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Research Article

PROTECTIVE EFFECT OF VITAMIN C IN SODIUM FLUORIDE (NAF) INDUCED TOXICITY IN FISHES: A STUDY WITH REFERENCE TO LIPID METABOLIC PROFILES AND LIVER MARKERS

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ABSTRACT
The current investigation was carried out to know the antilipidemic and hepatoprotective activity of vitamin C in sodium fluoride intoxicated fishes. Sodium fluoride (NaF) toxication is associated with oxidative stress and altered lipid metabolic profiles. 40 fishes were divided into 4 groups and treatment was given as per the experimental protocol. NaF caused an elevation in lipid metabolic profiles (LDL, VLDL, TG & TC) and liver markers (AST & ALT) enzymes. Whereas HDL level was decreased in Na F treated fishes. However, Vitamin C effectively reduced the elevation in lipid metabolic profiles and liver marker enzymes in NaF treated fishes. These experimental results strongly indicate that protective role of Vitamin C against toxic effects of NaF in fishes.

Keywords: *NaF, Vtiamin C, Lipid Metabolic Profiles, Liver Markers, Fishes*

INTRODUCTION
Fluoride is abundant in the environment and exists only in combination with other elements as fluoride compounds, which are constituents of minerals in rocks and soil (Edmunds and Smedley, 1996). Sources of fluoride include natural fluoride in food stuffs and water (fluoridated water usually at 1.0 mg/L) (Beltran and Szpunar, 1988).
Know day's pesticides and fertilizers are used to nourish the plants and food products. These chemicals have entered into the river, ponds and create pollution, which pose a great threat to aquatic animals including fishes. There are several reports regarding the effects of pesticides (Arunachalam *et al.*, 1985) on physiology of fish. The alteration in biochemical parameters in different tissues of fish is due to toxic effects of different heavy metals and pesticides have been reported by many workers (Virk and Sharma, 1999; Rawat *et al.*, 2002).
Sodium fluoride was the first fluoride compound used in the fluoridation of drinking water and it is still commonly used for that purpose to prevent dental caries (National Toxicology Program (NTP), 1990). In addition to the well-known effects of fluoride on the skeleton and on teeth, it exerts toxic effects on many other soft tissues and organs (Waldbott, 1978).
Vitamin C or Ascorbic acid is a cofactor for a number of metabolic enzymes and is one of the essential vitamins for humans (Rebouch, 1991). Under physiological conditions, Vitamin C functions as a potent reducing agent that efficiently quenches potentially damaging free radicals produced by normal metabolic respiration of the body (Arrigoni and De Tullio, 2002). Plants and almost all animals synthesize this vitamin. It reduces oxidative damage (Hsu and Guo, 2002). It acts as a free radical scavenger (Kleszczewska, 2001) and reduces the level of lipid peroxidation (Upasani *et al.*, 2001).
The present study was carried out to know the impact of vitamin C on the lipid metaboilic profiles and liver markers in NaF intoxicated fishes.

MATERIAL AND METHODS
Chemicals
All chemicals used in the current study are obtained from Sigma (St. Louis, O, USA), Fisher Scientific (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India) and Qualigens (Mumbai, India). Sodium fluoride (NaF) was purchased from Sigma Chemical Co., St. Louis, Mo., USA.

Animals

Fish Acclimation: Fishes were transported to laboratory under ordinary conditions. Fishes were divided into four groups, each containing ten fishes and placed in a fiber glass aquarium, size: 36cmx 18cm x 15cm containing ground water. Air pumps and filters were used to aerate the aquarium water by circulating it. All control and treated fish were fed with commercial pellet once a day. Water in aquaria was changed after every two days. Chemical analysis of water was done according to standard methods.

Channa punctatus ranging in length from 12-15cm and having a body weight of 30-35gm were obtained from the local fresh water resources and were maintained in large aquaria for 7 days to acclimatize them to the laboratory conditions. Chlorine free water was used which was changed at regular intervals. The values of some of the parameters of the water used were hardness 180ppm, pH 7.5±0.2, temperature 25±2c0.

The fishes were divided into 4 groups of 10 fishes in each group.

1. Normal Control (NC): Fishes are treated with normal ground water for 30 days.
2. NaF Control (NF C): Fishes are treated with NaF (100 ppm) for 30 days.
3. Vitamin C treatment (Vit c t): Fishes are treated with Vitamin C (100 ppm) for 30 days.
4. NaF + Vitamin C (NF + Vit C): Fishes received Na F (100 ppm) and Vitamin (100 ppm) for 30 days.

The blood samples from the treated as well as control fishes were collected directly after 30 days. The blood was collected in EDTA coated vials and serum used to estimated lipid metabolic profiles and liver markers enzymes.

Estimation of Serum Lipid Profiles and Liver Markers

Serum LDL, VLDL, HDL, TG & TC, AST, ALT, was done by using commercially available kits.

Statistical Analysis*:* Data was presented as mean ± SEM. One-way analysis of variance (ANOVA) with Turkey's significant difference post hoc test was used to compare differences among groups. Data were analyzed statistically by Graph Pad Prism 5.0 statistical software. P values <0.05 were considered significant.

RESULTS AND DISCUSSION

Results

Effect of Sodium Fluoride on Serum Lipid Metabolic Profiles

The administration of Sodium Fluoride for 30 successive days resulted in significant increases (P< 0.05) in serum LDL, VLDL, & TC, TG and AST, AST when compared with control group. But the HDL level was decreased in Na F treated fishes. Whereas in the combination treatment (Vitamin c + Na F) decreased these lipid metabolic profiles and liver markers significantly. However, HDL level also increased in the combination treatment group.

Discussion

The present study was an attempt to evaluate the toxic effect of sodium fluoride and possible ameliorative role of vitamin C in alleviating the toxicity of sodium fluoride when given to normal fishes. The following parameters lipogram picture (LDL, VLDL, HDL, TG & TC) and liver markers (AST & ALT) were estimated in serum.

Fluorosis is a well-defined clinical entity characterized by toxic effects of high-fluoride intake on teeth, bones and soft tissues (Krishnamachari, 1996).

Furthermore, the treatment with NaF caused changes in lipid metabolic profile and this is in accordance with the obtained results by Czernyet *et al.,* (2000). Many researchers suggested that the up regulation of lipid metabolic profiles seem to be one of the chief factors responsible for the rise in serum triglycerides and cholesterol. It appears that enzymes inhibited by fluoride, such as triglyceride lipase, unspecific esterase and pyrophosphates. Also, the obtained results of hyperlipidemia may be attributed to an increase in the synthesis of fatty acids in the liver. The administration of NaF for 30 successive days afforded significant increases (P< 0.05) in LDL, VLDL, TG & TC, when compared with control group. HDL level was decreased in Na F treatment fishes. The combination of Vitamin C with the NaF decreased significantly the elevated lipid metabolic profile levels and HDL level increased.

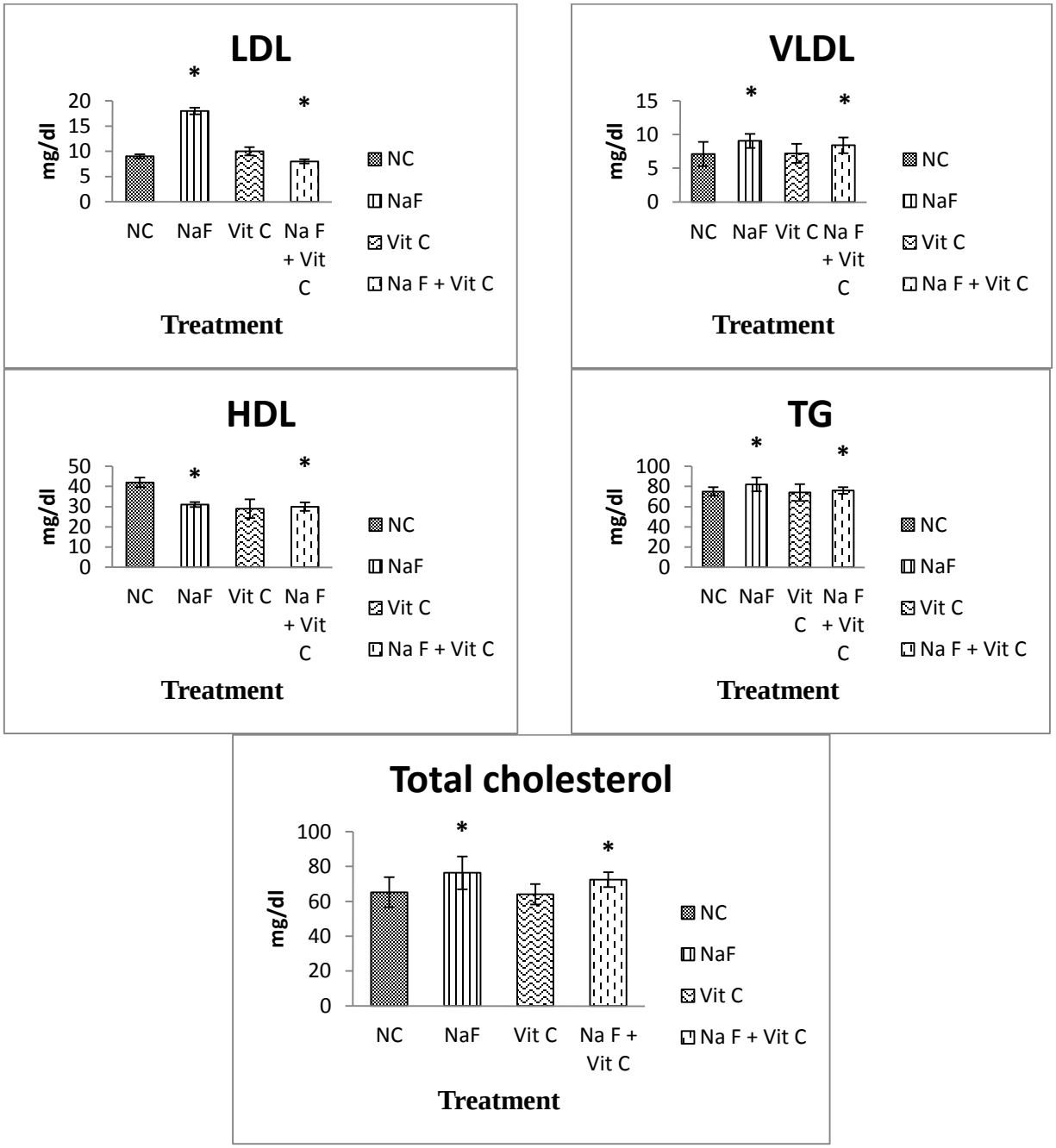

Figure 1: Effect of oral administration of Vitamin C on LDL, VLDL, HDL TG, TC, AST & ALT in Fluoride intoxicated rats. Values are significant compared to normal control (NC, * *P*<0.001) rats

Table 1: Effect of Vitamin C on AST & ALT in Na F toxicity rats

	AST	**ALT**
NC	36±2.6	41±3.2
NaF	48±4.8*	52±5.4*
Vit C	38±4.6	40±3.6
Na F + Vit C	50±6.2*	44±4.2*

All the values are mean, ± SD of six individual observations,
**significant at p< 0.001*

Increased oxygen radical generation and lipid peroxidation has been implicated in the pathogenesis- of many diseases and toxic action of a Na F (Bouaziz *et al.,* 2007). The observed abnormalities in lipid metabolic profiles may be due to over-production of very low density lipoprotein (VLDL) by the liver or to the decrease in removal of VLDL and LDL from the circulation (Tsutsumi *et al.,* 1995). The liver is the site of cholesterol and triglycerides synthesis. The observed decrease in total cholesterol, VLDL, LDL cholesterol, TG and other lipid metabolic profiles of serum yet desirably increasing the level of HDL implies that vitamin C on account of its effect on lipid profile may have a protective effect against NaF toxicity in fishes.

Vitamin C is considered to be an important antioxidant in extracellular fluid; it also guards against aqueous radicals in blood and protects plasma lipids from peroxidative damage caused by peroxyl radicals (Sies *et al.,* 1992).

Transaminases (AST and ALT) enzymes are a common mean of detecting liver damage. Alterations in these enzymes are reported in hepatic disease and in myocardial infarction (Hassoun and Stohs, 1995; Akila *et al.,* 1998). Fluoride toxicity caused elevated in the activities of transaminases (Qujeq *et al.,* 2002; and Shanthakumari *et al.,* 2004). The alterations in transaminases could be expected to occur associated with pathology involving necrosis of the liver. The increase in serum AST and ALT activities is in agreement with the findings of Qujeq *et al.,* (2002) who found that AST and ALT activities were significantly increased after treating with 10, 20 and 30 mg/kg NaF daily for 90 days.

Aspartate transaminase (AST) and alanine transaminase (ALT) activity was changed significantly by Fluoride exposure fishes after 30 days as compared to the control. ALT and AST are frequently used in the diagnosis of damage caused by pollutants in various tissues, such as liver, muscle, and gills (De *et al.,* 2000). It is generally accepted that increased activity of these enzymes in extracellular fluid or plasma is a sensitive indicator of even minor cellular damage (Palanivelu *et al.,* 2005). According to Harvey *et al.,* (1994) serum levels of ALT and AST may increase because of cellular damage in the liver and that high levels of these enzymes in serum are usually indicative of disease and necrosis in the liver of animals. Our present study indicates that increased ALT and AST activity in the serum of fishes caused mainly by leakage of these enzymes from liver cytosol into the bloodstream as a result of liver damage caused by fluoride exposure.

The similar result shows that the increased levels in AST and ALT observed in our fluoride exposed fish could alter protein metabolism (Chen *et al.,* 2013). However, with vitamin c supplementation in NaF toxicity fishes, we observed decreased activities of AST & ALT in serum. This show the hepatoprotective effect of Vitamin C.

From the results we concluded that Vitamin C administration decreased lipid metabolic profiles and serum markers in NaF treated fishes. Hence vitamin C has antilipidemic and hepatoprotective activity.

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REFERENCES

Akila G, Rajakrishnan V, Viswanathan P, Rajashekaran KN and Menon VP (1998). Effects of Curcumin on Lipid Profile and Lipid-Peroxidation status in experimental Hepatic-Fibrosis. *Hepatology Research* **11** 147–157.

Arrigoni O and De Tullio MC (2002). Ascorbic acid: Much more than just an Antioxidant. *Biochimica et Biophysica Acta* **1569** 1–9.

Arunachalam S, Palanichamy S and Balasubramanian MP (1985). Sublethal effects of carbaryl on food utilization and oxygen consumption in the air breathing fish Channa punctatus (Bloch). *Journal Environmental Biology* **6**(4) 279-286.

Beltrán ED and Szpunar SM (1988). Fluoride in toothpastes for children: suggestion for change. *Pediatric Dentistry* **10**(3) 185-188.

Bouaziz H, Ketata S, Jammoussi K, Boudawara T, Ayedi F, Ellouze F and Zeghal N (2006). Effects of sodium fluoride on hepatic toxicity in adult mice and their suckling pups. *Pesticide Biochemistry and Physiology* **86** 124–130.
Chen YJ, Cao YJ, Wang YJ, Jia YR, Xue YW and Li YY (2013). Effects of Fluoride on Growth, Body Composition, and Serum Biochemical Profile in a Freshwater Teleost, *Cyprinus carpio*. *Environmental Toxicology and Chemistry* **32**(10) 2315-2321.
Czerny B, Put A, Mysliwiec Z and Juzyszyn Z (2000). The influence of quarcetin on some parameters of lipid metabolism in rats chronically exposed to ammonium fluoride. *Fluoride* **33** 27–32.
De LA Torre FR, Salibian A and Ferrari L (2000). Biomarkers assessment in juvenile Cyprinus carpio exposed to waterborne cadmium. *Environmental Pollution* **109**(2) 227-278.
Edmunds WM and Smedley PL (1996). Groundwater geochemistry and health: an overview. In: *Environmental Geochemistry and Health,* edited by Appleton, Fuge and McCall (Geological Society Special Publication) **113** 91–105.
Harvey RB, Kubena LF and Elissalde M (1994). Influence of vitamin E on aflatoxicosis in growing swine. *American Journal of Veterinary Research* **55**(4) 572-577.
Hassoun EA and Stohs SJ (1995). Comparative studies on oxidative stress as a mechanism for the fetotoxic of TCDD, endrin and lindane in C57BL/6J and DBA/2J mice. *Teratology* **51** 186-192.
Hsu PC and Guo YL (2002). Antioxidant nutrients and lead toxicity. *Toxicology* **180**(1) 33–44.
Kleszczewska E (2001). Biological role of reactions of l-ascorbic acid with metals. *Postępy Higieny i Medycyny Doświadczalnej* **55**(1) 81–94.
Krishnamachari K (1996). Trace Elements in Human Nutrition and Health. WHO, Geneva 187–194.
National Toxicology Program (1990). NTP Toxicology and Carcinogenesis Studies of Sodium Fluoride (CAS No. 7681-49-4) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). *National Toxicology Program Technical Report Series* **393** 1-448.
Palanivelu V, Vijayavel K, Balasubramanian SE and Balasubramanian MP (2005). Influence of insecticidal derivative (Cartap Hydrochloride) from the marine polychaete on certain enzyme systems of the freshwater fish Oreochromis mossambicus. *Journal of Environmental Biology* **26** 191-196.
Qujeq D, Laghaie B, Gholipour A, Solimani N and Hassenzadeh S (2002). Effects of sodium fluoride on total serum protein levels and transaminase activity in rats. *Biomedicine & Pharmacotherapy* **56** 169–172.
Rawat DK, Bais VS and Agrawal NC (2002). A correlative study on liver glycogen and endosulfan toxicity in Heteropneustes fossilis (Bioch). *Journal of Environmental Biology* **23**(2) 205-207.
Rebouche J (1991). Ascorbic-Acid and Carnitine Biosynthesis. *American Journal of Clinical Nutrition* **54**(6S) 1147–1152.
Shanthakumari D, Srinivasalu S and Subramanian S (2004). Effect of fluoride intoxication on lipidperoxidation and antioxidant status in experimental rats. *Toxicology* **204** 214–228.
Sies H, Stahl W and Sundquist R (1992). Antioxidant Functions of Vitamins. Vitamins E and C, beta-carotene and other carotenioids. *Annals of the New York Academy of Sciences* **669** 7– 20.
Tsutsumi K, Inoue Y, Shime A and Murase T (1995). Correction of hypertriglyceridemia with low and high-density lipoprotein cholesterol by the novel compound No 1886, a lipoprotein lipase promoting agent in STZ induced diabetic rats. *Diabetes* **44** 414–417.
Upasani CD, Khera A and Balaraman R (2001). Effect of lead with Vitamins E, C, or Spirulina on malondialdehyde: conjugated dienes and hydroperoxides in rats. *Indian Journal of Experimental Biology* **39**(1) 70–74.
Virk S and Sharma RC (1999). Biochemical changes induced by Nickel and Chromium in the liver of cyprinus carpio (L). *Pollution Research* **18**(3) 217-222.
Waldbott GL (1973). *Health Effects of Environmental Pollutants,* 2nd edition (Mosby, St Louis).