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International Journal of All Research Education and Scientific Methods (IJARESM),
ISSN: 2455-6211, Volume 11, Issue 5, May-2023, Available online at: www.ijaresm.com

Biochemical Alteration Due To Accumulation of Fluoride in Cat Fish, *Clarias Batrachus*

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ABSTRACT

Fluoride is a natural component of the earth's crust and soil. Small amounts of fluorides are present in water, air, plants, and animals, which when enters the food chain causes toxic effects to the ecosystem due to its bioaccumulation. The fluoride ions act as enzymatic poisons, inhibiting enzyme activity and ultimately interrupting metabolic process such as glycolysis and synthesis of proteins. After oral uptake, water-soluble fluorides are rapidly and almost completely absorbed in the gastrointestinal tract. As soon as fluoride is absorbed, blood fluoride levels increase. The study deals with the acute exposure of sodium fluoride to *Clarias batrachus* under laboratory condition. Biological samples were collected after every 24 hours for estimation of biochemical parameters (Total protein, ACP, ALP, GOT, GPT). The percent fluoride accumulation and the bio-concentration factor were studied after 96 hr of exposure. The result indicates significant changes in the biochemical parameters. The statistical analysis was done using five-way ANOVA.

Keywords: Bioaccumulation, Biochemical alteration, Bio concentration factor

INTRODUCTION

Fluoride is a natural component of the earth's crust and soil. Small amount of fluoride is present in water, air, plants, and animals, which when enters the food chain causes toxic effects to the ecosystem (1). Aquatic life is continuously exposed to fluoride which tends to accumulate in the exoskeleton, bones and tissues of the fishes (2). The fluoride ions act as an enzymatic poison, inhibiting enzyme activity (3) and ultimately interrupting metabolic process such as glycolysis and synthesis of proteins (4). High concentration of fluoride causes fluorosis. After oral uptake, water-soluble fluorides are rapidly and almost completely absorbed in the gastro-intestinal tract. As soon as fluoride is absorbed, blood fluoride levels increase (at 10 minutes), reaching peak levels at 60 minutes. The rate of fluoride absorption from the stomach is directly related to the acidity (pH <3.5) of its contents. Absorbed fluoride is transported via the blood; with prolonged intake of fluoride from drinking-water, concentrations in the blood are the same as those in drinking-water, a relationship that remains valid up to a concentration in drinking-water of $10mgL^{-1}$. Distribution of fluoride is a rapid process. It is incorporated into exoskeleton and bones; there is virtually no storage in soft tissues. Incorporation into exoskeleton and skeletal tissues is reversible: after cessation of exposure, mobilization from these tissues takes place. Fluoride is excreted via kidneys (5, 6, 7, 8).

MATERIALS AND METHODS

1. Exposure of Toxicants

Healthy cat fishes *C. batrachas* were procured live from the local fish market and acclimatized for seven days in glass aquaria under laboratory conditions with continuous oxygen supply and fed daily (twice a day). After acclimatization fishes were subsequently divided into two groups i.e., control and experimental. Fishes of control group were exposed to normal tap water, whereas experimental group were exposed to sodium fluoride in water for short term duration of 96hrs with sub lethal concentration 300 ppm of NaF.

2. Sample Collection

(A) Water- Water is collected from the aquaria in plastic bottle for residual fluoride analysis after 96 hrs of treatment.

(B) Blood samples-Blood samples were collected in centrifuged tubes (4-5mL) at 24, 48, 72 and 96hr respectively by cutting the caudal peduncle. Further, it was centrifuged at 3000 rpm for 15 minutes and the collected serum was preserved at -20˚C until analysis.
(C) Tissue samples-Fishes were dissected at a regular interval of 24, 48, 72 and 96hr and liver and kidney tissues were extracted for analysis.

3. Samples Preparation

Water and serum samples were analyzed directly without pre-treatment whereas tissue samples were prepared by the method of Birkel and, (9). Tissues were homogenized and dried for 24hrs at 105˚C in a closed compartment; a weight of 200mg dry sample was dissolved in 2mL of 1:1 mixture of 11.6M perchloric acid and 14.3 M nitric acid and neutralized with citrate buffer to a pH 5.5 with a mixture of 7.8M sodium hydroxide and 1.0M trisodium citrate. The resulting solution sample thus obtained was used after appropriate dilution for recording the fluoride content.

4. Fluoride Analysis

(A) Quantitative analysis was done in the laboratory with the help of Thermo Fisher Scientific Orion 9609 BNWP ion selective fluoride electrode (APHA-AWWA-WPCF, 2005). Water Fluoride analysis was done with the help of direct determination method & Serum and tissue fluoride was estimated with the help of analyte addition method by adding TISAB in all samples.
(B) Calculations-*Water samples:* Direct determination method.

Serum & tissue samples: Analyte addition method using following equation:

$$C_U = C_S [(V_U / V_{S+}V_U)* 10^{\Delta E/S}]$$

Where: C_U =concentration of unknown sample, C_S = concentration of standard sample, V_U = volume of unknown sample, V_S = volume of standard sample$\Delta E = E_2 - E_1$= is the change in the electrode potential after addition, E_2= **mV** after addition of sample, E_1=**mV** before addition of sampleS = slope of the electrode

5. Enzyme Estimation

Tissue extracts were prepared in ice cold saline and kept under -20˚C until analysis. Biochemical analysis- Total protein (Biuret method), Alkaline phosphates (ALP) & Acid phosphates (ACP) (10), Gutamate oxaloacetate transaminases (GOT) &Glutamate pyruvate transaminases (GPT) were done by Reitman and Frankel method (11).

RESULTS

Total protein

Changes in the level of total protein in serum, liver and kidney of *C. batrachus* in unexposed and exposed group are shown in Fig.1. Results shows significant *(p<0.05)* decrease in the level of protein in serum in first 24 and 48 hours but there is gradual increase in the serum protein after72 and 96 hours, similarly there is significant decrease in the total protein in kidney has been observed after 24, 48 and 72 hrs of exposure, but conversely to serum protein total protein in liver significantly increase in fluoride exposed fishes.

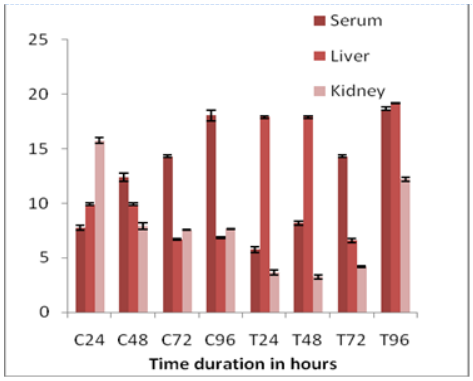

Fig.1 Effect of Naf On Total Protein (Mg/Ml), Mean (± Se) Values At P<0.05. Statistically Analyzed Using Costat Software

Antioxidant Enzymes

Activity of ACP, ALP, GOT and GPT enzymes in serum of *C. batrachus* in both control and exposed groups is shown in Fig- 2, 3, 4 & 5 respectively. Antioxidant enzyme activities increase in serum *(p<0.05)* during initial phase of exposure i.e., 24 hrs in comparison with control group, but in later phases exposure i.e., 48,72- & 96-hours enzyme activities further decrease, Similarly the activities of all four enzymes in liver and kidney exhibited a significant *(p<0.05)* increase in first 24 hours but there is gradual decrease in their activity in subsequent 96 hours.

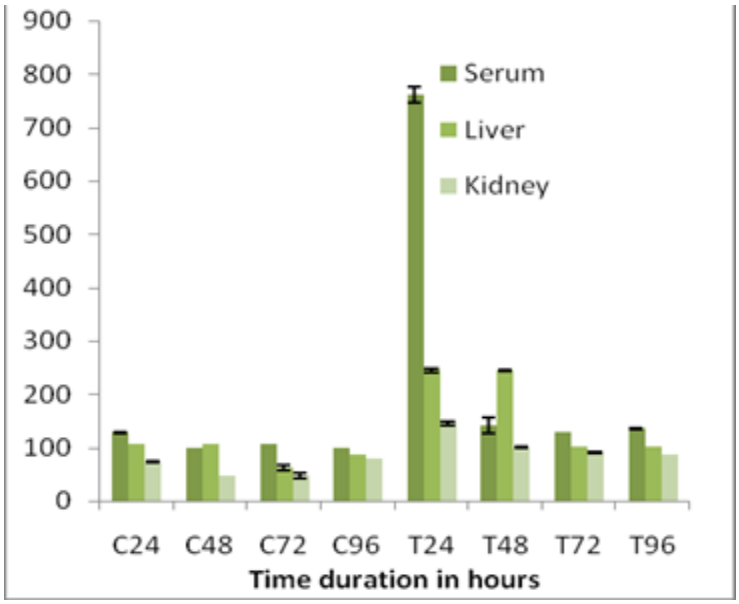

Fig.2 Effect of Naf on Activity of Enzyme ACP (IU/Mg Protein/Ml Serum, Means (± SE) Values at P<0.05. Statistically Analyzed Using COSTAT Software

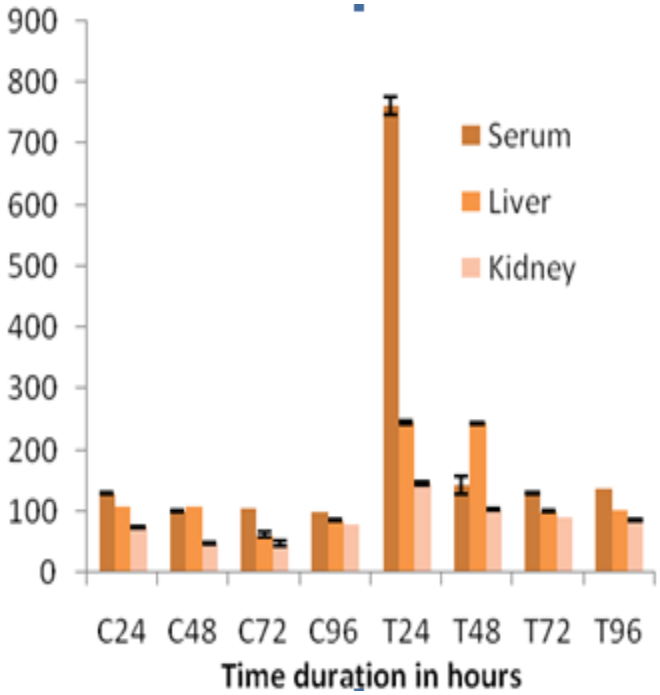

Fig.3 Effect of Naf on Activity of Enzyme ALP (IU/Mg Protein/Ml Serum, Means (± SE) Values at *P*<0.05 Statistically Analyzed Using COSTAT Software

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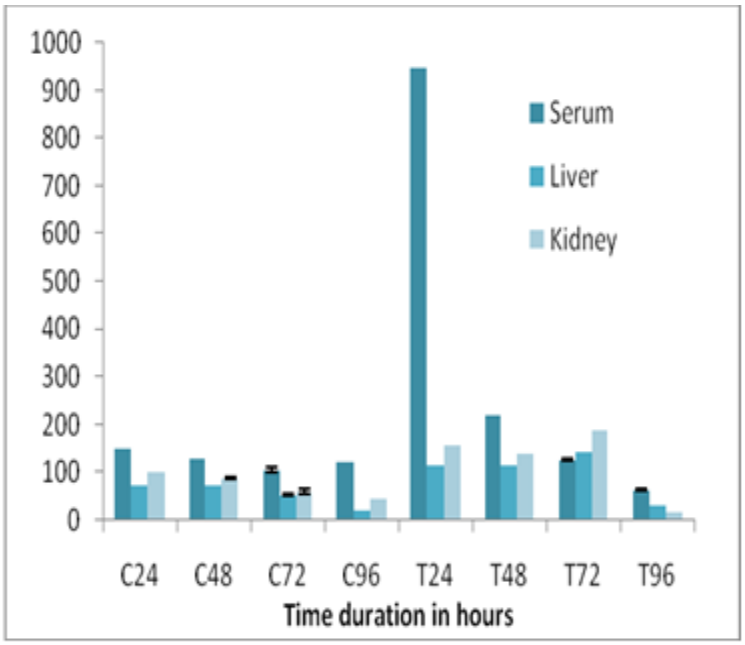

Fig.4 Effect of NaF on activity of enzyme GPT (IU/mg protein/ml serum, means (± SE) values at p<0.05. Statistically analyzed using COSTAT software

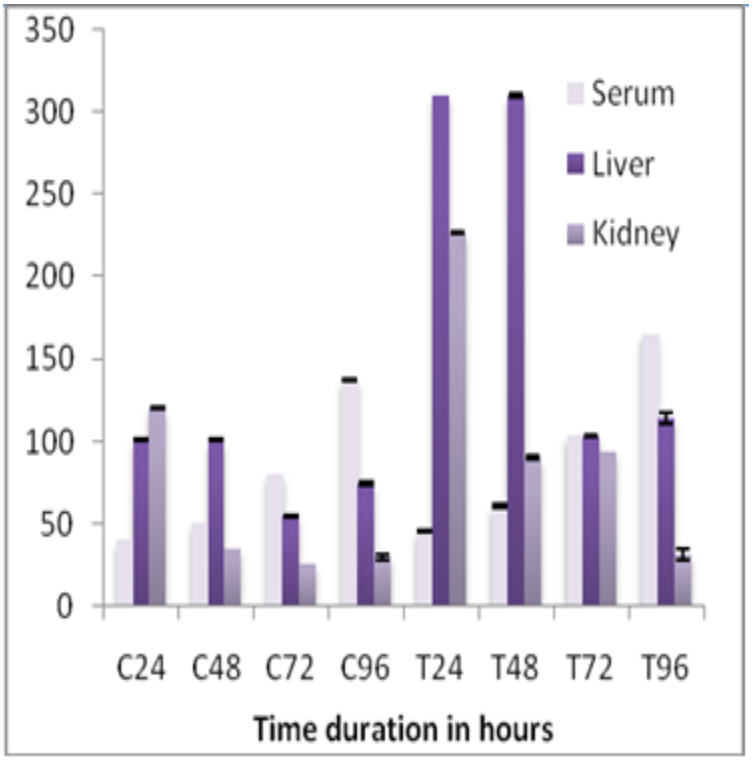

Fig.5 Effect of NaF on activity of enzyme GOT (IU/mg protein/ml serum, means (± SE) values at p<0.05.Statistically analyzed using COSTAT software.

Bio-concentration factor of fluoride

Bio-concentration factor of fluoride in serum, liver and kidney in 96 hours are shown in Fig.6. Concentration of fluoride in serum is lower than liver and kidney (Fig.7).

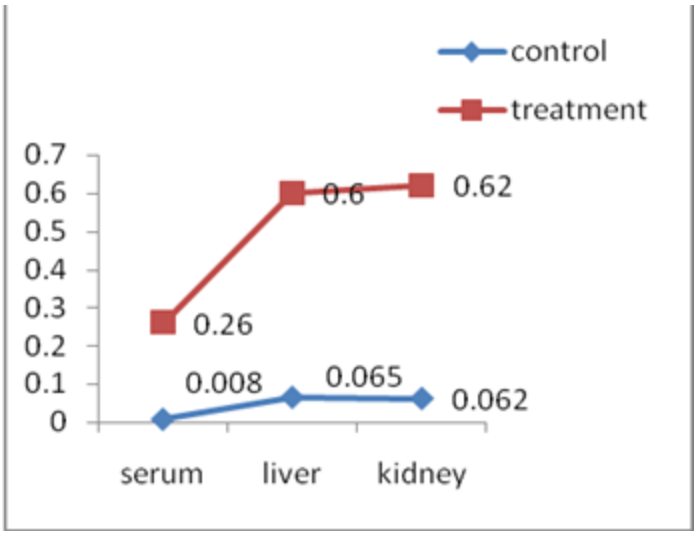

Fig 6 Bio Concentration Factor of Naf in Different Organ

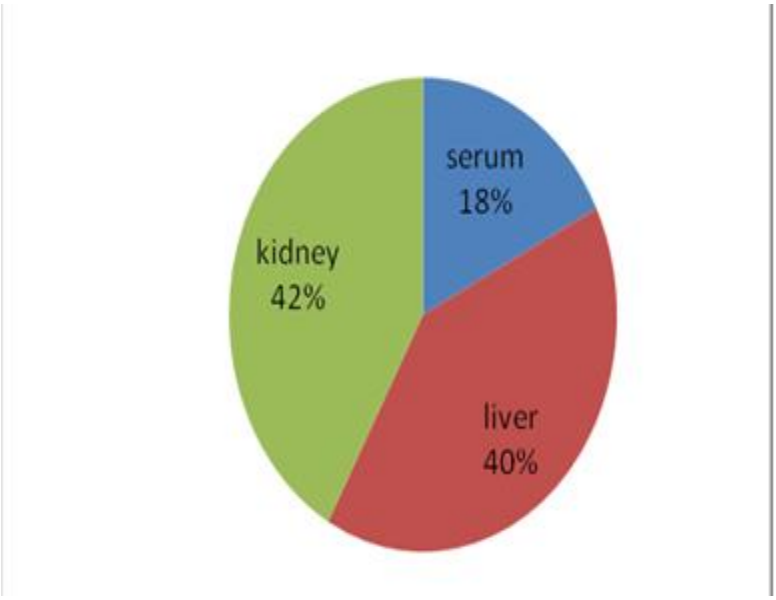

Fig 7. Concentration of fluoride in different organ (ppm)

DISCUSSION

As soon as fluoride is ingested and absorbed, serum fluoride levels increase (at 10 minutes), reaching peak levels at 60 minutes and return to its basal level after 11-15 hours [8]. Absorbed fluoride is transported via the blood; distribution of fluoride is a rapid process. It is incorporated into exoskeleton and bones; there is virtually no storage in soft tissues and usually excreted via kidneys.

Accumulation of fluoride in serum in first few hours leads to an increase in the biochemical parameters, which further decreases gradually after storage of fluoride in the soft tissues which is indicated by increased activity of various enzymes. The enzyme activity goes up when there is a toxic impact and the enzyme begin to counteract the toxic impact, subsequently the enzyme activity begins to drop either as a result of having partially or fully encountered the toxin or as a result of cell damage (12). Amongst the four enzymes, GOT in the most sensitive and protective enzymes against oxidative damage of serum, liver and kidney in fluoride exposed fishes. Fluoride is known to increases concentration of free radicals

by decreasing activities of free radical scavenging enzymes such as SOD, catalase, GOT, GST (13, 14). Due to increasing concentration of free radicals' fluoride causes oxidative tissue damage (15).

Decline in protein related to fluoride interfering with protein synthesis. But in subsequent hours serum protein increases, may be due to during stress conditions fish need more energy to detoxify the contaminants so that overcome stress and repair the damaged tissues.

ACKNOWLEDGMENT

The authors are grateful to Head, School of Studies in Life Science for providing lab facilities.

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