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Changes in some haematological parameters induced on exposure to a sub-lethal dose of fluoride (NaF) in the freshwater fish, *Channa punctatus* (BLOCH)

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Alterations in some haemato-biochemical parameters of a freshwater, air breathing fish, *Channa punctatus* under the stress of chronic, sub-lethal dose (10ppm) of fluoride (NaF) for 75 days were studied. The TEC, haemoglobin % and PCV steadily and significantly decreased, whereas the TLC was noted to increase significantly with an increase in the duration of exposure time. The MCV, MCH and MCHC decreased significantly with an increase in the exposure period. The plasma protein, lipid and glucose decreased progressively and significantly whereas the plasma cholesterol and triglyceride exhibited a significant increase upto 75days of exposure. The results indicate that fluoride in a chronic, sub-lethal dose of 10ppm brings about harmful alterations in different haemato-biochemical parameters, which can be used as a tool to assess the degree of pollution and the fish health.

Key words: Fluoride (NaF), *Channa punctatus*, haemato-biochemical parameters, sub-lethal dose.

INTRODUCTION

Fluoride is a well-known strong, hard anion and cumulative toxic mediator (1), which occurs naturally and is widely distributed in the rivers, lakes and seas of the world (2). Most rivers, streams, and ponds in India are severely polluted and serve as ‘open sewers’ because domestic sewage and Industrial wastes either untreated or partially treated are discharged into them. It is a most active damaging environmental pollutant and may be considered as genotoxic, neurotixic and mutagenic agent and it can be able to induce genotoxic, neurotoxic and mutagenic effects on fish (3,4). Fluoride is a naturally occurring compound in the earth’s crust in various forms including Fluorspar (CaF_2), Cryolite ($NaAlF_6$) and fluoropatite {$Ca_{10}F_2(PO_4)_6$} and enters surface and ground water through the natural and anthropogenic sources (5). Owing to expanded industrial emissions and commercial uses of F compounds, the concentration of F is increasing in both ground and surface waters. Fluorine (F) is a non-metal and has been included in the list of 14 elements recognized to be physiologically essential for the normal development and growth of human beings (6).

Research studies showed that inorganic fluoride ions are directly toxic to aquatic and terrestrial life (7,8). The toxic effects of elevated fluoride on various aquatic species are well documented (7), as are its harmful impacts on humans, livestock and plants (7,8). It also affects the vertebrates in their

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haematological parameters (9), morphological and behavioural parameters (10) and cellular architecture (11). Fluoride acts as enzymatic inhibitor disturbing enzyme activity, and finally, interfering metabolic processes such as glycolysis and synthesis of proteins (7). Usha Rani and Naik (5) have reported that fluoride effects carbohydrate metabolism and its effect on levels of blood glucose, liver and muscle glycogen in freshwater fish Carassius auratus. Gupta has observed that F decreased glucose and protein levels in blood and muscles of the fish (11).There are several reports on the toxic effects of metals on the haematological parameters of fishes (12-14). But the information's relating to the toxic effects of fluoride at a chronic and sub-lethal dose on the haemato-biochemical parameters of fishes is scanty. Therefore the present piece of investigation was undertaken to evaluate the changes in some haematobiochemical parameters in Channa punctatus due to sub-lethal fluoride intoxication.

MATERIALS AND METHODS

Apparently normal adult *Channa punctatus* weighing between 30-35gm and having a length of 12-15cm were collected from the local freshwater resources. They were acclimatized to laboratory conditions for 15 days. The water of the medium, both during acclimatization and experimental period, was replaced every alternate day. During this period the fishes were fed daily with rice bran and oil cake in 1:1 ratio. The physico–chemical characteristics of the water used was analysed according to APHA [15].

One hundred acclimatized fish, irrespective of sex were treated with a sub-lethal, chronic dose of 10ppm fluoride (NaF) concentration for 75 days. At the end of 15, 30, 45, 60 and 75 days, blood was drawn from the treated fishes as well as the control fishes by cardiac puncture using a heparinised hypodermic syringe. The blood from several fishes was pooled and then transferred to small vials, which were previously rinsed with heparin. The whole blood was used for the estimation of various haematological parameters. Then the collected blood sample was centrifuged at 10,000 rpm for 20 minutes to separate the plasma, which was used for the estimation of different biochemical parameters. The alterations in the values of the blood parameters after exposure of fishes for different exposure period as well as the control fishes were subjected to paired 't' test to ascertain, whether the alterations were significant or not, and the results are presented in Table No.1.

The blood form heparinized vial was used for estimation of R.B.C., W.B.C., and Hb% using the methods of Shah and Altindag [16,17] and Drabkin [18] respectively. The PCV was calculated by microhaematocrit or wintrobe's tube method [19]. The MCV, MCH and MCHC are reffered to as 'absolute' values and are calculated from TEC, Hb% and Ht%. The biochemical parameters like protein, cholesterol, triglyceride and lipid were estimated using the instrument (analyser) Kodak Ektachem DT System [20]. This system uses dry chemistry technology and dry chemical layer coated slides and the test results are automatically printed by an integral printer. The plasma glucose was estimated by O-Toluidine method of Cooper and Mc Daniel[21].

RESULTS AND DISCUSSION

The TEC increased upto 45days of exposure and thereafter decreased after 60 and 75days of exposure whereas the Hb% and PCV% showed a steady and significant decrease with an increase in the duration of exposure to 10ppm fluoride. Similar decrease in TEC, Hb% and PCV were noted when various fishes were treated with zinc (14), lead (22) and mercury (23). Apart from metals, pesticides and herbicides such as atrazinc(24) and monocrotophus (25) also significantly decreased the TEC, Hb% and PCV in fishes. The decrease in TEC, Hb% and PCV might be due to haemolysis of the blood forming cells, which fail to absorb required nutrients for normal erythropoisis. In contrast to the above findings Isikawa et.al., (26) and Alwan et.al., (27) observed an increase in TEC, Hb% and PCV of fishes exposed to mercury and aluminium respectively.

The MCV, MCH and MCHC decreased steadily with an increase in the exposure period to the chronic dose of 10ppm fluoride. Similar results have been reported in fluoride treated Channa punctatus (9). Ishikawa et.al.(26) were of the opinion that there were no significant difference among the mean haematological values when Tilapia was exposed to a sub-lethal dose of mercury. Alwan et.al,(27) observed a significantly lower MCV value in Tilapia zilli treated with aluminium, which is in agreement with the present investigation. In contrast to the present findings Alwan et.al, (27) observed a significant increase in MCH and MCHC compared to the control values in Tilapia zilli treated with aluminium. Adeyemo (22) reported an increase in MCV, MCH, and MCHC in Clarias gariepinus treated with lead.

The TLC exhibited a gradual and significant decrease with an increase in the exposure period to 10ppm fluoride. Ishikawa et.al, (26) and Ololade and Ogini (14) while working on the effects of mercury on Oreochromis niloticus, and zinc on Clarias gariepinus respectively noted a decrease in the TLC. In contrast to these findings Adeyemo (22) noted a gradual increase in the TLC of Clarias gariephinus treated with a sub-lethal dose of lead. Yaji and Auta (25) while working on the effect of monocrotophos and Ramesh et.al, (24) while working on the effect of atrazinc observed an increase in the TLC of fishes.

Table-1. Changes in some haematological parameters of *Channa Punctatus* on prolonged exposure to sub-lethal (10ppm) concentrations of fluoride.

Parameters	Control 0ppm	15 days	Control 0ppm	30 days	Control 0ppm	45 days	Control 0ppm	60 days	Control 0ppm	75 days
TEC ($10^6/mm^3$) % change	3.16± 0.16	3.36± 0.18 +6.32^{a}	3.16± 0.18	3.60± 0.06 +13.92^{a}	3.12± 0.06	3.42± 0.18 +9.16^{a}	3.08± 0.12	2.86± 0.12 -2.80^{b}	3.02± 0.02	2.46± 0.03 -8.14^{a}
Hb (g %) % change	10.26± 0.12	9.98± 0.24 -9.72 ^{a}	10.12± 0.16	9.86± 0.14 -9.74^{a}	10.02± 0.08	9.08± 0.08 -10.98^{a}	9.86± 0.16	8.64± 0.12 -12.37^{a}	10.02± 0.08	7.12± 0.06 -14.28^{a}
PCV % change	28.64± 0.92	28.04± 0.82 -2.09^{b}	28.24± 0.66	27.68± 0.86 -3.39^{b}	28.04± 0.76	26.48± 0.54 -5.56^{a}	27.78± 0.62	25.82± 0.48 -7.05^{a}	28.20± 0.58	24.08± 0.04 -8.54^{a}
MCV(μm^3) % change	102.28± 2.42	100.16 ±1.86 -2.07^{b}	101.72± 1.76	98.68± 2.32 -2.98^{b}	101.46± 1.38	96.24± 1.02 -5.13^{a}	100.78± 1.06	93.12± 0.98 -7.60^{a}	98.12± 0.98	90.10± 1.02 -9.18^{a}
MCH (pg) % change	30.68± 0.66	30.20± 0.52 -1.56^{c}	30.26± 0.60	29.46± 0.64 -2.64^{b}	30.12± 0.52	28.62± 0.48 -4.98^{b}	29.80± 0.48	27.54± 036 -7.83^{a}	30.08± 0.56	25.12± 0.42 -8.35^{a}
MCHC (%) % change	5.62± 0.24	5.52 ±0.32 -1.77^{c}	5.56± 0.22	5.48± 0.22 -1.80^{c}	5.48± 0.26	4.96± 0.22 -9.31^{a}	5.26± 0.24	4.28± 0.18 -18.63^{a}	5.40± 0.20	3.96± 0.16 22.12^{a}
TLC ($10^3/mm^3$) % change	8.54± 0.36	8.96± 0.28 +4.91^{b}	8.26± 0.42	9.02± 0.28 +9.20^{a}	8.08± 0.18	8.12± 0.24 +0.49^{c}	7.96± 0.08	7.16± 0.14 -10.05^{a}	8.02± 0.12	6.86± 0.08 -13.68^{a}
Total protein (gm/dl) %change	7.86± 0.18	7.62± 0.20 -3.05^{b}	7.54± 0.24	7.24± 0.16 -4.12^{b}	7.48± 0.18	6.68± 0.22 -10.69^{a}	7.68± 0.16	6.12± 0.08 -16.34^{a}	7.50± 0.12	5.62± 0.06 -20.42^{a}
Cholesterol (mg/dl) % change	52.68± 0.24	55.32± 0.38 +5.01^{a}	52.26± 0.22	58.24± 0.30 +11.44^{a}	52.24± 0.20	59.12± 0.24 +13.16^{a}	52.08± 0.12	60.42± 0.28 +16.01^{a}	52.54± 0.10	63.52± 0.24 +22.36^{a}
Triglyceride (mg/dl) % change	32.12± 0.38	33.48± 0.24 +4.23^{b}	31.88±0 .36	34.46±0. 42 +8.09^{a}	31.86±0 .30	35.68±0 .40 +11.98^{a}	31.48±0 .24	36.38±0 38 +15.56^{a}	31.98±0 .20	38.16± 0.20 +21.22^{a}
Total Lipid (mg/dl) % change	70.28± 1.12	68.64± 0.98 -2.23^{b}	69.64± 1.02	67.36± 0.96 -4.08^{b}	69.24± 0.86	65.86± 0.72 -5.80^{a}	69.30± 1.02	63.48± 0.62 -9.80^{a}	69.48± 1.06	61.20± 0.80 -12.28^{a}
Glucose (mg/dl) % change	58.24± 0.98	57.46± 0.92 -1.33^{c}	57.96± 0.88	56.36± 0.68 -2.82^{b}	57.76± 0.54	54.28± 0.36 -6.98^{a}	58.00± 0.62	52.24± 0.68 -10.02^{a}	57.98± 0.58	50.62± 0.58 -13.02^{a}

NS= Non significant, a= p>0.001, b=p>0.01,c=p>0.05

The decrease in TLC under stress may be due to tissue damage under different metallic stress.

The serum protein showed a steady and significant decrease with an increase in the duration of exposure to 10ppm fluoride. Various workers while working on the toxicity of mercury (23), zinc (14) and lead (22) also noted decrease in serum protein. This decrease might be due to malfunctioning of the liver and reduced protein synthesis or protein break down. Another reason for decrease in the value of serum protein may be due to excessive loss of protein due to nephropathy.

The plasma cholesterol and triglyceride contents increased steadily and significantly with an increase in the exposure period to 10ppm fluoride. Zaki et.al,(28) while working on the toxic effects of vanadium on Heteropneustes fossilis noted an increase in cholesterol and triglyceride. However Martinez et.al, (29) have reported a decrease in the cholesterol and triglyceride levels in Prochilodus lineatus exposed to lead. Increase in serum cholesterol and triglyceride presents a stressful condition in fish.

The serum lipid content showed a significant decrease with an increase in the exposure period at 10ppm fluoride. Shah (13) observed decrease in the lipid content of Tinca tinca treated with lead. Vutukuru (30) observed a decrease in the lipid content in Labeo rohita exposed to hexavalent chromium. Ramesh et.al, (24) while working on the toxic effects of atrazinc on Cyprinus carpio observed a decrease in plasma lipid content. In contrast to the above findings Zaki et.al, (28) noted a significant increase in the lipid content of Oreochromis niloticus exposed to lead. The decrease in plasma lipid may be due to their utilization in cell repair and tissue organization with the formation of lipoproteins.

There was significant decrease in the serum glucose level when the fish was treated with a 10ppm sub-lethal dose of fluoride for 75days. Several workers have reported an increase in the glucose level in different tissues of fishes due to metallic stress (28,29). Ramesh et.al, (24) noted a decrease in the glucose content of Cyprinus carpio under the stress of atrazinc, an herbicide. The changes in glucose content reflect the changes in carbohydrate metabolism under hypoxia and other stress conditions and it indicated the presence of stressful stimuli.

The present investigation exhibits the high toxic nature of fluoride on fishes and the fishes are very sensitive to the presence of even minute quantities of the pollutants. The present study also suggests that the alterations in the blood indices may be attributed to a defensive reaction against the toxicity of fluoride and may be due to the disturbances that occurred in both metabolic and haemopoitic activities of the fish exposed to fluoride.

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