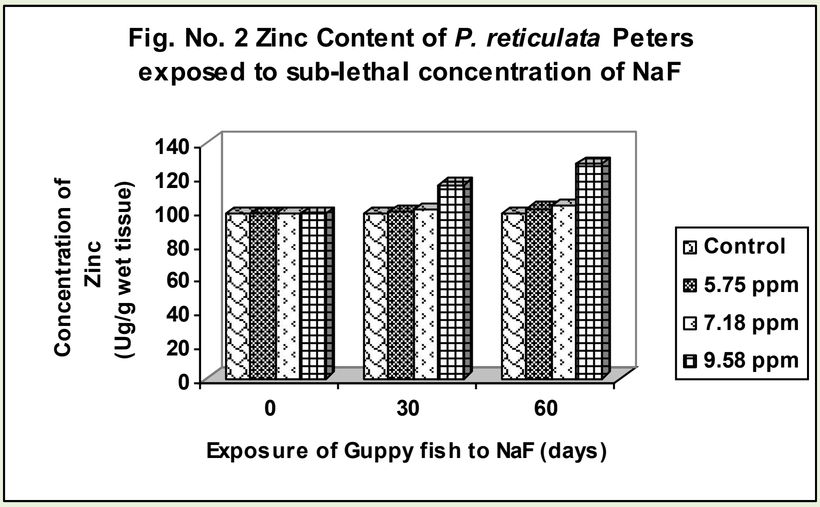
Fig. No. 1 Iron Content of *P. reticulata* Peters exposed to sub-lethal concentration of NaF





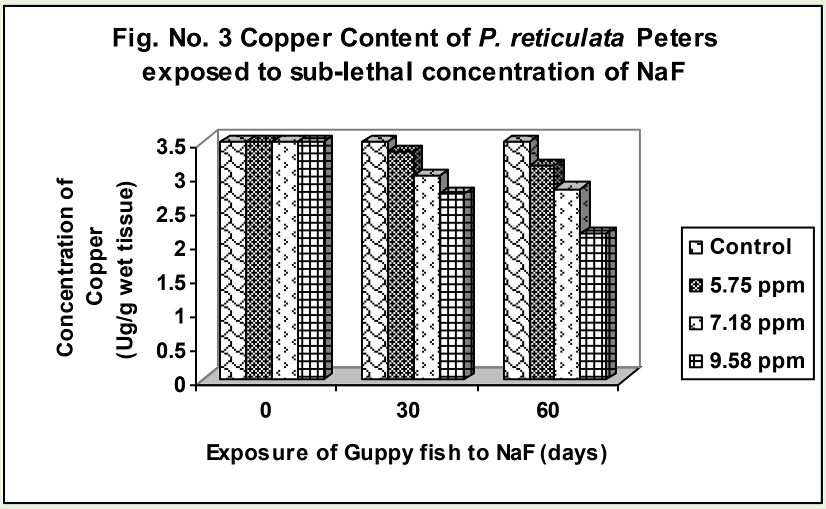


Table No. 1 Alteration in mineral content in muscle of *Poecilia reticulata* Peters exposed to sub-lethal concentrations of NaF

Trace Mineral	Period (days)	Control	Concentration of NaF (ppm)		
			5.75	7.18	9.58
Iron (µg per g wet tissue)	Initial	15.0 ±0.095	15.0 ±0.095	15.0 ±0.095	15.0 ±0.095
	30	15.0 ±0.095	13.0* ±0.026 (-13.33)	11.0* ±0.11 (-26.66)	9.1* ±0.085 (-39.33)
	60	15.0 ±0.095	9.5* ±0.016 (-36.66)	8.0* ±0.073 (-46.66)	4.6* ±0.081 (-69.33)
Zinc (µg per g wet tissue)	Initial	98.0 ±0.039	98.0 ±0.039	98.0 ±0.039	98.0 ±0.039
	30	98.0	99.5	100.4	115.0*

		±0.039	±0.018 (+1.53)	±0.026 (+2.44)	±0.058 (+17.34)
	60	98.0 ±0.039	101 ±0.135 (+3.06)	102.5 ±0.171 (+4.59)	128.0* ±0.077 (+30.61)
Copper (µg per g wet tissue)	Initial	3.5 ±0.08	3.5 ±0.08	3.5 ±0.08	3.5 ±0.08
	30	3.5 ±0.08	3.35 ±0.125 (-4.28)	3.0* ±0.091 (-14.28)	2.73* ±0.028 (-22.0)
	60	3.5 ±0.08	3.16 ±0.099 (-9.71)	2.81* ±0.193 (-19.71)	2.15* ±0.048 (-38.57)

Mean value of 5 observations, ± = Standard deviation ≤ 0.05, * Significant difference, Figure in parenthesis indicates significant % depletion (-) or % elevation (+) in the mineral content as compared to control. (Initial = '0' concentration)