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Morinda citrifolia L. (Noni) dietary supplementation ameliorates fluoride toxicity in the freshwater fish, *Cyprinus carpio* L.

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Fluoride is the organic and inorganic compound of the element fluorine, has detrimental effect to all animals including fish at higher concentration. Ground water with elevated concentration of fluoride is a major global problem including India. On the other hand, *Morinda citrifolia* (also called 'Noni' in Polynesia) is a medicinal plant, has antioxidative, anti-inflammatory and immunostimulatory properties. The ameliorating effect of *M. citrifolia* fruit extract dietary supplementation against fluoride induced toxicity on the growth performance, haematological parameters, and fluoride accumulation in the muscular tissue was investigated in the freshwater fish, *Cyprinus carpio* L. for 35 days in triplicate. One hundred and twenty acclimated *C. carpio* fingerlings were randomly allocated into five experimental groups (Groups I, II, III, IV and V) each with 24 fish divided into three replicates (eight fish/replicate). Group I (Control) and II (Toxic control or negative control) were provided with basal/control diet, whereas groups III, IV, and V were provided with 0.25% (2.5 g/kg), 0.50% (5.0 g/kg) and 0.75% (7.5 g/kg) *M. citrifolia* fruit extract supplemented diet respectively. All groups except for group I were exposed to 10% of the LC_{50} of fluoride. Selected growth and haematological parameters were estimated. The fluoride content in the muscular tissue of fish was also estimated following SPADNS method. Results revealed that exposure of sublethal concentration of fluoride significantly (P <0.05) reduced the growth performance and haematological parameters in Gr. II (toxic control) compared to the Gr. I (control). However, *M. citrifolia* dietary supplementation significantly (P <0.05) improved fluoride induced alteration of growth performance and haematological parameters of fish in the groups III, IV and V compared to Gr. II. *Morinda citrifolia* fruit extract dietary supplementation also significantly (P <0.05) decreased the accumulation fluoride in the muscular tissue of fish in groups III, IV and V compared to the Gr. II (toxic control). In conclusion, *M. citrifolia* dietary supplementation ameliorates the fluoride toxicity by preventing the accumulation of fluoride in the muscular tissue of the fish. The *M. citrifolia* dietary supplementation at 0.25% showed the best response, hence it is considered as the optimum dose.

Keywords: Aquatic pollution, Common carp, Dietary supplement, Trace elements

Fluorine is the most electronegative and highly reactive of all elements, form fluoride ion (F^-) by ionization. It occurs in 296 species of minerals but abundantly present in fluorite, fluorapatite, topaz and cryolite which are easily soluble in water, resulting in higher concentration in ground water[1]. Although fluoride has beneficial effect at lower concentration (0.5-1.0 mg/L) in growth and development of bone and teeth, but it causes dental- and skeletal fluorosis at higher concentration (>1.5 mg/L)[2]. Elevated concentration of fluoride in ground water is an endemic in different countries of the world, including India[3]. Fluoride contaminated ground water is prevalence in 23 out of 37 states and union territories of India[4] including West Bengal[5]. Having neurotoxicity of fluoride in the human development, several epidemiological studies placed it in the same category with toxic metals like lead, methyl mercury, arsenic, and polychlorinated biphenyls[6]. Several studies reported the detrimental effects of fluoride in animals including fish[7-13].

Morinda citrifolia L. (also called 'Noni' in Polynesia and 'Indian Mulberry' in India), a small evergreen tree of the family Rubiaceae is a well known medicinal plant in Southeastern Asia[14,15]. It is used in the treatment of many diseases like diabetes, high blood pressure, arthritis, hypertension, heart diseases, headache and fever[16]. *M. citrifolia* has antioxidant[17,18], anti-inflammatory[19-21], immunostimulatory[22] and antimicrobial[23] activities.

The common carp, *Cyprinus carpio* L (Cyprinidae) has been recognized as a suitable model organism for

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bioassay experiment by the Organization for Economic Cooperation and Development (OECD, 1992). It is widely distributed and cultivated in most part of the world including India[24]. It is also the 4th major species (7.7% of the total) produced in world aquaculture[25]. Considering the importance in commercial aquaculture and widespread distribution, *C. carpio* was selected as the model organism in the present study.

Owing to the non availability of specific drug to inhibit fluoride toxicity, diet editing in which ingested fluoride can be eliminated, and diet counselling in which ingestion nutrient rich diet, are the only alternatives[26,27]. Several plant based dietary supplements were used in the past to reduce fluoride toxicity[27,28]. However, till date there is no study using *M. citrifolia* fruit extract dietary supplements to ameliorate fluoride toxicity in animals. Hence in this study, we investigated the ameliorating effect of *Morinda citrifolia* fruit extract dietary supplementation against fluoride induced toxicity on the growth performance, haematological parameters, and fluoride accumulation in the muscular tissue in the freshwater fish, *Cyprinus carpio* L.

Materials & Methods

Experimental diet

The source of *Morinda citrifolia* L. fruit extract was the 'Noni capsules', manufactured by the Cosmic Nutracos Solutions Pvt. Ltd. The proximate composition of the noni fruit extract was analyzed before incorporation in the diet[29]. The *Cyprinus carpio* fingerlings can tolerate the *M. citrifolia* fruit extract up to 30 g/kg (3%) which was determined before start of the experiment as per the guidelines of Organization for Economic Cooperation and Development (OECD) for 14 days. From this range of tolerance, *M. citrifolia* fruit extract was incorporated at 0, 0.25% (2.5 g/kg), 0.50% (5.0 g/kg), and 0.75% (7.5 g/kg) level to prepare control or basal diet, 0.25, 0.50, and 0.75% *M. citrifolia* diet respectively. The ingredients as well as proximate composition[29,30] of the experimental diets is presented in Table 1.

Experimental design

The experimental study was conducted as per the internationally accepted laboratory animal use and care, and guidelines (guiding principles in the use of animals in toxicology, adopted by the Society of Toxicology in 1989), and as per the guidelines of the Institutional Animal Ethics Committee, University of Calcutta, West Bengal, India. In a previous study, the 96 h LC_{50} values of fluoride to *C. carpio* was estimated[31]. In this experiment 10% of the 96 h LC_{50} i.e. 67.5 mg/L of NaF was used as the sublethal dose. *C. carpio* fingerlings were purchased from a local fish market located at Naihati city, North 24 Parganas, West Bengal, India, and then brought to the laboratory in plastic bag with sufficient oxygen. The collected fingerlings were stocked in a large glass aquarium (150 L capacity) and acclimated for a total period of one month. During this period of acclimation, fingerlings were fed commercial diet at 3% of body weight daily and the water was continuously aerated. A total of 120 acclimated *C. carpio* fingerlings (mean weight: 7.011±0.30 g) of three month old were randomly allocated into five experimental groups (groups I, II, III, IV and V) each with 24 fish divided into three replicate (eight fish/replicate). Group I fish were provided with control diet and reared in fluoride free normal tap water (surface water), thus served as control group. Group II fish were also provided with control diet but they were exposed to 10% of the LC_{50} of fluoride (NaF @ 67.5 mg/L), therefore termed as toxic control

Table 1 — Experimental diets and ingredients with proximate composition (AOAC 1990)[29]

Ingredients (g/kg)	Basal or Control diet	*Morinda citrifolia* diet		
		0.25% (2.5 g/kg)	0.50% (5.0 g/kg)	0.75% (7.5 g/kg)
GNO cake[1]	600	600	600	600
Fish meal[2]	200	200	200	200
Rice bran[3]	100	100	100	100
Wheat flour	90	87.5	85	82.5
M. citrifolia[4]	-	2.5	5.0	7.5
Vit. & Min. mix	10	10	10	10
Proximate composition				
Dry matter	88.4±0.087^{b}	90.2±0.17^{a}	90.7±0.15^{a}	90.4±0.092^{a}
Crude protein	40.3±0.2^{ab}	40.6±0.068^{a}	40.2±0.22^{ab}	39.2±0.39^{b}
Fat	8.75±0.076^{c}	8.32±0.05^{c}	10.1±0.35^{b}	11.7±0.18^{a}
Fiber	6.22±0.15^{b}	6.87±0.15^{a}	6.67±0.088^{ab}	6.2±0.058^{b}
Ash	8.10±0.058^{c}	9.08±0.079^{ab}	8.8±0.061^{b}	9.22±0.067^{a}
Moisture	11.6±0.087^{a}	9.8±0.17^{b}	9.32±0.15^{b}	9.63±0.092^{b}
NFE[5]	25.1±0.28	25.5±0.78	24.8±0.036	23.9±0.082
GE (Kcal/100 g feed)[6]	413.208	414.953	424.947	430.148

[Composition of vitamin & mineral mixture(premix): Each 1 kg contains Vitamin A 8,00,000 IU, Vitamin D_3 80,000 IU, Vitamin E 0.6 g, Nicotinamide 1.2 g, Cobalt 2.2g, Copper 4.7g, Iodine 0.6 g, Iron 2.2 g, Magnesium 6.5 g, Manganese 3.3 g, Potassium 0.2 g, Sodium 0.04 g, and Zinc 10 g. [1]Ground nut oil (GNO) cake contains 55.43% proteins and 14.45% fat; [2]Contains 51.65% proteins and 7.6% fat; [3] 9.25% proteins and 8.3% fat; [4] 2.84% proteins, 2.5% fat, 4.5% ash and 7.1% moisture; [5]NFE (nitrogen free extract)=100-(protein+fat+ash+crude fiber); and [6]GE (gross energy): Estimated according to NRC (1993)[30] as 4.64, 9.44 and 4.11 Kcal/gm for protein, fat and carbohydrate respectively. Values are in mean±SE, (n=3 per sample). Different superscripts in a row are differ significantly (P <0.05)]

group or negative control group. Whereas, groups III, IV, and V were provided with 0.25, 0.50 and 0.75% *M. citrifolia* fruit extract supplemented diet respectively, and were exposed to 10% of the LC_{50} of fluoride (NaF @ 67.5 mg/L). The experiment was continued for a total period of 35 days. Water was aerated continuously with the replacement of water in every alternate day. Fish were provided feed @ 3% of their body weight at 9.00 h daily[32].

Physicochemical parameters of water

The physicochemical parameters of water were measured as per the method provided elsewhere[33] and the values were in the optimum level (Dissolve Oxygen 7.44±0.049 mg/L, Free carbon dioxide 10.22±0.053 mg/L, Total alkalinity 92.33±1.452 mg/L, Total hardness in $CaCO_3$ 126±2.081 mg/L, Water temp. 25.66±0.440°C and pH 7.76±0.145.

Fluoride content in water and fish muscle

The SPADNS spectrophotometric method was followed to estimate the fluoride content in water as well as in fish muscle[33].

Haematological parameters

After the end of the 35 days of feeding trial, blood was collected by cutting the caudal peduncle using a sharp knife, after anaesthetized with clove oil. The blood was collected in a watch glass containing EDTA (ethylene diamine tetra acetic acid). The erythrocyte (RBC) and leukocyte (WBC) of blood were counted using an Improved Neubauer haemocytometer. Whereas, haemoglobin (Hb) content of the blood was measured using Sahli's haemoglobinometer[34].

Statistical analysis

Oneway Analysis of Variance (ANOVA) followed by Tukey's post hoc test was conducted to compare the means between experimental groups using Statistical Package for Social Science (SPSS) version 20.

Results & Discussion

The results of the present study showed that sublethal concentration of fluoride adversely affected all the growth parameters by significantly (P <0.05) reducing weight gain, weight gain%, specific growth rate (SGR), and protein efficiency ratio (PER), and significantly (P <0.05) increasing feed conversion ratio (FCR) in Gr. II compared to the Gr. I (control) (Table 2). The result of the present study is in agreement with several previous studies in which fluoride negatively affected the growth performances in fish[35-37]. Fluoride inhibited the growth performances in fish through the anorexia and reduction of feed intake[35], higher accumulation in bones[36], and inhibition of enzymatic activity[37,38].

However, *M. citrifolia* dietary supplementations significantly (P <0.05) improved the fluoride toxicity induced alteration of growth performance in groups III, IV and V compared to Gr. II (Table 2). *Morinda citrifolia* fruit extract also enhances the growth performance in white leg shrimp[16]. *M. citrifolia* dietary supplementation possibly enhanced the growth performance of fish via increasing the digestive enzyme activity[16]. In addition, the nutrients rich nature of *M. citrifolia* positively affected the overall metabolism resulting in the higher growth performance in *M. citrifolia* fed groups (groups III, IV and V) compared to the Gr. II[14]. Other plant based dietary supplement like *Arthrospira platensis* (spirulina) also improves the fluoride induced alteration of growth performance in fish[39].

The result of the present study showed that exposure of sublethal concentration of fluoride altered

Table 2 — Growth performance of fish fed *Morinda citrifolia* diet and exposed to sublethal concentration of fluoride

	Group I	Group II	Group III	Group IV	Group V
Initial wt.(g)	7.11±0.0967	6.81±0.0681	7.17±0.0978	6.8±0.127	7.02±0.246
Final wt.(g)	$7.84±0.101^{ab}$	$7.2±0.0628^{b}$	$7.9±0.1^{a}$	$7.3±0.131^{ab}$	$7.51±0.252^{ab}$
Wt. Gain(g)	$0.73±0.00593^{a}$	$0.392±0.00608^{c}$	$0.732±0.00436^{a}$	$0.503±0.00677^{b}$	$0.494±0.00664^{b}$
Wt. gain%	$10.3±0.101^{a}$	$5.75±0.143^{c}$	$10.2±0.118^{a}$	$7.4±0.125^{b}$	$7.06±0.17^{b}$
SGR(%/day)	$0.279±0.00267^{a}$	$0.16±0.00328^{c}$	$0.277±0.00173^{a}$	$0.203±0.00328^{b}$	$0.195±0.00367^{b}$
FCR	$3.41±0.0339^{c}$	$6.1±0.153^{a}$	$3.43±0.0399^{c}$	$4.73±0.0809^{b}$	$4.96±0.117^{b}$
PER	$0.723±0.00709^{a}$	$0.404±0.00987^{c}$	$0.729±0.00819^{a}$	$0.523±0.00888^{b}$	$0.504±0.0122^{b}$
Survivality(%)	$100±0^{a}$	$83.3±4.17^{b}$	$100±0^{a}$	$95.8±4.17^{ab}$	$91.7±4.17^{ab}$

[Weight gain: Final weight – Initial weight; Weight gain %: (Final weight-Initial weight)/ Initial body weight × 100; Specific Growth Rate (SGR): 100 × (ln final weight-ln initial weight) /days; Feed Conversion Ratio (FCR): Consumed Feed / Final weight-Initial weight); Protein Efficiency Ratio (PER): Weight Gain/ Protein intake; Survivality (%): Total number of fish survived/Total number of fish stocked x 100. Values are in mean±SE, (n=3 per sample). Different superscripts in a row are differ significantly (P <0.05)]

the haematological parameters in Gr. II by significantly (P <0.05) decreasing the haemoglobin and erythrocyte count, and significantly (P <0.05) increasing the leukocyte count compared to Gr. I (control) (Table 3). The occurrence of higher number of morphologically abnormal erythrocyte (poikilocytosis) was also recorded in Gr. II compared to Gr. I. This is in consistency with several previous studies which have reported the detrimental effect of fluoride in haematological parameters in fish[28,39,40] and in mice[13]. Fluoride decreased the erythrocyte count by suppressing the erythropoiesis via interacting with iron of haemoglobin, and also inhibiting globin synthesis[13,41]. Fluoride reduces fluidity and increases the permeability of plasma membrane, resulting in the abnormal shape of the erythrocyte or poikolocytosis[42]. As a foreign material, fluoride caused the increase of leukocyte count whose main function is to defend the infectious agents and foreign materials. Fluoride induced toxicity in haematological parameters include alteration of bone marrow by the production of superoxide radical via fanton reactions[43].

Morinda citrifolia dietary supplements, however, restored the haematological parameters in groups III, IV and V by significantly (P <0.05) increasing the erythrocyte count, haemoglobin content and leukocyte count compared to Gr. II (Table 3). The increased leukocyte count by *M. citrifolia* dietary supplement in the present study demonstrates the immuno-stimulatory activity of *M. citrifolia* fruit extract. The antioxidant rich nature of *M. citrifolia* fruit extract possibly neutralized the fluoride induced excessive production of superoxide radical, thus ameliorates fluoride toxicity. In addition, *M. citrifolia* fruit extract containing vitamin E and methionine probably mitigates the fluoride induced alteration of haematological parameters[23,43]. There is no study about the effect of *M. citrifolia* fruit extract dietary supplementation in haematological parameters in animals, to compare the results of the present study. However, other plant based dietary supplement like *Arthrospira platensis* (spirulina) alleviates the fluoride toxicity induced alteration of haematological parameters in fish[28,39] and in Wistar albino rat[43].

Table 3 — Haematological parameters of fish fed *Morinda citrifolia* diet and exposed to sublethal concentration of fluoride

	Gr. I	Gr. II	Gr. III	Gr. IV	Gr. V
RBC ($\times10^6$cells/mm^3)	225± 1.76^b	158± 1.86^e	255± 1.45^a	214± 1.15^c	204± 1.15^d
WBC ($\times10^3$cells/mm^3)	189± 2.15^d	230± 0.809^c	341± 2.77^a	242± 0.794^b	240± 0.521^b
Hb (g/dL)	6.8±0.115^b	5.6±0.115^c	7.6±0.115^a	7±0.115^{ab}	6.73±0.176^b

[RBC, Red Blood Corpuscles; WBC, White Blood Corpuscles; and Hb, Haemoglobin. Values are in mean±SE, (n=3 per sample). Different superscripts in a row are differ significantly (P <0.05)]

Table 4 — Fluoride content in the experimental waters and in the fish muscle

	Gr. I	Gr. II	Gr. III	Gr. IV	Gr. V
Water (mg/L)	0.813±0.008^b	36.2±1.01^a	35.7±0.441^a	35.7±0.882^a	36.8±0.167^a
Fish (mg/kg)	93.9±1.05^c	220±1.43^a	36.9±0.698^d	210±1.21^b	213±1.22^b

[Values are in mean±SE, (n=3 per sample). Different superscripts in a row are differ significantly (P <0.05)]

The present study recorded the significantly (P <0.05) high accumulation of fluoride in the muscular tissue of Gr. II compared to Gr. I (Table 4). Cao *et al.*[11] also reported the accumulation of fluoride in the muscular tissue of *C. carpio* depending on the concentration and the time of exposure. The fluoride of the water was accumulated in the muscular tissue of fish via dissolve route[38]. The *M. citrifolia* dietary supplementation significantly (P <0.05) reduced the fluoride accumulation in the muscular tissue of fish in groups III, IV and V compared to Gr. II (Table 4). The ascorbic acid and calcium are known to decrease the absorption of fluoride[44]. *M. citrifolia* containing ascorbic acid and calcium content probably reduced the absorption of fluoride resulting in the less content of fluoride in the muscular tissue of the fish in the groups III, IV and V. In addition, the protein and amino acid content of the *M. citrifolia* fruit extract also prevented the accumulation of fluoride in the muscular tissue[45,46], and thus restored the fluoride induced alteration of growth performance as well as haematological parameters of fish in groups III, IV and V. The outcome of the present study is in agreement with James *et al.*[47] where a plant based dietary supplement like *A. platensis* (spirulina) prevents the accumulation of copper in carp. Similarly, *A. platensis* dietary supplementation reduces the accumulation of fluoride in the muscular tissue of a freshwater fish, common carp (*C. carpio* L.)[39]. Abdel-Rahman *et al.*[48] also reported the prevention of heavy metal accumulation in fish using another plant based dietary supplement, Indian lotus leaves.

Conclusion

The *Morinda citrifolia* L. (Noni) fruit extract is rich with antioxidants which neutralized the fluoride induced excessive production of superoxide radical, thus ameliorates fluoride toxicity. In addition, nutrient (essential amino acids, ascorbic acid, and calcium) rich nature of *M. citrifolia* fruit extract dietary supplement

reduced the accumulation of fluoride in the muscular tissue, and thus restored the fluoride induced alteration of growth performance as well as haematological parameters of fish. The *M. citrifolia* dietary supplement at 0.25% showed the best response, thus it is considered as the optimum dose.

Conflict of Interest

Authors declare no competing interests.

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