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## Effect of Diazinon on Catalase Antioxidant Enzyme Activity in Liver Tissue of *Rutilus rutilus*

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**Abstract:** Diazinon as an organophosphate toxin applied widely in agricultural gardens of Iran, so every year huge volume of diazinon enter to aquatic ecosystems. In this study, antioxidant enzyme activity (Catalase) were assessed in liver of *Rutilus rutilus* weighing about  $7.0 \pm 1.0$  g following exposure of fish to 20, 40 and  $60 \mu\text{g L}^{-1}$  concentration of diazinon at  $22.0 \pm 1.0^\circ\text{C}$  and acceptable water quality conditions. At the first,  $\text{LC}_{50}$  96 h was determined 4.5 ppm. We have 3 treatments with 3 replicate and 1 controls (without diazinon). In each aquarium (Volume: 8 L) were 10 fishes. The result of measuring catalase activity in liver with special kit did not show significant differences between treatment groups and control ( $p > 0.05$ ). Also, regression curve did not show significant correlation between increasing toxin concentration and catalase activity changes. The results showed that in same concentration, catalase activity in time 24 h significantly was higher than times 48 and 96 h ( $p < 0.05$ ). Finally, the results did not show special relationship between different concentrations of diazinon and catalase activity in fish liver, that is maybe was result of parameters such as antioxidant enzymes conjugative operation, enzymes professional reaction against special pollutant, rapid reactions and acute phase reaction and antioxidant adaptation against pollution and antioxidative reaction outbreak in target organ.

**Key words:** Catalase, antioxidant, *Rutilus rutilus*

### INTRODUCTION

Human destructive influence on the aquatic environment is in the form of sublethal pollution, which results in chronic stress conditions that have a negative effect on aquatic life (Adedeji *et al.*, 2008). Studies have shown that many of pesticides not reach to target pest and many of them enter to environment (EPA, 2005). Diazinon is an organophosphate insecticide that used to control insects in agricultural ground, households and urban settings. It is mobile and moderately persistent in the environment and does not bioaccumulation (Pehkonen and Zhang, 2002). Due to its chemical properties and widespread use, diazinon is frequently found in point sources and non-point sources in urban and agricultural areas (EPA, 2003). This toxin after application easily enters to current and underground water resources. Today, many organizations represented diazinon as a dangerous poison for almost animal (EPA, 2005).

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The acute toxicity of chemicals may be easily evaluated in a short term test and death represents unequivocal end points (Segner and Braubeck, 1990). Oxidative stress is defined as a disruption of the prooxidant antioxidant balance in favor of the former, leading to potential damage almost study showed that it is a result of one of three factors: (1) an increase in Reactive Oxygen Species (ROS), (2) an impairment of antioxidant defense systems, or (3) an incapacity to repair oxidative damage (Dorval and Hontela, 2003). Antioxidants are substances capable decreasing of oxidative stress harmful effects. Antioxidant system in many organisms, such as fish including Super Oxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxides (GPx) and Glutathione Reductase (GR) that a liquidator or compensating oxygen species deliver response act. Pesticides may induce oxidative stress, leading to generation of free radicals and cause lipid peroxidation (Kehrer, 1993; Lackner, 1998). In the antioxidant defense system, chemical reactions produced neutral by internal systems are or xenobiotic metabolism. This system includes enzymatic reactions also remove chemicals electrophilic or metabolites organic and reduced organic peroxides. Antioxidant defense systems affected by contact with chemicals and therefore, can antioxidant defense systems as biological markers for environmental pollution monitoring used (Rosa *et al.*, 2005). Therefore, the use of diazinon in the agriculture and possibility of enter to aquatic ecosystem specially Caspian Sea watershed and then impact on roach fish (*Rutilus rutilus*) as a valuable species fishery in the fish food chain and potential harmful effects on the fish survival was the most important reason of this research. The research hypothesis was that concentrations of specific toxins Diazinon have an immune system influence the roach fish and fish reaction leading to the different levels of toxins and changes in performance and in particular antioxidant catalase levels and this change probably increased levels of catalase in the liver as the main place antioxidant. The purpose of this study was investigation of influence the sublethal concentration of Diazinon on catalase antioxidant enzyme activity in liver of roach fish and thus, investigation of anti antioxidant turnover as an indicator in order to offer input this toxin to aquatic ecosystem (EPA, 2005).

## MATERIALS AND METHODS

### Fish Preparation and Adaptation

This Study carried out at January 2009 in Iran. First, fish cathead of Syjeval fish hatchery ponds located in Torkaman port at Golestan province and stocked in bags containing water and oxygen and transferred to Laboratory Islamic Azad University. In Laboratory for adaptation of fish with environmental conditions, fish stocked at aquarium (with volume 110 L) and aeration with air pump 24 h before experiment. After this adaptation period, fish with similar mean weight separation and survival test was performed later with three replication: 10 fish in each replication, fish density  $1 \text{ g L}^{-1}$ , aeration constant at all time, 96 h experiment period, photo period 12-16 h, stop feeding 48 h before experiment, not exchange water test (water static) and recorded fish mortality in each 24 h, if the above conditions, mortality rate is less than 5%, therefore fish were suitable for determination of lethal range and appropriate  $LC_{50}$ .

### Lethal Range and Acute Toxicity Experiment

First determination of lethal range and the following test to determine acute toxicity and  $LC_{50}$  using the OECD standard guideline (OECD, 1992). To this purpose, 10 fish groups in three replication transferred to aquariums with similar water quality conditions to survival test. Then 7 concentration of toxin at geometric progression added to the aquarium and

mortality rate recorded. After determining lethal range than  $LC_{50}$  of Diazinon in roach (*Rutilus rutilus*) fingerling determined (within 4 days, times 24, 48, 72 and 96 h with fish exposed to the toxin levels, mortality were recorded). During the experiments was one treatment as a control group and 48 h before the test until the end of test, stopped fish feeding.

#### **Treatments and Replications Design**

Fish categorized in the 4 treatment each with 3 replications. The first group with 10 fish in each replication as a control group (without Diazinon) and 3 treatments with concentrations of 20, 40 and 60  $\mu\text{g L}^{-1}$  Diazinon with 10 fish in each replication. For experiment 110 L glass aquarium with the dimensions 0.23×1.4×0.25 m was used, the water volume of about 80 L. Random sampling done in each treatment and replication at 24, 48 and 96 h after placing the fish was exposed to Diazinon.

#### **Preparation of Different Diazinon Toxin Concentrations**

At this stage, toxin volume necessary for preparation of treatments was calculated by  $C_1V_1 = C_2V_2$  ratio that at first mother solution containing the toxin prepared and then take of it's with regard to concentrations.

#### **Measuring Water Quality Parameters**

During experiment, dissolved oxygen, pH, hardness, electrical conductivity and temperature controlled and recorded in all aquariums and will try to water quality conditions for all the replication is similar. During experiment, oxygen levels (about 6 mg) and temperature (about 22°C) was kept constant.

#### **Measuring Antioxidant Catalase**

Catalase activity measured by a special kit (Cayman Chemical Catalase Kit) for this purpose, after sampling fish in each treatment, liver tissue samples transferred to the laboratory of Veterinary Medicine faculty, Tehran University and amount of catalase activity calculated with using of prooxidation activity this enzyme unitage nmole/min/mL and special activity unitage nmole/min/mg protein, respectively, test sensitivity was about 0.25 to 4 nmol/min/mL.

#### **Statistical Analysis**

For drawing graphs of results applied of Excel 2003 software. Also for the data statistical comparison between different treatments and control, data were analyzed by one-way ANOVA test followed by Tukey test to determine the effect of the treatment. The level for accepted statistical significance was  $p < 0.05$ .

## **RESULTS**

#### **Lethal Test (Acute Toxicity)**

With progressive increase in diazinon concentration and different times in terms of fish mortality, the results showed that 50% of fish tested were died at 4.5  $\text{mg L}^{-1}$  diazinon after 96 h, in other words, the rate of diazinon  $LC_{50}$  for 7 to 8 g fingerling was about 4.5 mg (96 h (Diazinon) *Rutilus rutilus*: 4.5 ppm  $LC_{50}$ ). Also, correlation between the concentration of diazinon and fish mortality rate under acute toxicity test was significant ( $p < 0.05$ ,  $r = 0.9576$ ) and with increasing of diazinon concentrations, mortality increased.

### Catalase Activity Measured in Fish Liver

Catalase activity measurement in different Diazinon concentrations (20, 40 and 60  $\mu\text{g}$ ) and the desired times (24, 48 and 96 h) using a special kit as shown in Fig. 1-4. After 24 and 48 h the fish exposed to different concentrations of diazinon, the highest specific activities of liver catalase were measured in treatment with 60  $\mu\text{g L}^{-1}$  (3.545  $\mu\text{mol}/\text{min}/\text{mg}$  protein) and 40  $\mu\text{g L}^{-1}$  Diazinon (0.279  $\mu\text{mol}/\text{min}/\text{mg}$  protein), respectively. Statistical analyzed indicated that after 24 h, differences between 60 and 40 was significance ( $p < 0.05$ ) and higher catalase activity in 60 treatment as well as shown in (Fig. 1). Also not significant difference was observed between different treatments with control ( $p > 0.05$ ). However, after 48 h, the amount of liver catalase activity at control treatment was higher than were 20 and 60 treatments (Fig. 2). Statistical analyze indicated that was not significant difference between 60 treatment and control ( $p > 0.05$ ).

Comparison of mean obtained from different treatments showed that highest liver catalase activity seen in treatment 20 and later times control treatment and 60 and 40. Statistical analysis showed that the rate of liver catalase specific activity in different treatments to control was not significantly different ( $p > 0.05$ )(Fig. 3).

Catalase activity fluctuation in fish liver during experiment (24, 48 and 96 h) was considerable, so that similar concentrations much higher catalase activity at various times during the 24 h is that has difference significant to periods of 48 and 96 h (Fig. 4).

Statistical analyze showed that the catalase activity measured in control group at all measurement times (24, 48 and 96 h) were not significant differences ( $p > 0.05$ ), while the

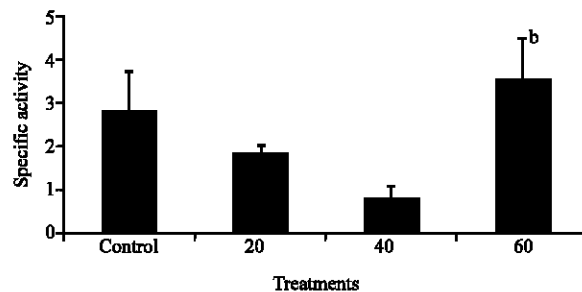


Fig. 1: Catalase enzyme specific activity in the liver of fish after 24 h exposure of different concentrations of diazinon (Mean±SEM). Different letters (a, b) indicate significant difference between treatments ( $p < 0.05$ )

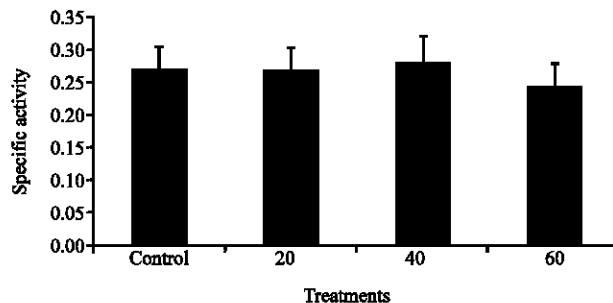


Fig. 2: Catalase enzyme specific activity in the liver of fish after 48 h exposure of different concentrations of diazinon (Mean±SEM)

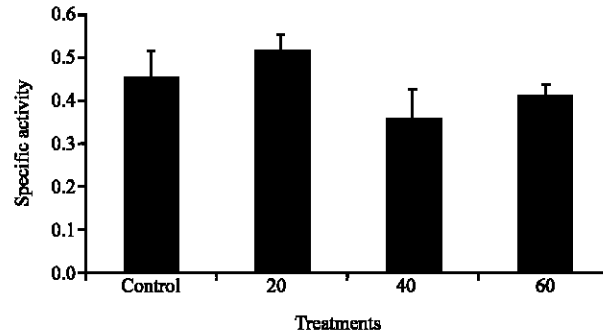


Fig. 3: Catalase enzyme specific activity in the liver of fish after 96 h exposure of different concentrations of diazinon (Mean±SEM)

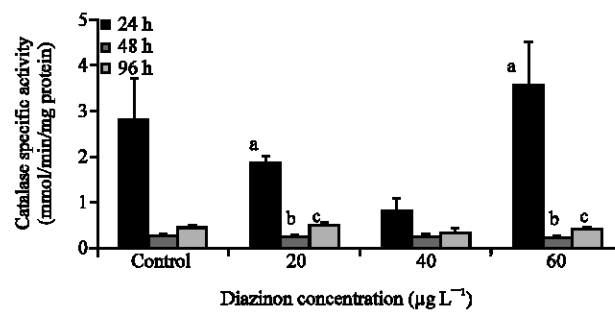


Fig. 4: Catalase specific activity variations over time, 24, 48 and 96 h (Mean±SEM). Different letters indicate significant difference between treatments ( $p < 0.05$ )

concentration  $20 \mu\text{g L}^{-1}$ , between catalase activity values measured between 48 and 24 times with 96 significant difference was observed ( $p < 0.001$ ); while between 48 with 96 h difference was no significance ( $p > 0.05$ ). This trend not seen in concentration  $40 \mu\text{g L}^{-1}$  diazinon and catalase activity level under 40 concentrations in times 24, 48 and 96 h together were not significantly ( $p > 0.05$ ). Review the statistical level of catalase activity in the fish liver that exposure to  $60 \mu\text{g L}^{-1}$  diazinon showed that the level activity at 24 h with 96 and 24 with 48 was a significant difference ( $p < 0.05$ ) but compared to catalase activity level in same concentration at 48 with 96 was not significant ( $p > 0.05$ ).

### Fish Investigation

At initial investigation, selected fish were healthy for experiment and their mean total length was 11 cm and average weight of about 7 to 8 g, respectively. The fish in control until the end of study were alive and have a normal behavior.

### Water Quality Parameters

Measurements of water quality of treatments showed that averages of Ec, DO, temperature, pH and hardness was about 410 to 430 micro Siemens  $\text{cm}^{-1}$ , 6.4 to 6.9  $\text{mg L}^{-1}$ . The 22 to 23°C, 8.28 to 8.43, 90 to 95 mg of carbonate calcium per liter, respectively. In general, the results of water quality parameters measured during the experiment showed that water quality was relatively constant.

## DISCUSSION

In this study,  $LC_{50}$  diazinon was about  $4.5 \text{ mg L}^{-1}$ . In other studies, obtained  $LC_{50}$  Diazinon 96 h levels for European eel (*Anguilla anguilla*)  $0.01 \text{ mg L}^{-1}$  (Sancho *et al.*, 1992), for Guppy (*Poecilia reticulata*) 8 and zebra fish (*Brachydanio rerio*)  $8 \text{ mg L}^{-1}$  (Keizer *et al.*, 2001). In a study done by Svoboda *et al.* (2001) obtained  $LC_{50}$  96 h for Common carp (*Cyprinus carpio*)  $12 \text{ mg L}^{-1}$  (Svoboda *et al.*, 2001) and for golden carp (*Carassius auratus*)  $4 \text{ mg L}^{-1}$  and for *Brachydanio rerio*  $8 \text{ mg L}^{-1}$  (Eliser, 2000). Sensitivity of fish to diazinon may be due to the difference in diazinon uptake and act to prevent enzyme acetylcholine esterase and neutralization by the different fish species, moreover, depending on water quality conditions can vary  $LC_{50}$  results, especially amount of water hardness is very importance. Also in addition to fish species, size or weight of the fish in different studies can make comparison difficult (Oh *et al.*, 1991). Be noted that the amount of polluting materials and pesticides  $LC_{50}$  including diazinon in various fish the numerous factors including age, fish, fish species, physiological status of fish when testing, source of fish supply, the consumer diazinon (powder, emulsion), hard water and other is different. Therefore, in many cases the amount of antioxidants in specific organ such as a liver increased while other organs such as brain remain constant. Many scientists believe in some fish, antioxidant enzymes activities severely affected fish growth stage, especially to sexual maturity (Jena *et al.*, 1998). In this study, after 24 h of placing fish exposed to different concentrations of diazinon, the highest specific activity of liver catalase was determined in concentration  $60 \text{ } \mu\text{g L}^{-1}$  diazinon and the level of catalase activity in 40 and 20 was lower than control. But after 48 h, the highest specific activity were measured at treatment with  $40 \text{ } \mu\text{g L}^{-1}$  diazinon. The level of catalase activity has significance fluctuation during measured times. Furthermore, results showed that in a similar concentration, catalase activity level at 24 higher than 48 and 96 h and the difference was significant ( $p < 0.05$ ) while the control at measurement times (24, 48 and 96 h) despite the same trend but differences were not significant. About this trend can be raised two hypotheses: First while diazinon is the most effect in the early 24 on the catalase activity (acute effects) and then over time, catalase enter to adaptation phase and catalase activity level is reduced to it that the statistical differences between the treatment 24 h with 48 and 96 h can be acceptable. The second hypothesis that somewhat contrasted in the first hypothesis is that trend of reduced catalase activity in similar concentrations (e.g.,  $20 \text{ } \mu\text{g L}^{-1}$ ) at different times, in control was seen. Therefore, can not indicate the logical relationship between the concentration of diazinon and catalase activities and why all the treatments over the reduced catalase activity should be searched for other reasons.

Liver is a uniform organ that most antioxidant activity of enzymes such as catalase and superoxide dismutase it is seen. This can be because the position of the liver and multiple oxidation reactions produce free radicals is maximum (Gul *et al.*, 2004). Many toxins and pollutants can be produced through free radicals and antioxidant system changes lead to scavenge ring of activated oxygen species and finally damage to the body. In this subject, the types of antioxidant enzymes for example super oxide dismutase (SOD), catalase (CAT) and glutathione S-transferase can be protect living organisms against adverse effects of ROS. Dutta *et al.* (1997) showed that the sublethal concentration of diazinon ( $15$  to  $75 \text{ } \mu\text{g L}^{-1}$ ) can cause various complications in fish gill *Lepomis macrochirus* and with increase the concentration of diazinon, significant gill complications was seen.

Oh *et al.* (1991) suggested that 3 factor as the reason for diazinon selective toxicity of different fish species: different acetylcholine esterase inhibition, detoxification and

absorption. The three factor response factor differences toxicity to fish different chemical concentrations are different. Result of several studies have shown that necessarily when fish affected by pollutant, not active antioxidant system and the other hand, changes in activity of these enzymes according to type and nature of pollutants in a particular organ and provides incidence rate in many cases their activities in a low at organ and other organ are high (Rosa *et al.*, 2005).

Tetyana *et al.* (2005) in their study on goldfish (*Carassius auratus*) concluded that the level of catalase activity in the brain exposed to pollutant of 3- amoni 1,2,4-triazole were reduced with increased concentrations of pollutant and leads to oxidation stress and lipid peroxidation that stimulated this effect was highly specialized tissue and increase of other antioxidant enzymes.

Today, numerous studies have shown that toxins, organic and metal pollutants can result in stimulating production process ROS, decreased antioxidant enzymes activities and thus oxidation damage in aquatic organisms in acute tests are the other hand, can prevent or reducing toxicity of ROS by antioxidant enzymes considered (Pinto *et al.*, 2003). Although, the chronic experiments, activities of antioxidant enzymes typically have increased in animals, but should be noted that antioxidant enzymes changes according to species and type of aquatic pollutants is different and effect of pollutants on antioxidant levels depend to investigated organ (Oruc *et al.*, 2004). May be values obtained in antioxidants vary which can due to different environmental conditions (such as water temperature or level of available oxygen) and species differences such as biological rhythms and reproductive cycles. Some of these differences can be due to the different defense systems between different tissues and organs. For example, glutathione peroxidase, super oxide dismutase and glutathione reductase activity in gill and digestive system of bivalve *Perna perna* is the same, but in bivalve *Methylyus edulis*, higher values of these enzymes gill has been reported. Also compared with digestive glands, the level of catalase activity in the gill is 3 times less (Almedia *et al.*, 2007).

The other hand, many researches have shown that may be each antioxidant enzymes have more obvious role in certain types of pollution and their reaction is more organ specific in body. Some research has shown that the level of antioxidant activity of some enzymes by aquatic age will be different, so in many aquatic animals, antioxidant enzymes activity decreased with increases of age, but this trend about all antioxidant enzymes is not true (Rosa *et al.*, 2005). It is remarkable that the defense system antioxidant activity changes against pollutants are two distinct phases: an acute phase usually in early hour of exposure to contaminants in fish exposed to update (usually 24 h early) and a adaptation phase that according to the species and type of contaminants can take several days (Tetyana *et al.*, 2005).

## CONCLUSION

In general, with according to this study results can be suggested that the lethal range of diazinon value obtained for 7 to 8 g of fish (*Rutilus rutilus*) indicates that this material is quite a significant toxicity and entering the water ecosystems can have undesirable effects the fish and about the roach fish (*Rutilus rutilus*) that is on of the valuable species of Caspian is considered to be true. The results were also proved that the concentrations of the diazinon used (20, 40 and 60  $\mu\text{g L}^{-1}$ ) no significant influence on the catalase activity in the liver of fish and reasons for certain changes had required further studies in this area. The general conclusion must be stated that measuring simultaneously and combined of



antioxidant enzymes in several target organs could be due to more specific results, the other hand, understanding the mechanisms of body defense system against diazinon and reactive agent also has problems the need for more studies and is more accurate.

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