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SCAVENGING EFFECT OF VITAMIN C AGAINST SODIUM FLUORIDE RESIDUE ACCUMULATION IN FRESHWATER FISH, AMUR CARP (*CYPRINUS CARPIO HAEMATOPTERUS*)

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ABSTRACT: The present study was conducted to observe the scavenging effect of vitamin C against sodium fluoride (NaF) residues in the freshwater fish Amur carp (*Cyprinus carpio haematopterus*). The experiment included 5 groups i.e., G_1 (control); G_2 (NaF exposure at 100 ppm); G_3 (vitamin C supplemented diet at 100 ppm); G_4 (both NaF exposure at 100 ppm and vitamin C supplemented diet at 100 ppm), and G_5 (NaF exposure at 100 ppm for 60 days and then vitamin C supplemented diet at 200 ppm for the last 30 days). Fortnightly, 5 fish from each group were randomly collected and dissected for tissue collection to estimate fluoride accumulation. Results showed that fluoride gets accumulated in all NaF exposed groups (G_2, G_4, and G_5) but the residual content was highest in G_2 from 0^{th} to 90^{th} DPT (day post treatment). In G_5, the residual content was similar to G_2 from 0^{th} to 60^{th} DPT but at 75^{th} DPT and 90^{th} DPT, NaF concentration in exposed fish tissues was significantly lesser than those in G_2. In G_3, residual concentration was significantly less than the estimated content in G_2 throughout the experimentation period (from 0^{th} to 90^{th} DPT). While studying the fluoride accumulation in tissues of the exposed fish, residue levels were found in the order of gill > liver > intestine > kidney > muscle > gonads. Bio-concentration factor in the different tissues of experimental fishes of group G_2 was higher than G_4 group at different days post treatment. These findings revealed clearly that vitamin C has the potential to reduce the F accumulation and has a scavenging property against the free radicals in freshwater fishes.

Keywords: Amur carp; Fluoride accumulation; Scavenging effect; Vitamin C.

INTRODUCTION

The condition of water resources directly reflects the image of its fishery. The stress on aquatic environment as a result of increased industrialization, which aids urbanization, is becoming very high thus reducing the availability of clean water. Polluted water is a great threat to the aquatic organisms, plants, humans, and climate, and is exacerbated by global warming.[1] The continuous discharge of effluents from industries, factories, etc. without being treated is increasing the elemental load in the water of diverse reservoirs. Eventually, when their concentration crosses the permissible limits, these elements pose a great threat to the aquatic life. The upper limit for the concentration of fluoride (F) in drinking water recommended by the World Health Organization (WHO) is 1.0–1.5 mg/L,[2] although WHO also allows for lower Country Standards to be set, such as the 0.6 mg/L set by Senegal. The level of fluoride in drinking water is higher than the permissible limit of 1.0–1.5 mg/L in about 25 countries all around the world.[3] Aquatic pollutants ultimately affect the highest consumers, like human beings, by entering their body through the food chain by the bioaccumulation process.

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According to the earlier research, vitamin C has an ameliorative effect against F toxicity[4,5] and its various sources, including *Emblica officinalis* (Amla), *Mangifera indica* (Mango), *Averrhoa carambola* (Star fruit), and *Tamarindus indicus* (Imli), etc. have the potential to ameliorate F-induced oxidative stress. Amur carp has a higher growth rate in comparison to the other major carps resulting in it being commonly cultured in composite freshwater aquaculture systems. It also has a wide range of feed acceptability which makes it more vulnerable to coming in contact with the contaminants present in soil and water.[6] Keeping this in view, the present study was under taken to investigate the effect of vitamin C on the bioaccumulation of F in the key organs of sodium fluoride-exposed freshwater fish, Amur carp (*Cyprinus carpio haematopterus*).

MATERIALS AND METHODS

Experimental fish: The experimental fish (number=150, average weight=170 g) were collected from the Instructional Fish Farm and then stocked in fibre-reinforced-plastic (FRP) tanks in the Wet Lab of College of Fisheries, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. Fish were acclimatized for 10 days prior exposure to sodium fluoride.

Experimental design: Fish were randomly divided into five groups comprising of 30 fish in each group. Each group was set in triplicate with 10 fish in each replicate. The experimental groups were designated as control (G_1), NaF exposure at 100 ppm (G_2), supplemented diet with vitamin C at 100 ppm only (G_3), NaF exposure at 100 ppm along with vitamin C supplemented diet at 100 ppm (G_4), and NaF exposure at 100 ppm for first 60 days then fed with vitamin C at 200 ppm supplemented diet for the last 30 days (G_5). The exposure experiment was conducted for 90 days. Fluoride concentration in each tank was monitored on alternate days throughout the experiment and replenished accordingly to ensure the required F concentration (i.e., 100 ppm). This was performed by using a spectrophotometer at 620 nm absorbance[7, 8] and the groundwater was taken as the control, whose F content was found to be 0.05 ppm. During the experiment, fish were fed at 5% of total body weight twice a day (the 5% was split into two doses).

Experimental diet: The experimental diet was formulated using locally available ingredients such as rice bran and mustard oil cake in 1:1 ratio. Both ingredients were mixed thoroughly by adding water and then made into pellets in the fish feed mill unit established at the College of Fisheries, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar (India). Following which, pellets were shade dried and the feed for G_1 and G_2 was not supplemented with Vitamin C. L-ascorbic acid (Vitamin C) was used at the rate of 100 ppm for group G_3 and G_4 and 200 ppm for G_5. The feed pellets were stored in air tight containers and labeled as per the experimental groups and the feed containers were kept in a cool dry place away from sunlight and heat for the further use.

Sampling schedule and F estimation: Three fish from each group were randomly taken at the 0, 15^{th}, 30^{th}, 45^{th}, 60^{th}, 75^{th}, and 90^{th} DPT and then were dissected to collect key organs such as gills, liver, intestine, kidney, muscle, and gonads for studying the residual accumulation. F residues were estimated in the collected tissues according to the Birkel method[9]and these are expressed as µg F/g dry tissue. Briefly,

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the tissues were initially homogenized and dried for 24 hours at 105ºC. Then, 200 mg of a dry tissue sample was weighed in a closed compartment weighing balance and dissolved in 2 mL of a 1:1 mixture of 11.6 M perchloric acid and 14.3 M nitric acid. Following which, the samples were neutralized with citrate buffer to a pH 5.5 with a mixture of 7.8 M sodium hydroxide and 1.0 M trisodium citrate.[10] The absorbance of the samples was taken at 620 nm using a spectrophotometer.

RESULTS

F residue accumulation: The record of F residues in the studied tissues (gills, liver, intestine, kidney, muscle, and gonads) of fish in the various experimental groups at different DPT is presented in Tables 1A–1E. No significant amount of F residues was found in groups G_1 and G_3 from 0^{th} to 90^{th} DPT.

In gills: The F residues in gills of groups G_2 and G_4 increased significantly from 15^{th} to 90^{th} DPT with values ranging from 108.36±17.52 to 663.21±27.63 and 63.52±21.90 to 247.15±24.58 µg F/g, respectively. In G_5 group, residual content increased significantly from 15^{th} to 60^{th} DPT with values varying between 101.42±37.41 and 412.15±28.59 µg F/g and then showed a significant decrease at 75^{th} and 90^{th} DPT with values of 384.37±34.28 and 310.38±22.19 µg F/g, respectively, being recorded. However, the residual content was higher in groups G_2 and G_5 than in the G_4 group (Table 1A).

Table 1A. Average (mean±SD) fluoride (µg/g) levels in the gills in the various experimental groups at different days post treatment

Tissue	Group	Days post treatment (DPT)*						
		0^{th} DPT	15^{th} DPT	30^{th} DPT	45^{th} DPT	60^{th} DPT	75^{th} DPT	90^{th} DPT
Gills	G_1	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
	G_2	0.00^{aA}	108.36 ± 17.52^{bC}	238.74 ± 30.46^{cC}	326.45 ± 25.74^{dC}	418.25 ± 20.45^{eC}	545.30 ± 32.51^{fD}	663.21 ± 27.63^{gD}
	G_3	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
	G_4	0.00^{aA}	63.52 ± 21.90^{bB}	101.37 ± 28.56^{cB}	155.65 ± 36.48^{dB}	193.80 ± 19.65^{eB}	215.31 ± 27.35^{fB}	247.15 ± 24.58^{gB}
	G_5	0.00^{aA}	101.42 ± 37.41^{bC}	225.61 ± 25.45^{cC}	320.26 ± 31.14^{dC}	412.15 ± 28.59^{fC}	384.37 ± 34.28^{eC}	310.38 ± 22.19^{dC}

*Different lower case (small) letters (a, b, c, d, e, f, and g) indicate a significant ($P<0.05$) difference between days within a particular group whereas different upper case (capital) letters (A, B, C, and D) indicate a significant ($P<0.05$) difference between the groups at a particular DPT.

In liver: The residual F content in liver of groups G_2 and G_4 increased significantly from 15^{th} to 90^{th} DPT with values ranging from 86.36±32.18 to 408.23±28.44 and 47.13±25.66 to 177.22±33.27 µg F/g, respectively. In group G_5, residual content increased significantly from 15^{th} to 60^{th} DPT with values varying between

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81.45±22.46 and 252.31±34.28 and then the values decreased significantly at 75th and 90th DPT. These values at 75th and 90th DPT were 215.56±30.15 and 182.86±32.08 µg F/g, respectively. The residual content was highest in the group G_2 followed by the G_5 and G_4 groups (Table 1B).

Table 1B. Average (mean±SD) fluoride (µg/g) levels in the liver and the intestine in the various experimental groups at different days post treatment

Tissue	Group	Days post treatment (DPT)*						
		0th DPT	15th DPT	30th DPT	45th DPT	60th DPT	75th DPT	90th DPT
Liver	G_1	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
	G_2	0.00^{aA}	86.36 ± 32.18^{bC}	137.42 ± 23.76^{cC}	184.62 ± 26.63^{dC}	255.56 ± 19.34^{eC}	324.60 ± 20.65^{fD}	408.23 ± 28.44^{gC}
	G_3	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
	G_4	0.00^{aA}	47.13 ± 25.66^{bB}	73.56 ± 31.08^{cB}	105.67 ± 35.52^{dB}	130.38 ± 30.45^{eB}	154.08 ± 24.61^{fB}	177.22 ± 33.27^{gB}
	G_5	0.00^{aA}	81.45 ± 22.46^{bC}	130.12 ± 28.19^{cC}	178.75 ± 29.37^{dC}	252.31 ± 34.28^{fC}	215.56 ± 30.15^{eC}	182.86 ± 32.08^{dB}
Intestine	G_1	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
	G_2	0.00^{aA}	65.91 ± 24.30^{bC}	114.56 ± 31.16^{cC}	187.45 ± 19.53^{dC}	256.72 ± 34.09^{eC}	312.47 ± 22.37^{fD}	388.64 ± 32.65^{gC}
	G_3	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
	G_4	0.00^{aA}	41.34 ± 21.43^{bB}	68.53 ± 26.38^{cB}	98.67 ± 26.72^{dB}	126.36 ± 35.20^{eB}	151.62 ± 30.12^{fB}	182.24 ± 25.46^{gB}
	G_5	0.00^{aA}	62.53 ± 20.24^{bC}	115.08 ± 23.55^{cC}	178.57 ± 31.35^{dC}	246.45 ± 24.28^{fC}	205.61 ± 25.60^{eC}	174.18 ± 28.76^{dB}

*Different lower case (small) letters (a, b, c, d, e, f, and g) indicate a significant ($P<0.05$) difference between days within a particular group whereas different upper case (capital) letters (A, B, C, and D) indicate a significant ($P<0.05$) difference between the groups at a particular DPT.

In intestine: The residual F content in intestines of groups G_2 and G_4 increased significantly from 15th to 90th DPT with values ranging from 65.91±24.30 to 388.64±32.65 and 41.34±21.43 to 182.24±25.46 µg F/g, respectively. In group G_5,

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the residual content increased significantly from 15th to 60th DPT with values varying between 62.53±20.24 and 246.45±24.28 µg F/g, then decreased significantly at 75th and 90th DPT. The values at 75th and 90th DPT were 205.61±25.60 and 174.18±28.76 µg F/g, respectively. The residual content was highest in the group G_2 followed by G_5 and G_4 groups (Table 1B).

In kidney: The residual F content in kidneys of groups G_2 and G_4 increased significantly from 15th to 90th DPT with values ranging from 52.77±25.43 to 305.35±30.77 and 36.19±23.40 to 163.37±35.16 µg F/g, respectively. In group G_5, the residual content increased significantly from 15th to 60th DPT with values varying between 55.46±35.26 and 205.48±21.52 µg F/g and then the values decreased significantly at 75th and 90th DPT. These values at 75th and 90th DPT were 183.36±27.78 and 155.18±24.62 g F/g, respectively. The residual content was highest in the group G_2 followed by G_5 and G_4groups (Table 1C).

Table 1C. Average (mean±SD) fluoride (µg/g) levels in the kidney in the various experimental groups at different days post treatment

Tissue	Group	Days post treatment (DPT)*						
		0th DPT	15th DPT	30th DPT	45th DPT	60th DPT	75th DPT	90th DPT
Kidney	G_1	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
	G_2	0.00^{aA}	52.77 ± 25.43^{bC}	110.45 ± 30.23^{cC}	152.23 ± 33.60^{dC}	198.64 ± 20.26^{eC}	253.56 ± 34.09^{fD}	305.35 ± 30.77^{gC}
	G_3	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
	G_4	0.00^{aA}	36.19 ± 23.40^{bB}	51.05 ± 34.11^{cB}	83.61 ± 31.47^{dB}	106.28 ± 28.63^{eB}	131.67 ± 22.48fB	163.37 ± 35.16^{gB}
	G_5	0.00^{aA}	55.46 ± 35.26^{bC}	116.31 ± 28.81^{cC}	159.65 ± 25.36^{dC}	205.48 ± 21.52^{fC}	183.36 ± 27.78^{eC}	155.18 ± 24.62^{dB}

*Different lower case (small) letters (a, b, c, d, e, f, and g) indicate a significant (P<0.05) difference between days within a particular group whereas different upper case (capital) letters (A, B, C, and D) indicate a significant (P<0.05) difference between the groups at a particular DPT.

In muscles: The residual F content in muscle of groups G_2 and G_4 increased significantly from 15th to 90th DPT with values ranging from 42.37±15.40 to 256.35±25.18 and 33.17±24.52 to 156.23±20.32 µg F/g, respectively. In group G_5, the residual content increased significantly from 15th to 60th DPT with values varying between 46.11±20.45 and 163.35±22.14 µg F/g and then these values decreased significantly at 75th and 90th DPT. These values were 134.19±24.28 and 110.64±15.46 µg F/g, respectively. The residual content was highest in the group G_2 followed by the G_5 and G_4 groups (Table 1D).

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Table 1D. Average (mean±SD) fluoride (µg/g) levels in the muscles in the various experimental groups at different days post treatment

Tissue	Group	Days post treatment (DPT)*						
		0th DPT	15th DPT	30th DPT	45th DPT	60th DPT	75th DPT	90th DPT
Muscles	G_1	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
	G_2	0.00^{aA}	42.37 ± 15.40^{bC}	71.62 ± 12.48^{cC}	112.48 ± 20.55^{dC}	155.75 ± 23.61^{eC}	211.72 ± 21.15^{fC}	256.35 ± 25.18^{gD}
	G_3	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
	G_4	0.00^{aA}	33.17 ± 24.52^{bB}	46.31 ± 22.39^{cB}	73.09 ± 18.46^{dB}	97.67 ± 20.35^{eB}	121.63 ± 16.25^{fB}	156.23 ± 20.32^{gC}
	G_5	0.00^{aA}	46.11 ± 20.45^{bC}	75.48 ± 19.12^{cC}	114.62 ± 25.07^{dC}	163.35 ± 22.14^{fC}	134.19 ± 24.28^{eB}	110.64 ± 15.46^{dB}

*Different lower case (small) letters (a, b, c, d, e, f, and g) indicate a significant ($P<0.05$) difference between days within a particular group whereas different upper case (capital) letters (A, B, C, and D) indicate a significant ($P<0.05$) difference between the groups at a particular DPT.

In gonads: The residual F content in gonads of groups G_2 and G_4 increased significantly from 15th to 90th DPT with values ranging from 34.58±12.25 to 175.49±15.36 and 25.18±20.18 to 134.27±20.16 µg F/g, respectively. In group G_5, residual content increased significantly from 15th to 60th DPT with values varying between 36.31±18.22 and 105.83±16.35 µg F/g and then the values decreased significantly at 75th and 90th DPT. The values at these two intervals were 91.27±15.30 and 85.44±21.08 µg F/g, respectively. The residual content was highest in the group G_2 followed by G_5 and G_4 groups (Table 1E).

When these values in all the tissues, in different groups were compared at different time intervals, from 15th to 60th DPT, the values of groups G_2 and G_5 were significantly higher than G_4 but the difference between the G_2 and G_5 groups was non-significant. At 75th and 90th DPT, the residual content was highest in the group G_2 followed by the G_4 and G_5 groups.

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Table 1E. Average (mean±SD) fluoride (µg/g) levels in the gonads in the various experimental groups at different days post treatment

Tissue	Group	Days post treatment (DPT)*						
		0th DPT	15th DPT	30th DPT	45th DPT	60th DPT	75th DPT	90th DPT
Gonads	G_1	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
	G_2	0.00^{aA}	34.58 ± 12.25^{bC}	63.72 ± 11.09^{cC}	84.16 ± 15.31^{dC}	109.52 ± 18.45^{eC}	141.26 ± 22.60^{fD}	175.49 ± 15.36^{gD}
	G_3	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
	G_4	0.00^{aA}	25.18 ± 20.18^{bB}	42.36 ± 16.26^{cB}	70.16 ± 21.68^{dB}	91.53 ± 22.24^{eB}	107.35 ± 25.56^{fC}	134.27 ± 20.16^{gC}
	G_5	0.00^{aA}	36.31 ± 18.22^{bC}	67.51 ± 12.72^{cC}	89.45 ± 20.44^{dC}	105.83 ± 16.35^{fC}	91.27 ± 15.30^{eB}	85.44 ± 21.08^{dB}

*Different lower case (small) letters (a, b, c, d, e, f, and g) indicate a significant ($P<0.05$) difference between days within a particular group whereas different upper case (capital) letters (A, B, C, and D) indicate a significant ($P<0.05$) difference between the groups at a particular DPT.

Bio-concentration factor: The bio-concentration factor of accumulation of F in the different fish tissues of groups G_2 and G_4 is shown in Tables 2A and 2B. The accumulation of F ions was highest in gill tissues followed by tissues of liver, intestine, kidney, muscle and gonad. The result of the bio-concentration factor shows that accumulation of F was highest in the group of G_2 as compared to the G_4 group and it was time dependent as the highest values of residues were recorded at 90th DPT in all the organs examined in both the groups G_2 and G_4 (Tables 2A and 2B).

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Table 2A. Bio-concentration factor in the different tissues of the G2 experimental group at different days post treatment (DPT)

Group	DPT	Tissue					
		Gills	Liver	Intestines	Kidney	Muscles	Gonads
G2	0	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
	15	1.08 ± 0.17^{dB}	0.86 ± 0.32^{cB}	0.66 ± 0.24^{bB}	0.53 ± 0.25^{bB}	0.42 ± 0.15^{aB}	0.35 ± 0.12^{aB}
	30	2.39 ± 0.31^{dC}	1.37 ± 0.24^{cC}	1.15 ± 0.31^{bC}	1.10 ± 0.30^{bC}	0.72 ± 0.13^{aC}	0.64 ± 0.11^{aC}
	45	3.26 ± 0.26^{eD}	1.85 ± 0.27^{dD}	1.87 ± 0.20^{dD}	1.52 ± 0.34^{cB}	1.12 ± 0.21^{bD}	0.84 ± 0.15^{aD}
	60	4.18 ± 0.20^{eE}	2.56 ± 0.19^{dE}	2.57 ± 0.34^{dE}	1.99 ± 0.20^{cE}	1.56 ± 0.24^{bE}	1.09 ± 0.18^{aE}
	75	5.45 ± 0.33^{dF}	3.25 ± 0.21^{cF}	3.12 ± 0.22^{cF}	2.54 ± 0.34^{bF}	2.12 ± 0.21^{bF}	1.41 ± 0.23^{aF}
	90	6.63 ± 0.28^{fG}	4.08 ± 0.28^{eG}	3.89 ± 0.33^{dG}	3.05 ± 0.31^{cG}	2.56 ± 0.25^{bG}	1.75 ± 0.15^{aG}

*Different lower case (small) letters (a, b, c, d, e, f, and g) indicate a significant ($P<0.05$) difference between days within a particular group whereas different upper case (capital) letters (A, B, C, D, E, F, and G) indicate a significant ($P<0.05$) difference between the groups at a particular DPT.

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Table 2B. Bio-concentration factor in the different tissues of the G4 experimental group at different days post treatment (DPT)

Group	DPT	Tissue					
		Gills	Liver	Intestines	Kidney	Muscles	Gonads
G4	0	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
	15	0.63 ± 0.22^{cB}	0.47 ± 0.26^{bB}	0.41 ± 0.21^{bB}	0.36 ± 0.23^{aB}	0.33 ± 0.25^{aB}	0.25 ± 0.21^{aB}
	30	1.01 ± 0.29^{cC}	0.74 ± 0.31^{bC}	0.69 ± 0.26^{bC}	0.51 ± 0.34^{aB}	0.46 ± 0.22^{aB}	0.42 ± 0.16^{aC}
	45	1.56 ± 0.37^{cD}	1.06 ± 0.36^{bD}	0.99 ± 0.27^{bD}	0.84 ± 0.31^{aC}	0.73 ± 0.19^{aC}	0.70 ± 0.22^{aD}
	60	1.94 ± 0.20^{cE}	1.30 ± 0.30^{bD}	1.26 ± 0.35^{bE}	1.06 ± 0.29^{aD}	0.98 ± 0.20^{aD}	0.92 ± 0.22^{aE}
	75	2.15 ± 0.27^{dF}	1.54 ± 0.25^{cE}	1.52 ± 0.30^{cF}	1.32 ± 0.23^{bE}	1.22 ± 0.16^{aE}	1.07 ± 0.26^{aF}
	90	2.47 ± 0.25^{dG}	1.77 ± 0.33^{cF}	1.82 ± 0.20^{cG}	1.63 ± 0.35^{bF}	1.56 ± 0.20^{aF}	1.34 ± 0.20^{aG}

*Different lower case (small) letters (a, b, c, d, e, f, and g) indicate a significant ($P<0.05$) difference between days within a particular group whereas different upper case (capital) letters (A, B, C, D, E, F, and G) indicate a significant ($P<0.05$) difference between the groups at a particular DPT.

DISCUSSION

Bioaccumulation of toxicants in the fish body negatively affected the diverse tissues investigated which resulted in disturbing the various physiological activities or processes of the fish and hampered growth and development.[11-13] In the present study, the accumulation of F was found to be highest in the gills followed by the liver, intestine, kidney, muscle, and gonads. In an earlier study,[14] the maximum accumulation of F residues was observed in the tissues of bone followed by gills and muscles. The present findings are in agreement with the findings reported by Cao et al.[13] These researchers have also observed the maximum accumulation F residues in the order of gills > liver > brain > kidney > muscle > intestine of freshwater fish (Amur carp). In the present investigation, the accumulation of F residues in the

different tissues increased with increasing the duration of F exposure. Similarly, Cao et al.[13] and Shi et al.[15]have also done studies on freshwater fishes, *Cyprinus carpio* (common carp) and *Acipenser baerii* (Siberian sturgeon), respectively, and concluded that F accumulation in fish tissues was time dependent. In fact, F accumulation and severity of F toxicity are proportional to the duration of F exposure.[16,17]

The concentration of toxicants in gills reflects the level of contaminant in the water whereas the concentration in liver and kidney represents the storage of contaminant.[18,19] Gills in fish are more often recommended as environmental indicator organs of water pollution than any other fish organs as it is the primary organ to get exposed to toxicant before it enters the fish body from water.[20-22] The relatively high concentration of F in the gills could be in response to the function of the gill as an ion regulator.[13] Once the gills are damaged, the functions of gas exchange and regulation of osmotic pressure could be impacted leading to negative health effects.[13]

On prolonged exposure, rise in F concentrations in liver may be due to its function in metabolism, storage, redistribution, and detoxification of toxicants.[23] High residual concentration in the kidneys of exposed fish may be related to the role it plays in excretion. The metal concentration in muscle tissue is important because it is the chief edible portion of fish that plays an important role in human nutrition.[24] Muscle and gonadal tissues accumulated the least residues because they do not come in direct contact with toxicants and they do not have active sites of detoxification, the primary site for which is the liver.[25] The differences in the level of accumulation in the different fish organs are primarily attributed to the differences in the physiological role of each organ.[26]

The study observed lesser F residues in the G_4 group compared to the G_2 group whereas in the G_5 group, the residual level decreased from 75th to 90th DPT on feeding the F-exposed fish with vitamin C supplemented feed. This indicates that the F uptake is a function of its concentration in the aquatic environment, exposure time, and water temperature.[27,28] Subsequently, although fluoride may be eliminated as fluoride ions via the excretory systems,[29] F tends to be accumulated in the tissues of the exposed organism. The variation in prevalence and severity of F effects in animals living in the same conditions is much more dependent upon the presence of calcium (Ca) and vitamin C micro-nutrients in their foods and frequency of F intake[30-32] and its consistency of exposure.[33] The presence and level of vitamin C act as an important entity to ameliorate the ill effects of fluoride.[34] It has been reported in previous studies that F exposure can cause oxidative stress and vitamin C can either inhibit the production of free radicals or scavenge them. Mittal et al.[35] have reported that the co-administration of DMSA (meso-2,3-dimercaptosuccinic acid) with vitamin C led to a more pronounced depletion of F from the blood and soft tissues compared to the individual effects of DMSA and Vitamin C. In the present study also, the decline in the level of F residues found in the different organs of exposed fish could be due to the interruption of free radical chain reactions by ascorbate radicals.[36]

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CONCLUSION

It can be concluded from the present investigation, that a dose of vitamin C of 100 ppm, given to protect against NaF toxicity, did not fully eliminate F residues from the exposed fish tissues but significantly reduced the accumulation of F in the tissues thus proving that Vitamin C has anti-oxidant properties against free radicals and that supplementation with vitamin C can be used as a treatment in aquaculture in F-affected areas.

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REFERENCES

1 Adejumoke IA, Babatunde AO, Abimbola OP, Tabitha AA, Adewumi DO, Toyin OA. Water pollution: effects, prevention, and climatic impact. In: Glavan M, editor. Water challenges of an urbanizing world [book on internet]. Chapter 3. London, Intech Open; 2018. pp. 33-53. Available from: https://doi.org/10.5772/intechopen.72018

2 Adler P, Armstrong WD, Bell ME, Bhussry BR, Buttner W, Cremer HD., et al. Fluorides and human health. World Health Organization Monograph Series No. 59. Geneva: World Health Organization, 1970.

3 Madhusudhan N, Basha PM, Rai P, Ahmed F, Prasad GR. Effect of maternal fluoride exposure on developing CNS of rats: Protective role of *Aloe vera*, *Curcuma longa* and *Ocimum sanctum*. Indian J Exp Bio 2010;48:830-6.

4 Shanmugam KR, Reddy SA. Protective effect of vitamin C in sodium fluoride (NaF) induced toxicity in fishes: a study with reference to lipid metabolic profiles and liver markers. Indian J Fund App Life Sci 2015;5(4):1-5.

5 Shanmugam KR, Reddy AS.Effect of Vitamin C on haematobiochemical parameters in sodium fluoride (NAF) intoxicated *Channa punctatus* fishes. Indo Am J Pharma Res 2016;6(4):5320-3.

6 Narasimhacharya AVRL, Vasant RA. Medicinal plants as antioxidants in fluoride induced oxidative stress. Research Signpost 2012;267-87.

7. Balaaji VB, Shashank R. Fluoride estimation and reduction in Indian water samples, using spectrometer method with optimum alumina, pH and optimum time of operation. International Conference on Biology, Environment and Chemistry. IPCBEE Vol.1. Singapore; IACSIT Press; 2011.

8 Yin F, Yao Y, Liu CC, Wen ML. Developments in the analysis of fluoride 1997-1999. Fluoride 2001;34(2):114-25.

9 Birkel JM. Direct potentiometric determination of fluoride in soft tooth deposits. Caries Res 1970;4:243-55.

10 Mathur M. Estimation of fluoride content in liver of swiss albino mice after chronic administration of sodium fluoride. International Journal of Basic and Applied Medical Sciences 2013;3(1):300-3. Available from: https://www.cibtech.org/J-MEDICAL-SCIENCES/PUBLICATIONS/2013/Vol_3_No_1/JMS...49-062...Manisha%20Mathur...Estimation...Fluoride.pdf

11 Dallinger R, Prosi F, Segner H, Back H. Contaminated food and uptake of heavy metals by fish. A review and a proposal for further research. Oecologia 1987;73:91-8.

12 Korai AL, Sahato GA, Kazi TG, Lashari KH. Lead concentrations in fresh water, muscle, gill, and liver of *Catlacatla* (Hamilton) from Keenjhar Lake. Pak J Anal Environ Chem 2008;9:11-19.

13 Cao J, Chen J, Wang J, Wu X, Li Y, Xie L. 2013. Tissue distributions of fluoride and its toxicity in the gills of a freshwater teleost, *Cyprinus carpio*. J Aquat Toxico 2013;130:68-76.

14 Gikunju JK, Githui K, Maitho TE. Fluoride levels in bore-hole water around Nairobi. Fluoride 1992;25:111-4.

15 Shi XT, Zhuang P, Zhang LZ, Feng GP, Chen LQ, Liu JY, Wang RF. Growth inhibition of siberian sturgeon (*Acipenser baerii*) from dietary and waterborne fluoride. Fluoride 2009;42:137-41.
16 Choubisa SL. Fluoride in drinking water and its toxicosis in tribals, Rajasthan, India. Proc Natl Acad Sci India Sect B Biol Sci 2012;82(2):325-30.
17 Choubisa SL. Some observations on endemic fluorosis in domestic animals of southern Rajasthan (India). Vet Res Commun 1999;23(7):457-65.
18 Romeoa M, Siaub Y, Sidoumou Z, Gnassia-Barelli M. Heavy metals distribution in different fish species from the Mauritania coast. Sci Total Environ 1999;232:169-75.
19 Rao LM, Padmaja G. Bioaccumulation of heavy metals in M cyprinoids from the harbor waters of Visakhapatnam. Bull Pure Applied Sci 2000;19:77-85.
20 Obasohan EE, Oronsaye JAO, Eguavoen OI. A comparative assessment of the heavy metal loads in the tissues of a common catfish (*Claria sgariepinus*) from Ikpoba and Ogba Rivers in Benin City Nigeria. Afr Sci 2008;9:13-23.
21 Yilmaz F. The comparison of heavy metal concentrations (Cd, Cu, Mn, Pb, and Zn) in tissues of three economically important fish (*Anguilla anguilla*, *Mugil cephalus* and *Oreochromis niloticus*) inhabiting Koycegiz Lake-Mugla (Turkey). Turk J Sci Technol 2009;4:7-15.
22 Kamaruzzaman BY, Rina Z, John BA, Jalal KCA. Heavy metal accumulation in commercially important fishes of south west Malaysian coast. Res J Environ Sci 2011:5:595-602.
23 Bawuro AA, Voegborlo RB, Adimado AA. Bioaccumulation of heavy metals in some tissues of fish in lake Geriyo, Adamawa State, Nigeria. J Environ Pub Health 2018. Article ID:1854892. Available from: https://doi.org/10.1155/2018/1854892
24 Rajeshkumar S, Li X. Bioaccumulation of heavy metals in fish species from the Meiliang Bay, Taihu Lake, China. Toxicol Rep 2018;5:288-95.
25 Abdel-Tawwab M, El-Sayed GO, Shady SH.Growth, biochemical variables, and zinc bioaccumulation in Nile tilapia, *Oreochromis niloticus* (L.) as affected by water-born zinc toxicity and exposure period. Inter Aq Res 2016;8:197-206.
26 Karuppasamy R. Evaluation of Hg concentration in the tissue of fish *Channa punctatus* (Bloch.) in relation to short and long-term exposure to phenyl mercuric acetate. J Plat Jubilee A.U. 2004;40:197-204.
27 Neuhold JM, Sigler WF. Effects of sodium fluoride on carp and rainbow trout. Trans Am Fish Soc 1960;89:358-70.
28 Nell JA, Livanos G. Effects of fluoride concentration in seawater on growth and fluoride accumulation by Sydney rock oyster (*Saccostrea commercialis*) & flat oyster (*Ostrea angasi*) spat. Water Res 1988;22:749-53.
29 Kessabi M. Metabolism and toxicological biochemistry of fluorine: A review. Rev Med Vet 1984;135:497-510.
30 Choubisa SL. Natural amelioration of fluoride toxicity (fluorosis) in goats and sheep. Curr Sci 2010;99(10):1331-32.
31 Choubisa SL, Mishra GV, Sheikh Z, Bhardwaj B, Mali P, Jaroli VJ. Food, fluoride, and fluorosis in domestic ruminants in the Dungarpur district of Rajasthan, India. Fluoride 2011;44(2):70-6.
32 Choubisa SL. Fluorotoxicosis in diverse species of domestic animals inhabiting areas with high fluoride in drinking waters of Rajasthan, India. Proc Natl Acad Sci India Sect B Biol Sci 2013;83(3):317-21.
33 Choubisa SL, Mishra GV, Sheikh Z, Bhardwaj B, et al. Toxic effects of fluoride in domestic animals. Adv Pharmacol Toxicol 2011;12(2):29-37.
34 Choubisa SL, Choubisa L, Choubisa D. Reversibility of natural dental fluorosis. Int J Pharmacol Bio Sci 2011;5:89-93.
35 Mittal M, Chatterjee S, Flora SJS. Combination therapy with vitamin C and DMSA for arsenic–fluoride co-exposure in rats. Metallomics 2018;10(9):1291-306.
36 Nimse SB, Pal DK. Free radicals, natural antioxidants, and their reaction mechanisms. RSCAdv 2015;35(5):27986-8006.