Trends in Fisheries Research
An International Peer-Reviewed Journal
UGC Approved Journal No: 63495
www.sciencejournal.in

AMELIORATIVE POTENTIAL OF VITAMIN C IN SODIUM FLUORIDE (NAF) INDUCED TOXICITY IN FISHES: A STUDY WITH REFERENCE TO ANTIOXIDANT ENZYMES

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ABSTRACT
A study was undertaken to investigate the effect of Vitamin C on the toxicity of NaF in fishes. The fishes were divided into four groups and treatment was given for 30 days as per the experimental protocol. Antioxidant enzymes like Super oxide dismutase (SOD), catalase (CAT), ascorbic acid (AA), uric acid (UA) and thiobarbituric acid reactive substances (TBARSs) levels are estimated in liver tissue. Antioxidant enzymes decreased and TBARSs levels increased in NaF intoxicated fishes. However, these toxic effects of NaF were significantly reduced when the fishes were treated with the Vitamin C. This indicated that Vitamin C might play a role in reducing the toxic effect of NaF in fishes.

KEYWORDS: Antioxidant enzymes, Fishes, NaF, Vitamin C.

INTRODUCTION

Fluoride in small doses has influence by inhibiting dental caries while in higher doses it causes dental and skeletal fluorosis (Shanthakumari *et al.*, 2004). Fluoride enter the body through drinking water, food, toothpaste and other dental products; drugs and fluoride dust and fumes from industries using fluoride containing salt and hydrofluoric acid (Shulman and Wells, 1997). Recent studies revealed that fluoride induces excessive production of oxygen free radicals, and might cause the depletion of antioxidant enzymes (Chlubek, 2003).Increased free radical generation and lipid peroxidation are proposed to mediate the toxic effects of fluoride on soft tissues (Rzeuski *et al.*, 1998).

Antioxidants are substances that are efficient to trap ROS and so maybe competent of decreasing oxidative damage. The use of antioxidants as therapeutic agents to treat diseases. The administration of antioxidant compounds have many protective effects. Exogenous sources of antioxidants are those that can be obtained from the diet. These can include Vitamin E (tocopherols), ascorbic acid (Vitamin C), carotenoids and flavonoids. In addition to dietary antioxidants, endogenous antioxidants also play a vital role in regulating ROS production and can be either enzymatic or non-enzymatic.

The present study was an attempt to evaluate the toxic effect of sodium fluoride and possible ameliorative role of Vitamin C in fishes.

MATERIALS AND METHODS

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.

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4. NaF + Vitamin C (NF + Vit C): Fishes received Na F (100 ppm) and Vitamin (100 ppm) for 30 days.
The liver tissues are collected after the 30days of treatment.

RESULTS

Effect of Sodium Fluoride on hepatic antioxidant enzymes SOD, CAT, ascorbic acid and uric acid levels decreased in NaF intoxicated fishes. Administration of Vitamin C alone caused significant ($P < 0.05$) increase in the activities of SOD, CAT, ascorbic acid and uric acid levels compared to control rats. In addition, a significant recovery relating to SOD, CAT, ascorbic acid and uric acid was observed in response to the treatment with Vitamin C in NaF fishes. (Table 1).

Table 1. Effect of Vitamin C on the antioxidant enzymes in NaF intoxicated fishes

	NC	NaF	Vit C	Na F + Vit C
SOD (Superoxideanion reduced/mg rotein/min)	14.06±1.612	8.02±1.214	15.86±2.418	12.84±1.82
CAT (µm of hydrogen peroxide degraded /mg protein/min)	28.02±2.14	14.62±1.48	32.04±0.32	22.14±2.86
AA (mg ascorbic acid/g wet weight of the tissue)	1.12±0.212	0.82±0.012	2.4±0.384	1.42±0.814
UA (µm of uric acid/g wet weight of the tissue)	1.6±0.10	1.02±0.036	1.8±0.246	1.4±0.028
MDA (µm of malondialdehyde /gram)	98.12±4.628	180.28±6.216	94.06±4.82	126.04±6.264

Our results indicated that the levels of thiobarbituric acid reactive substances(TBARSs) is significantly ($P < 0.05$) increased in liver of fishes treated with NaF (Table 1). Treatment with Vitamin C alone caused significant ($P < 0.05$) decrease in liver TBARS compared to control. In addition, Vitamin C supplementation in NaF fishes reduced the elevation of TBARS. (Table 1)

DISCUSSION

Fluorosis is a well-defined clinical entity characterized by toxic effects of high-fluoride intake on teeth, bones and soft tissues (Krishnamachari, 1976).

This is the first report on the effect of vitamin c in sodium fluoride intoxicated fishes. The present study exhibits the toxic effects of sodium fluoride on antioxidant enzymes in fishes. The present investigation also suggests sodium fluoride alters antioxidant enzymes, however Vitamin C supplementation protected the fishes from NaF intoxication. Hence, Vitamin C may be used as protective agent against many toxic substances in aquatic conditions.

SOD is an important antioxidant enzyme found in nearly all living cells exposed to oxygen. CAT is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS).Ascorbic acid is a potent water-soluble antioxidant enzyme involved with radical chain-breaking and regeneration of tocopherol from tocopheroxy radicals. Uric acid, a important antioxidant for humans, birds, reptiles, and some primate species, that scavenges singlet oxygen, peroxy radicals, and hydroxyl radicals and has been studied extensively in many physiological and pathological systems.

In the present study, SOD, CAT, AA and UA activities are decreased in Naf intoxicated fishes. It is proposed that there is an increased oxidative stress in Fluorosis and decreased in SOD and CAT in the red blood cells (RBC) of fluoride-in

toxicated rats (Shivarajashankara *et al.*, 2001).In another study, decreased SOD and CAT activities in rat upon ingestion of sodium fluoride for 14 days were observed (Vani and Reddy, 2000). The decreased activities of SOD and CAT may be a response to increased production of hydrogen peroxide and superoxide by excessive production of free radicals. Many reports suggested that NaF supplementation causes excessive production of toxic free radicals which scavenges free radicals thus antioxidant enzymes came to rescue, hence these antioxidant enzymes activities decreased in NaF fishes (Liu et al., 2003). However Vitamin C treatment recovered all these antioxidant enzymes near normal levels.

Some studies suggest the use of antioxidants and antioxidant rich foods for the management of fluorosis (Susheela, 1999) and also for the beneficial effects of antioxidants as antidotes for fluoride toxicity (Chinoy *et al.*, 1994). Therefore, the purpose of the present study is to find out the ameliorating potential of Vitamin C on sodium fluoride-induced reactive oxygen species (ROS) and lipid peroxidation. Treatment with Vitamin C significantly increased SOD, CAT, AA and UA in NaF intoxicated fishes. The increased lipid peroxidation in NaF in fishes as found in the present study may be due to the inefficient anti-oxidant system. The elevated lipid peroxidation is responsible for the formation of lipid hydroperoxides in membrane and would result in damage of the membrane structure and inactivation of membrane bound enzymes. Elevated oxidative stress due to this imbalance leads to liver tissue injury through oxidized proteins and augmented levels of lipid peroxidation, as indicated by increased TBARS contenting NaF in the present study. Vitamin C treatment significantly reduces this enhanced lipid peroxidation. The anti-oxidant compounds and other pharmacological compounds of plants may inhibit the production of free radicals, and reduced the products of lipid peroxidation (Table 1).

CONCLUSION

From the above study, we conclude that Vitamin C supplementation protected liver tissue in NaF intoxicated fishes. To the best of our knowledge this is the first reported data on the effect of Vitamin C in Na F fishes and also with reference to the antioxidant enzymes. However, further research is needed, for the better understanding of the mechanism of action of vitamin C by which it modulates anti-oxidant enzymes in toxicity condition.

ACKNOWLEDGEMENTS

The authors are thankful to the University Grants Commission, New Delhi (UGC SERO, Hyderabad) for the sanction of Minor Research Project.

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