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Changes in Ascorbic Acid in Liver, Muscle and Gills Due to Dimethoate Induced in Freshwater Fish *Puntius ticto*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In the present investigation, ascorbic acid content was observed in the liver, muscle and gills in the *Puntius ticto* fishes, exposed to sub lethal concentration of dimethoate. Ascorbic acid is a key antioxidant in the liver, gill and muscles of fish. Intrinsic properties of ascorbic acid beyond acting as

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an antioxidant are important in its role as a key molecule in the metabolism of fish. The present research work deals with the evaluation of the ascorbic acid on dimethoate induced in *Puntius ticto*. The dimethoate pesticide EC 30% was administered directly in the water contained in the aquarium at the dose of 1.3 ppm (1/5), 0.63 ppm (1/10) and 0.43 ppm (1/15) respectively for 96 hrs hours after the dose calculation through LC50. Thereafter, ascorbic acid was administered to each pesticide treated group. All over the experiment the ascorbic acid content gradually increased in the liver, muscle and slightly decreased in the gills of the intoxicated fishes.

Keywords: *Puntius ticto*; dimethoate; ascorbic acid; liver; muscle; gills.

1. INTRODUCTION

"The accumulated pesticide interacts with biomolecules and alters the physiology of organisms. The toxic compounds exerts stress to organism and organism responds to it by developing necessary potential to counteract that stress" [1]. "The exorbitant use of pesticides has resulted in sedate problems as well as health hazard" [2]. "Many pesticides are known incites of oxidative stress by directly producing reactive oxygen species (ROS) and interfere the natural antioxidant or oxygen free radical scavenging system" [3]. "Pesticides disturb the prooxidant-antioxidant system of the cells, as a result of that leading to the generation of free oxygen radical and reactive oxygen species causing oxidations in chain". [4] "The biomolecules in the cell (lipids, proteins, polysaccharides and nucleic acids) are likely substrates for ROS" [5]. "Such an effect may be at cellular or molecular level but finally it would lead to physiological, pathological and behavioural disorders that may prove conclusive to the organisms" [6]. "Biological intricate antioxidant system includes antioxidant enzymes and non-enzymatic antioxidants such as ascorbic acid and vit E acting against intracellular oxidative stress" [7]. "Ascorbic acid has potential role to reduce the activity of free radical induced reactions" [8]. "Ascorbic acid is a very priceless scavenger in biological system. It can reduce the toxic effects of environmental toxicants" [9]. "Therefore, increasing the bioavailability of ascorbic acid may reduce the effects of environmental toxicants in fish. Ascorbic acid has a simple chemical structure and small molecular weight in invasion of its high density and negative charge due to the presence of acid and carbonyl groups. Ascorbic acid is a water soluble free radical scavenger. Moreover, it regenerates Vitamin E in cell membrane in combination with GSH or compounds capable of donating reducing equivalents. Ascorbic acid changes to ascorbate radical by donating an electron to lipid radical in order to end up the lipid peroxidation chain reaction. The pairs of ascorbate radicals

react rapidly to produce one molecule of ascorbate and one molecule of dehydroascorbate. The dehydroascorbate does not have any antioxidant capacity. Hence, dehydroascorbate is converted back to ascorbate by addition of two electrons" [10]. "The last stage of the addition of two electrons to the dehydroascorbate has been proposed to be carried out by oxidoreductase. Its forerunner is glucose" [11]. "Biochemical studies on the organisms inhabiting polluted aquatic habitats indicate the boundary of physiological stress experienced by them and also their efforts to resist the toxic effect of the pesticides. Under the pesticidal stress, the energy demand in the organisms becomes high" [12].

"Dimethoate has become the most become a wide area used class of pesticides in the world replacing the persistent problematic organophosphate compounds. Dimethoate is widely used because of its low toxicity to mammals and high sensitivity for insects compared with other organophosphate pesticides. The literatures regarding the changes in the ascorbic acid content during pesticidal stress on animals are very inadequate" [13]. Therefore, in the present investigation effect of dimethoate on the ascorbic acid content in the liver, gill and muscles of the fish, *Puntius ticto* has been used because of their wide availability and suitability for toxicity testing.

2. MATERIALS AND METHODS

The live specimens of *Puntius ticto* were collected from Shivana river near Dhoregaon, 26 km away from Aurangabad (M.S.) and brought to the laboratory. The fishes were maintained in glass aquaria and were acclimatized for four weeks. After acclimatization healthy fishes, showing normal activities were selected for biochemical estimations. The test fishes, *Puntius ticto* were exposed to three sublethal concentrations of dimethoate for 30 days. Simultaneously, a control aquarium was also



maintained. On 30th day's exposure, fishes from each experimental group were sacrificed, liver, gill and muscle were dried in oven at 75 0C to 80 0C till constant weight was obtained and blended into dry powder. These powders were used for the estimation of biochemical components such as ascorbic acid. The methods applied for estimations are as follows [14].

2.1 Estimation of Ascorbic Acid

Ascorbic acid estimation was carried out by the method of [15]. 1.0 ml supernatant was taken in test tube from the homogenate which was already centrifuged for protein estimation. In these test tubes 0.25 ml aliquot of hydrazine reagent was added. The reaction mixture was kept in boiling water bath for 15 minutes. It was cooled and 3.0 ml ice cold 85 % H₂ SO₄ was added drop wise with constant stirring. The reaction mixture was kept at room temperature for 30 minutes. O.D. was taken at 530 nm. A 1.0 ml 10% TCA similarly treated was used as blank, while ascorbic acid was used as standard. Amount of ascorbic acid in different tissues was calculated from standard graphical values. It was expressed as mg of ascorbic acid per 100 mg of dry tissue.

3. RESULTS

In the present investigation, changes in the biochemical constituents in body tissues of test fishes, *Puntius ticto* were exposed to dimethoate for long term (30 days) exposure at different sublethal concentrations have been recorded for ascorbic acid. Dimethoate induced changes in

biochemical constituents which have been represented in the form of percentage in alterations of biochemical constituent. The data were supported to various statistical analysis and the variance, standard deviation and standard error of the mean were calculated. Students' t test was used to find out significance.

3.1 Ascorbic Acid

The ascorbic acid plays an important role in detoxification of foreign bodies or toxicant in metabolic process. The main site to synthesize the ascorbic acid is the liver. The ascorbic acid content was analyzed in both control and experimental fish tissues.

Ascorbic acid recorded in control group of fishes, *Puntius ticto* were 3.9216 mg in liver, 3.8006 mg in gill and 2.51 mg in muscle. The fishes, *Puntius ticto* were exposed to three sublethal concentrations of dimethoate for long term (30 days) exposure, showed that there were significant increase in the level of ascorbic acid content in liver and muscle at 1.3 ppm, 0.65 ppm and 0.43 ppm dimethoate exposure. In liver increases recorded were -113.1321%, -102.8474 % and -92.5628 % as compared with their control value. In muscle increases recorded were -192.8286 %, -168.7251 % and -136.587 % as compared with their control values. Where as in case of gill there was significant decrease in the level of ascorbic acid content at 1.3 ppm, 0.65 ppm and 0.43 ppm exposure. In gill decrease recorded were 53.0608 %, 35.0201 % and 32.8977 % as compared with their control values. These variations are recorded in Table 1 and Fig. 1.

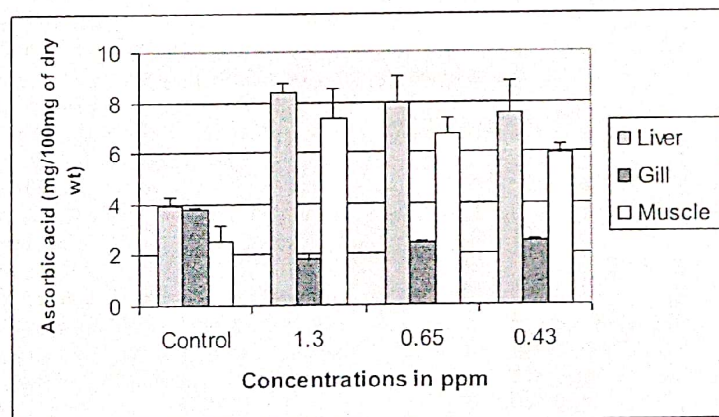


Fig. 1. Effect of dimethoate on ascorbic acid content in different tissues of freshwater fish, *Puntius ticto* after exposure to sublethal concentrations for 30 days



Table 1. Effect of dimethoate on ascorbic acid content in different tissues of freshwater fish, *Puntius ticto* after exposure to sub lethal concentrations for 30 days

Sr. No.	Tissues	Control	1.3 ppm (1/5)	% changes	0.65 ppm (1/10)	% changes	0.43 ppm (1/15)	% changes
1	Liver	3.9216±0.3492	8.3583**±0.3492	-113.1321	7.955*±1.0478	-102.8474	7.5516*±1.2594	-92.5626
2	Gill	3.8006±0.0698	1.784***±0.2095	53.0608	2.4696***±0.0698	35.0201	2.5503***±0.0698	32.8977
3	Muscle	2.51±0.605	7.35*±1.21	-192.8286	6.745*±0.605	-168.7251	5.9383*±0.3492	-136.587

1 The values are expressed in mg/100 mg dry weight (mean ± S.D).

2. ± indicates S.D.

3. *P < 0.005, **P < 0.01, ***P < 0.001

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4. DISCUSSION

"In recent times, due to excessive utilisation of pesticides for the better yield of crops has caused ill-effects to the aquatic ecosystem. The biomagnification of these pesticides through various food chains has reached the human food chain causing them various types of metabolic disorders as well as disease in them. The disease burden in the long duration of exposure is the cause of cancer as well" [16-21]. "In the aquatic ecosystem, fishes are the best indicators of the toxicant exposure; hence the biochemical and histopathological parameters are the best to evaluate the pesticide toxicity" [22-27].

"Ascorbic acid is an important intercellular antioxidant and is involved in the self-defense mechanism of fish. It works as an antitoxic agent heavy metal" [28].

"Ascorbic acid plays a role directly related to homeostatic mechanism and essential for wound healing and regeneration Gould" [29]. "Several investigators have reported protective effect of ascorbic acid against the toxicity of various environmental chemicals" [30]. Due to this factor, ascorbic acid content must have been increased in certain tissues of *Puntius ticto* under dimethoate condition stress.

According to Wagh and Khillare [31] observed ascorbic acid content is normally much higher in liver than muscles in *Puntius ticto*. Similar results have been obtained during the present investigation. As suggested by Bhusari [32] studied effect of endosulfan, ekalux and sevimol on brain, liver, kidney, muscle and gut of *Barbus ticto* and found that ascorbic acid level decreased in brain and gut, increased in liver, kidney and muscles. Davane [33] studied effect of dimethoate, thiodon and carbaryl on biochemical composition of *T. sandkhol* and found that ascorbic acid content increased in liver and muscles and decreased in gill tissue. Elevated ascorbic acid levels have been reported in the liver of fish exposed pulp and paper mill effluent [34] and also to pesticides, such as deltamethrin [35]. Datta and Kaviraj [36] reported decreased ascorbic contents in the kidney and increased in the liver of the fish, *Clarias gariepinus* exposed to fenvalerate.

In present study, the ascorbic acid content was increased in muscle and liver where as decreased in gills in treated fishes, *Puntius ticto* during chronic exposure. This increase in

ascorbic acid content in tissue is dependent upon concentration of dimethoate. In certain tissue the ascorbic acid content decreased, it may be due to shifting of ascorbic acid to the tissues like intestine, muscle, kidney and liver due to increase demand of energy and fatigue retardant suggested by [37] in *Etropolis maculates*. Similarly in poikilotherms ascorbic acid has been shown to act as fatigue retardant. It is observed that liver, kidney are the actual site of detoxification having large amount of ascorbic acid these observation are in agreement with [38,39].

5. CONCLUSION

Ascorbic acid is one of the most important defense systems against free radicals and peroxides that are generated during cellular metabolism [40]. From the present studies, it is concluded that the ascorbic acid content was increased in muscle, liver and decreased in gills in the treated fishes, *Puntius ticto* and during chronic exposure due to dimethoate. Dimethoate is highly toxic to fish as its administration results in augmentation of ascorbic acid content, because ascorbic acid synthesis was elevated to fulfill the energy demand to mitigate any stress condition.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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