JEB™

ISSN: 0254-8704 (Print)
ISSN: 2394-0379 (Online)
CODEN: JEBIDP

DOI : <http://doi.org/10.22438/jeb/38/5/PRN-127>

Efficiency of ascorbic acid against oxidative stress-induced dimethoate toxicity in fish, *Capoeta capoeta* [Guldenstaedt, 1773]

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Key words

Ascorbic acid,
Capoeta capoeta,
Dimethoate toxicity,
Oxidative stress,
Sialic acid

Publication Info

Paper received : 25.02.2016
Revised received : 18.05.2016
Re-revised received : 16.08.2016
Accepted : 09.11.2016

Abstract

Aim: The main objective of the present study was to evaluate the effect of ascorbic acid against oxidative stress-induced by dimethoate in *Capoeta capoeta*.

Methodology: The fish caught in Kars Creek were randomized in four equal groups and acclimatized in separate tanks. The fish were kept in tanks for 10 days as follows: Group I (control) was kept in normal water, Group II, III and IV were kept in separate tanks containing 2 mg l⁻¹ dimethoate and 2 mg l⁻¹ dimethoate plus 100 mg l⁻¹ ascorbic acid and ascorbic acid. The level of total sialic acid, total antioxidant status and total oxidant status in fish serum were analyzed.

Results: Total sialic acid level of Group III was found to be lower than the level of Group II ($P < 0.05$), there were no statistically important differences between these two groups in total antioxidant status and oxidant status. In gills, intensive cartilage degenerations and mononuclear cell infiltrations in group II and III were observed. It was found that toxic effects of dimethoate in group III decreased.

Interpretation: Ascorbic acid in fish exposed to dimethoate reduced the level of total sialic acid and degenerations in lamellae of gills, it had no significant effect on the oxidative stress response.

Introduction

It is generally anticipated that the pesticides used to control unwanted organisms in agricultural practices might trigger emergence on functional loss of the metabolic activities and cell membrane permeability of fish in the aquatic environment (Aktar *et al.*, 2009).

Dimethoate is an organophosphate pesticide used as insecticide and has harmful effects on various tissues and organ systems of organisms in contaminated aquatic ecosystems (Pandey *et al.*, 2009; Singh, 2013). Fish have antioxidant defense system components to block oxidant substances originating from pesticide (Poljšak and Fink, 2014). The most important feature of the antioxidative reaction against oxidants is the substances involved in this reaction sequence form a synergy against free radicals (Nimse and Pal, 2015). Analysis of total antioxidant status exposes the impact of non-enzymatic antioxidants such as bilirubin, uric acid, ascorbic acid and proteins containing thiol groups. Analysis of total oxidant status reveals the effect of oxidant-induced hydrogen peroxide and lipid peroxide (Erel, 2004; Erel, 2005).

Sialic acid is a derivative of N-acetyl neuraminic acid and is found as a structural component of receptors and terminal side chains in oligosaccharides. Factors that cause health problems in organisms, affect the level of total sialic acid level (Varki and Schauer, 2009). Water-soluble ascorbic acid (Vitamin C) can not be synthesized in aquatic organisms (Wang *et al.*, 2003). Researchers have reported the protective role of ascorbic acid against various pesticides in fish (Korkmaz *et al.*, 2009; Dubey *et al.*, 2014). *Capoeta capoeta* is a freshwater fish commonly consumed by the settlers of Kars and vicinity region (Karakus and Gey, 2006). However, study related to role of ascorbic acid and dimethoate toxicity on *Capoeta capoeta* is meagre.

In view of the above, the carried out to present study was assess the protective role of ascorbic acid against dimethoate on total sialic acid, total antioxidant status and total oxidant status in *Capoeta capoeta*.

Materials and Methods

In the present study, thirty two samples of *Capoeta capoeta* weighing 120 -170 g were caught with fishing net at 1-5 m depth from Kars Creek (Turkey). The fish in water tank (300 l) were allowed to adapt to the laboratory environment for 10 days and then they were randomly divided into four groups. Afterwards, the fish were kept in tanks for 10 days as follows: Group I (control) was maintained in untreated water. Groups II, III and IV were maintained in separate tanks containing 2 mg l⁻¹ dimethoate (Cansagor Dimethoate 40EC), 2 mg l⁻¹ dimethoate plus 100 mg l⁻¹ ascorbic acid and 100 mg l⁻¹ ascorbic acid (Sato *et al.*, 1982), respectively. Blood samples from the dorsal aorta of anaesthetized (75 mg l⁻¹ MS 222) fish from each group were

obtained at the end of the treatment period. The gill samples to show pathophysiological changes in fish were fixed in formaldehyde solution.

Biochemical analysis : Serum level of total antioxidant status and total oxidant status was determined by commercial kits (Rel Assay, Gaziantep, Turkey) and by spectrophotometric method which was related to the total amount of oxidant molecules present in the sample. In total antioxidant status analysis, the antioxidative effect of sample against potent-free radical reactions, initiated by the produced hydroxyl radical, was measured. The results were expressed as mmol Trolox eq l⁻¹.

For total oxidant status, the assay was calibrated with hydrogen peroxide and the results were expressed as $\mu\text{mol H}_2\text{O}_2 \text{ eq.l}^{-1}$ (Erel, 2004; Erel, 2005). Total sialic acid was estimated by the spectrophotometric method of Sydow (1985). Statistical analysis was performed by IBM SPSS version 21.0 for Windows. Significant differences among the groups were determined by one-way analysis of variance (ANOVA). To determine the differences among groups, the Tukey test (post hoc) from multiple comparison tests was made out, results were calculated as mean $\pm$ standard deviation.

Histopathological examinations : The gills of fish were fixed in 10% formaldehyde for 24-48 hrs. The gill was decalcified with Osteodec (Bio-Optica, Italy). 5 μ thick sections were prepared from paraffin blocks and stained with hematoxylin – eosin. Stained sections of gills were observed under a light microscope (Olympus BX51).

Results and Discussion

It was observed that nutritional activities and movements of fish exposed to dimethoate during the experimental practices slowed down, and in total four fish two from group II and two from group III died on 10th day.

No significant difference was detected in level of total oxidant status and total antioxidant status between different groups. Fish have delicate gill surface and high proportion of polyunsaturated fatty acids (PUFA) (Mendes *et al.*, 2009). For this reason, oxidant and antioxidant systems in tissues and cells of fish might be different from other organisms. In the present study, serum level of total antioxidant status and total oxidant status of *Capoeta capoeta* exposed to 2 mg l⁻¹ dimethoate did not show any significant oxidative damage.

Serum total sialic acid level increased in fish exposed to dimethoate as compared to control fish, whereas level of total sialic acid in fish exposed to dimethoate plus ascorbic acid significantly decreased in comparison to control fish (P<0.05). Sialic acids have antioxidative property and is responsible for removal of superoxides from vascular system of living organisms (Varki *et al.*, 2009). In the study, the probable reason for low total

sialic acid levels in dimethoate treated fish can be decreased the level of superoxide radicals due to sialic acid.

Gill samples from control fish and those exposed to ascorbic acid were either normal or showed minimal degenerative changes (Fig. 1, 3). The toxic effects of dimethoate were mainly observed by mononuclear cell infiltrations. The prominent degeneration of hyaline cartilage tissue in gills were only observed in dimethoate exposed fish (Fig. 2). Hyaline cartilage and lamellas of gills of fish exposed to dimethoate plus ascorbic acid exhibited less degeneration and mononuclear cell infiltration than fish exposed to dimethoate (Fig. 4). Histopathological changes, as a result of the toxic effects of pesticides have also been reported by Singh (2014). Dimethoate

is strongly hepatotoxic and severely affects histology, carbohydrate and protein metabolism of *Cyprinus carpio* liver (Singh, 2013).

Jothinarendiran (2012) showed decomposition of epithelium tissue and edema in the gill following exposure to dimethoate in *Channa punctatus*. Dubey *et al.* (2014) observed vacuoles in liver cells of *Clarias batrachus*, exposed to dimethoate. In the present histopathological study, certain disorders were detected such as cytoplasmic vacuolization with pyknotic nuclei in hepatic cells. However, fish supplemented with Vitamin C along with dimethoate showed recover towards normalcy in their histomorphological changes after in comparison to dimethoate induced group. These findings indicate that

Fig. 1 : Gills of control group. In gill tissue, the cells that constitute the gill epithelial (black arrow) and hyaline cartilage (white all arrow) are seen. Also, vessel wall and nucleated red cells (white short arrow) are seen (HE, X20)

Fig. 2 : Gills of fish exposed to dimethoate, vessel wall with enlarged diameter inside primer and secondary lamella (white arrow). Gill epithelial cells, hyaline cartilage degeneration linked to the epithelium, and mononuclear cell infiltrations (black arrow) (HE, X10)

Fig. 3 : Fish exposed to ascorbic acid are observed with their primary and secondary epitheliums (arrows) (HE, X10)

Fig. 4 : Fish exposed to dimethoate plus ascorbic acid showed hyalin cartilage (arrows) and lamella (black arrow) necrotic area (white tall arrow) settled into the tissue among the lamellae were observed. Mononuclear cell infiltration (white short arrow) were reduced (HE, X10)

Table 1 : The level of serum TSA, TOS and TAS in *Capoeta capoeta* applied DM and ascorbic acid treatment

Parameters	Group I	Group II	Group III	Group IV
TAS (mmol Trolox eq l ⁻¹)	1.15 ± 0.05	1.07 ± 0.16	1.09 ± 0.12	1.11 ± 0.2
TOS (μmol H ₂ O ₂ eq l ⁻¹)	6.87 ± 0.52	7.58 ± 0.95	7.42 ± 0.62	7.98 ± 1.11
TSA (mg dl ⁻¹)	69.97 ± 5.26 ^a	136.36 ± 9.08 ^c	122.82 ± 9.64 ^b	71.04 ± 6.45 ^a

Group I: Control group; Group II: Dimethoate group; Group III: Dimethoate plus ascorbic acid group; Group IV: Ascorbic acid group. ^{a, b, c} Values with different letter indicates significant differences (P<0.05)

ascorbic acid, in terms of microscopic changes may be protective against dimethoate toxicity.

As fish can not synthesize ascorbic acid, it must obtain with supplementations or food. Ascorbic acid has important functions, such as lymphocyte transformation, preventing oxidation of molecules used in the metabolic pathways, increasing chemotactic activity in neutrophils, collagen synthesis, hydrogen transport, regular growth and nucleic acid synthesis (Wang *et al.*, 2003). The type of collagen which increased in fish exposed to ascorbic acid were identified as glycoproteins containing sialic acid groups (Lisowska, 2002; Yavuz, 2001).

Dubey *et al.* (2014) reported that *Clarias batrachus* exposed to 0.45 μg l⁻¹ dimethoate increased the level of the hepatic glutamate oxaloacetate transaminase, glutamate pyrophosphate transaminase, acid phosphatase, and alkaline phosphatase enzymes were increased after 30 and 60 days compared to control fish, however protein content of the fish decreased. Inyang *et al.* (2016) observed changes of the levels of lactate dehydrogenase (LDH), creatinine kinase (CK) and amylase in *Clarias lazera* with treatment of 2.50, 3.00 and 3.50 ppm dimethoate for 30 days and found to elicit profound changes in all the enzymes tested. It has been reported that enzymes tested may be more useful biomarkers of sublethal effect of dimethoate in *Clarias lazera* (Inyang *et al.*, 2016). Dubey *et al.*, (2014) concluded that ascorbic acid (50 mg l⁻¹) had protective effects against dimethoate toxicity. In the present study, serum total sialic acid level of ascorbic acid treated group were found to be significantly lower than fish exposed to dimethoate and dimethoate plus ascorbic acid group. Serum total sialic acid level of fish exposed to dimethoate plus ascorbic acid was also found to be significantly lower than fish exposed to dimethoate only.

Based on these findings it is suggested that decreased levels of serum total sialic acid in ascorbic acid exposed fish might be related to the active role of sialic acids in the synthesis of glycoproteins. ascorbic acid showed a protective role by reducing the level of serum total sialic acid and histological changes in gill tissue of fish exposed to dimethoate.

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