http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 33(2): 113-120 (2016)

Su Ürünleri Dergisi (2016)
DOI: 10.12714/egejfas.2016.33.2.04

RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Effects of vitamin C on oxidative stress parameters in rainbow trout exposed to diazinon

Alireza Mirvaghefi* • **Mohsen Ali** • **Hadi Poorbagher**

Department of Fisheries, Faculty of Natural Resources, University of Tehran, Karaj PO Box 4314, Iran
**Corresponding author: mani_a777@yahoo.com*

Received date: 23.01.2016 *Accepted date: 04.03.2016*

How to cite this paper:
Mirvaghefi, A., Ali, M. & Poobagher, H. (2016). Effects of vitamin c on oxidative stress parameters in rainbow trout exposed to diazinon. *Ege Journal of Fisheries and Aquatic Sciences,* 33(2): 113-120. doi: 10.12714/egejfas.2016.33.2.04

Abstract: : Diazinon (Organophosphorus insecticide) can cause reactive oxidative stresses during metabolism of aquatic organisms. The study presented here aimed to investigate the antioxidant effect of vitamin C on antioxidant enzyme activities and lipid peroxidation in rainbow trout exposed to subchronic dosage diazinon. A total number of 360 Oncorhynchus mykiss were allocated into four treatment groups (with three replicates): control; diazinon (0.1 mg L 1); vitamin C (300 mg kg 1 in diet) plus diazinon (0.1 mg L-1), and vitamin C (1000 mg kg-1 in diet) plus diazinon (0.1 mg L-1). After two and four weeks, blood samples were collected and superoxide dismutase, catalase enzymes, total antioxidant capacity and malondialdehyte index were measured. The specimens exposed exclusively to diazinon showed a significant decrease in superoxide dismutase and total antioxidant capacity activities and also an increase in catalase activity and malondialdehyde index compared to those of the control group. The changes in oxidative stress parameters may be related to destructive free oxiradicals such as superoxide and hydroxyl radicals produced in diazinon metabolism. In conclusion, vitamin C moderated activity of the antioxidant enzyme, increased the total antioxidant capacity, and decreased cellular damages caused by destructive lipid peroxidation process in presence of diazinon.

Keywords: *Diazinon, antioxidant, vitamin C, enzyme, malondialdehyde, catalase*

INTRODUCTION

Organophosphorus pesticides are used in agriculture to control pests. Organophosphorus pesticides decompose fast and are less stable than chlorate pesticides causing them to be consumed more than other pesticides. Diazinon is an organophosphorus pesticides that is used against various insects, household and garden pests (Virtue and Clayton, 1997), also, for pests of vegetables, tobacco, feedstuff and ornamental house plants (Bailey et al., 2000). The average residence times (half-life) of diazinon in lakes and rivers is approximately 30 days (range: 14-184 days) and 39 days, respectively (Arthur et al., 1983; Jarvinen and Tanner, 1892). Diazinon is an organic, ingestional and dermal contact toxin. Their lipophilic feature and solubility in lipid facilitates its passing through the cell membrane. This toxin can enter the fish body through the epithelium, skin and digestive system, and owing to its lipophilic characteristics, it is transferred from the blood to other tissues (Vale, 1998). The metabolism of diazinon results in formation of metabolites that may upset the balance between antioxidant factors and production of free radicals. The occurrence of oxidative stress caused by diazinon has already been demonstrated in trout, tilapia, and carp (Uner et al., 2006).

Diazinon is decomposed in the fish body through transformation into diazoxon in the phase I enzyme activities such as cytochrome enzymes (CYP1A2 & CYP3A2) during desulphurization process (Schlenk, 2005). Carp, guppy, and zebra fish are all able to transform diazinon into diazoxon (Keizer et al., 1995). Also, in Dace and Yellow Tail, hydroxy diazinon and hydroxyl methyl diazinon are both the main metabolites having hydroxyl factor (Fujii and Asaka, 1982; Schlenk, 2005). In the initial phase of diazinon metabolism, an active polar group like hydroxyl (OH-) is produced in isopropyl hydroxyl pyrimidine structure (IMP), which is one of the metabolites of this toxin. This compound is electrophilic and has the capacity to form conjugated compounds and defecated. On the other hand, these compounds are likely to turn into oxygen free radicals including hydroxyl and superoxide anion and able to target the cellular structures (Fujii and Asaka, 1982).

Generally, organophosphorus pesticides can increase lipid peroxidation through direct influence on the plasma membrane (Hazarika et al., 2003). Malondialdehyde (MDA) is produced during lipid peroxidation caused by an organophosphate pesticide like diazinon and is regarded as an index of oxidative stress induction. MDA damages the cells during the lipid peroxidation (Doba et al., 1985).

To overcome toxicological stress, fishes have enzymatic defense mechanisms such as secretion of specific antioxidant defense enzymes including superoxide dismutase (SOD),

catalase (CAT) that form the cellular antioxidant defense system being responsible for scavenging of free radicals (El-Gendy et al., 2010). In this respect, the SOD enzyme is considered as the main defense factor against superoxide anion radicals and is somehow the first line of defense against oxidative stresses. This enzyme is responsible for accelerating the transformation of superoxide anions into molecular oxygen and hydrogen peroxide (Das et al., 1997; McCord and Fridovich ,1969). The CAT enzyme is found in cells of aerobic organisms and decomposes hydrogen peroxide to water and oxygen (Aebi, 1984).

In addition, there are non-enzymatic antioxidant defense mechanisms involving different chemical groups, e.g. vitamins, carotenoids, amino acids and peptides which are established in various cellular structures to deal with anti-oxidative effects. These factors prevent production of radicals and play an important role in maintaining homeostasis in aquatic organisms (Zama et al., 2007).

The all antioxidant factors of the body of an organism, either intracellular enzymes or antioxidant nutritional compounds (non-enzymatic factors), are called total antioxidant capacity (TAC) (Mahfouz et al., 2009).

Vitamin C (ascorbic acid) is one of the non-enzymatic antioxidant factors both in extracellular (interstitial and intercellular fluids) and intracellular fluids (cytosol), being able to neutralize many oxyradicals (Bigard, 2001). The characteristics of this vitamin as reviving factor to nullify wide variety of produced free radicals during the pesticide metabolism has been proven (Tsao, 1997). For instance, inclusion of vitamin C to the diet of Oncorhynchus mykiss may neutralize the revival of O2-, OH and H2O2 free radicals and prevent damages of oxidative stresses (Verlhac and Gabaudan , 1997; Verlhac et al., 1998). Also, vitamin C prevents the lipid peroxidation process through inhibiting reactive oxygen species in aqueous phase (Frei et al., 1988; Jialal et al., 1990). Effects of pesticides on enzymatic mechanisms of fishes (e.g. MDA index, SOD and CAT activities) have extensively been investigated (Durmaz et al., 2006; Isik and Celik, 2008; Monterio et al., 2009).

Most bony fishes are however unable to synthesize vitamin C as they lack L glunolacton oxidase enzyme to convert glucose, which makes it an essential vitamin needed to be supplied with food (Moreau et al., 1999; Verlhac and Gabaudan ,1997). In general, supplying vitamin C to enhance resistance of fishes against environmental stresses has become an effective way through influencing the biochemical parameters of the blood (Fabiana et al., 2007). In other words, the role of ascorbic acid is to reduce and balance biochemical function during the oxidative stresses which can result in reduction of ROS through various physiological methods in the body of organisms (Buettner and Moseley, 1993).

Organophosphorus pesticides are used in agriculture to control pests. Many flowing water resources contain diazinon that may cause a serious threat to fish health exposed to this pesticide. Diazinon can cause reactive oxidative stresses (ROS) during metabolism of aquatic organisms. The present study evaluated the activity of antioxidant enzymes and lipid peroxidation during oxidative stress in rainbow trout. Therefore, the present study aimed to reduce the adverse effects of environmental toxins in trout farming using an antioxidant compound. In this respect, this study investigated the antioxidative effects of dietary vitamin C in Oncorhynchus mykiss exposed to subacute dosages of diazinon using SOD and CAT activities, total antioxidant capacity (TAC) and MDA index of blood serum.

MATERIAL AND METHOD

The chemicals were purchased from Sigma and Merck (Sigma, Merck Co., Germany). The spectrophotometer, Unico version UV-2100, sudatorium or Ben Murray (Shimaz Co.), and centrifuge (Genofuge M16) were used in this study.

Experimental condition

A total number of 360 rainbow trouts (Oncorhynchus mykiss) with an average weight and length of 121 ± 18 g and 22.9 ± 1.6 cm (± SD), respectively, were purchased from a fish farm. In this study, the 360 Oncorhynchus mykiss (mean weight: 121 ± 18 g) were allocated into four groups (with three replicates). The animals were acclimatized to the tanks for a week with 10% of water was changed every day. The physicochemical properties of water during the experiment period were: temperature, 12.5 ± 1 °C, pH, 7.8 ± 0.1, dissolved oxygen, 8 ± 0.5 ppm and water hardness, 205 ± 16 (mg L 1 CaCO3). Diazinon (Partovnar® Co., Iran), in emulation form (60%) and soluble in 40% zylon were used in preparing the treatments. A stock solution of 10 ppt was prepared (Koprucu et al. 2006) to make a final solution of 0.1 mg L 1 diazinon as sub acute concentration, which was 10% of diazinon's LC50 in O. mykiss (Eisler 1998). Vitamin C was provided in encapsulated powder form and stable in water with chemical structure of L-ascorbic acid-2-phosphate (Tiger C-35, Tiger Co., China). The chemical formula and molecular weight of vitamin C were C6H9O9P and 256.11 g mol 1, respectively. The 300-L tanks (containing 30 specimens) were treated as follows: (1) control (no diazinon), (2) exposed to diazinon (0.1 mg L 1), (3) exposed to diazinon (0.1 mg L 1) and vitamin C (300 mg kg 1 in the diet), (4) exposed to diazinon (0.1 mg L 1) and vitamin C (1000 mg kg 1 in the diet). The experiment was run for four weeks and the animals were fed at the rate of 2% of their mean body weight.

Preparation of blood samples

The clover powder solution (100 ppm) was used to anesthetize the specimens and collect the blood samples. Thirty fish in each group were sampled. The blood samples were taken from the base of the pelvic fin using a 2-mL syringe, transferred to a 2-mL Eppendorf tube and centrifuged immediately at 4500 rpm for 15 minutes to separate the serum from the blood. Then, the samples were maintained at 70 °C.

Biochemical analysis

Superoxide dismutase (SOD)

The Marklund method was used to measure superoxide dismutase of the blood serum (Marklund and Marklund, 1974). In this method, the activity of superoxide dismotase enzyme is assessed colorimetrically using pyrogallol auto-oxidasion process in the presence of hydrogen peroxide (H2O2). With increasing the amount of superoxide dismutase enzyme in the serum, the process of pyrogallol auto-oxidation is decreased. This assessment was measured spectrophotometrically at 420 nm.

Catalase (CAT)

The Goth method was used to measure the activity of catalase enzyme in the blood serum (Goth, 1991). In brief, the serum and hydrogen peroxide were mixed for 10 minutes (at room temperature), then Amonium molybdate ((NH4) MO7O24)) was used to stop the oxidation and determine the catalase enzyme activity level. An increase of CAT activity decreases the light absorption at 410 nm.

Total antioxidant (TAC)

The FRAP (Ferric-reducing ability of plasma) assay was used to examine total antioxidant capacity level as proposed by Koracevic et al. (2001). Briefly, the standard solution of complex Fe-EDTA and peroxide hydrogen (H2O2) produces hydroxyl radicals during fentone reaction. The reactive oxygen causes the release of thiobarbituric acid (TBA), which is a reactive acid. The production of TBA is measured spectrophotometrically at 532 nm.

Malondialdehyte (MDA)

The Ledwozyw method was used to measure MDA index (Ledwozyw, 1986). In this method, an increase of MDA level increases the level of TBA inhibition, and in turn decreases the color production, which is measured spectrophotometrically. One mL of serum samples was mixed with two mL Tri-chloro acetic acid, thiobarbituric acid (TBA) and hydrochloric acid in acidic condition and then diluted to 200 mL using distilled water. Next, the tubes containing the solution were settled in sudatorium for 30 min. Then they were transferred in vitro until they were cold. Later, the samples were centrifuged for 10 min (300 rpm) and the supernatant solution was carefully decanted. Finally, absorption was read at 535 nm.

Statistical Analysis

A significant difference among the specimens treated with vitamin C and diazinon in SOD, CAT, TAC and MDA was examined using a one-way ANOVA followed by the Tukey test. Normality and homogeneity of variance were examined using the Kolmogorov-Smirnov goodness-of-fit and the Levene tests, respectively. The analyses was performed in SPSS version 16.

RESULTS

SOD

After two weeks, SOD decreased significantly in the specimens treated with diazinon compared to the control group ($P < 0.05$). Also, during the fourth week the reduction of SOD in diazinon treated group (DZN) showed significant difference with that of the control group ($P < 0.05$). (Fig. 1). No significant difference was detected between the specimens received vitamin C300+diazinon and diazinonin in SOD after two weeks and four weeks ($P > 0.05$) (Fig. 1). There was a significant decrease in SOD in those treated with vitamin C1000+diazinon compared to those received only diazinon after two weeks ($P < 0.05$) and the fourth week ($P < 0.05$) (Figure 1).

CAT

There was a significant difference in CAT between the specimens received diazinon compared with the control group after two and four weeks ($P < 0.05$). No significant difference was detected between the specimens received vitamin C300+diazinon and diazinonin in CAT after two week ($P > 0.05$) and four week ($P > 0.05$) (Figure 2). On the other hand, no significant difference was detected in CAT between the control group and the specimens received vitaminC1000+diazinon after four weeks ($P > 0.05$) (Figure 2). Also there was a significant decrease in CAT between diazinon group and vitaminC1000+diazinon group after four weeks ($P < 0.05$).

TAC

At the end of the second week, the specimens received diazinon showed a significant decrease in TAC compared to the control group ($P < 0.05$) (Figure 3). Also, TAC increased significantly in the specimens treated with vitaminC300 and C1000+ diazinon compared to the DZN group ($P < 0.05$).While After four weeks, there was no significant difference in TAC between the treatments diazinon, vitaminC300+diazinon and the control group (Figure 3). On the other hand, there was a significant difference ($P < 0.05$) between the DZN group and the treatment VitaminC1000+diazinon in the fourth week in TAC showing an increase of TAC in VitaminC1000+diazinon compared to the DZN group (Figure 3).

MDA

After two and four weeks, the specimens received diazinon showed a significant increase in MDA compared with that of the control group ($P < 0.05$) (Figure 4). While, after two weeks, there was no significant difference between vitaminC1000+diazinon and the control group ($P > 0.05$). Also, after four weeks, there was no significant difference between vitamin C1000+diazinon and the control group in MDA ($P > 0.05$). However, there was a significant difference between vitaminC300+diazinon and the control group within the two week ($P < 0.05$) but after four weeks no significant difference was detected between these two groups ($P > 0.05$) (Figure 4).

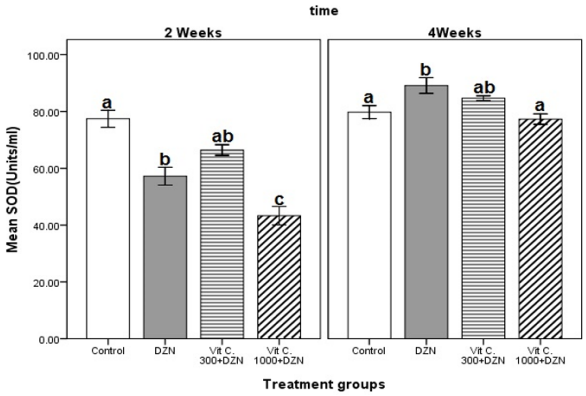

Figure 1. Changes in Superoxide dismutase (SOD) activity level in serum samples of fish treated with diazinon (0.1 mg L 1) and vitamin C (300 and 1000 mg kg 1 per diet) after two and four weeks. Thirty fish in each group were sampled. Different alphabetic letters (a, b, c, d) show significant difference (P < 0.05) and similar alphabetic letters show lack of significance difference among groups.

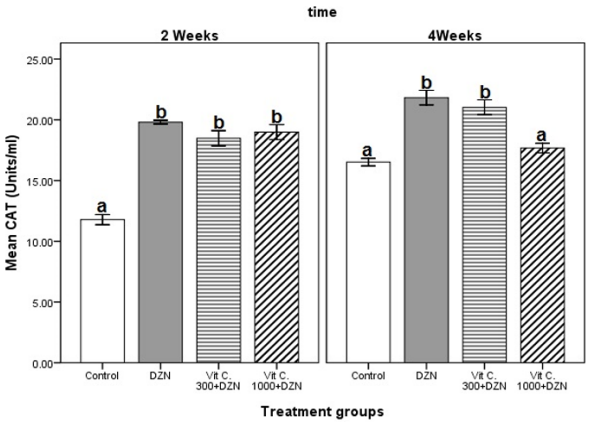

Figure 2. Changes in catalase (CAT) activity level in serum samples of fish treated with diazinon (0.1 mg L 1) and vitamin C (300 and 1000 mg kg 1 per diet) after two and four weeks. Thirty fish in each group were sampled. Different alphabetic letters (a, b, c, d) show significant difference (P < 0.05) and similar alphabetic letters show lack of significance difference among groups

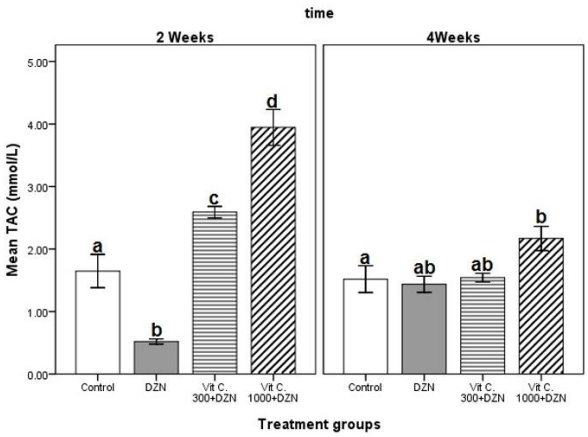

Figure 3. Changes in total antioxidant capacity (TAC) in serum samples of fish treated with diazinon (0.1 mg L 1) and vitamin C (300 and 1000 mg kg 1 per diet) after two and four weeks. Thirty fish in each group were sampled. Different alphabetic letters (a, b, c, d) show significant difference (P < 0.05) and similar alphabetic letters show lack of significance difference among groups.

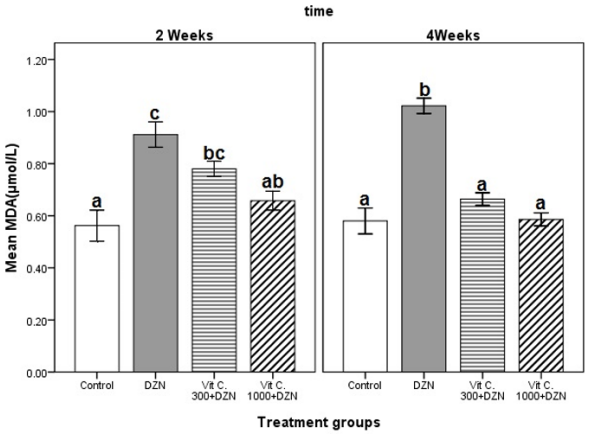

Figure 4. Changes in malondialdehyde(MDA) index level in serum samples of fish treated with diazinon (0.1 mg L 1) and vitamin C (300 and 1000 mg kg 1 per diet) after two and four weeks. Thirty fish in each group were sampled. Different alphabetic letters (a, b, c, d) show significant difference (P < 0.05) and similar alphabetic letters show lack of significance difference among groups

.

DISCUSSION AND CONCLUSION

The present study investigated oxidative effects of inducing subacute doses of diazinon on the activities of SOD and CAT enzymes, TAC and MDA index. In addition, it examined effects of dietary vitamin C in 300 and 1000 mg kg 1 on activity of biochemical factors in *Oncorhynchus mykiss* during the exposure to diazinon pesticide. The results showed that diazinon induction (0.1 mg L 1) could decrease the activity level of SOD due to the increase of superoxide anion radicals O2- as a metabolite produced by diazinon, and the reason is using SOD for neutralizing oxidative factors. The role of geobiotics especially pesticides in causing oxidative stress has already been proven. Halliwell and Gutterdige (1984) found that these compounds could produce the final product of O2- during the oxidation-reduction reactions (redox) in cellular metabolism processes. Also, decrease of SOD levels in presence of diazinon in *Oncorhynchus mykiss* with the concentrations of 0.5-1 ppm in 24, 48 and 72 hours were reported (Isik and Celik, 2008). In a similar study, the effect of diazinon with concentrations of 1, 0.1 ppm on Nile tilapia (*Oreochromis niloticus*) during a 2-week period indicated a decrease in SOD

level (Durmaz et al., 2006).The study presented here indicated that after four weeks, SOD level in samples treated with dizinon increased significantly compared to the second week. However, this difference was not significant in comparison with control group. Pesticides, including organophosphorous pesticides, disable and inhibit antioxidants enzymes which are inhibitors of SOD activity (Naqvi and Vaishnavi, 1993). This increase may be due to continuous production of superoxide anion O2- in cells, and induction and re-stimulation of cells for enzyme production. In this respect, Oruc (2010) believes that the excessive increase of O2- free radical production in the sample tissues of *Oreochromis niloticus* in dealing with chlorpyrifos organophosphorus toxins caused significant increase in SOD level in a 30-day period.

The CAT level increased in the treated fish dealing with diazinon in the second and fourth weeks. This result indicates an increase in the level of peroxide hydrogen (H2O2), which may be the result of SOD enzyme activity to neutralize super oxide radicals. According to Xing et al. (2012) induction of sub-acute dose of Atrazine and chlropyrifos either separately or combined caused an increase in CAT activity level in the liver tissues and gills of *Cyprinus carpio* after 40 days. In another similar study, an increase was reported in activity level of catalase enzyme during the use of diazinon in heart tissues and red blood cells of rats (Akturk et al., 2006). Furthermore, based on a study prescribing the oral use of diazinon to mice could increase the concentration of oxygen free radicals and in turn change the biochemical parameters of the blood (Abdou and El-Mazody, 2007).

In the present study, total antioxidant level of serum in fish treated with diazinon compared with control group after two weeks. This decrease may be the result of continuous production of free radicals in diazinon metabolism. In other words, decrease of TAC level occurs after scavenging process and inhibiting free radicals through enzymatic and non-enzymatic factors of antioxidant defense system and using these factors. In another work, it was observed that subacute diazinon affected liver hepatocytes cells in *Oncorhynchus mykiss* which resulted in decrease of TAC level (Banaee et al., 2011). In a similar work, diazinon induction in examined mice caused decrease of total antioxidant in muscle tissues of treated samples (Amirkabirian et al., 2007).

In general, based on the studies, excessive production of free radicals during the detoxification process of pesticides could cause decrease in total antioxidant capacity in liver cells. In other words, when oxidative process increases through reactive oxygen species (ROS), the antioxidant capacity level decreases (Monterio et al., 2006; Turkez and Togar, 2011).

In addition, the findings of this study showed that TAC level increased in the end of fourth week in comparison with second week, however the difference was not significant in comparison with control group. The reason for this compensation in TAC level could be attributed to the increase in catalase and superoxide dismutase levels in the fourth week, as subacute doses of diazinon has improved the chance for compensatory and adaptive responses for the cells to reach a kind of homeostasis. According to some studies, the increase of TAC caused by oxidative stresses in biological systems could be due to the increase in antioxidant activities via cellular defense mechanism in order to reach homeostasis and somehow adaptation in dealing with antioxidative stresses (Castillo et al., 2006; Kohen et al., 2000).

In the current study was investigating the amount of cellular damages, called lipid peroxidation, during diazinon induction which was measured by MDA index. Based on the measurements of MDA levels in serums of samples during two and four weeks, the amount of this index increased in DZN in comparison with control group, which indicated the increase in lipid peroxidation level of cells. This increase could be the result of production of hydroxyl radicals (OH) as a powerful oxidizing agent and key factor in the beginning of lipid oxidation process which was produced in diazinon metabolism.

Based on previous studies, awareness of the destructive process of lipid peroxidation could be possible through measurement and investigation of the products such as Aldehydes, acetone and MDA produced in this process. Meanwhile, increase of MDA level could indicate the increase of lipid peroxidation and damages to cell membrane (De Zwart et al., 1999; Valavanidis et al., 2006). One of the important factors in occurrence of lipid peroxidation is organophosphorus pesticides which could increase lipid peroxidation through affecting plasma membrane. For example, decomposition of diazinon can cause the production of oxygen free radicals especially superoxide anions and strong hydroxyl radicals and in turn the increase of MDA level, which was proved by Abdou and El-Mazoudy (Abdou and El-Mazoudy, 2007; Hazarika et al., 2003).

In the present study, SOD and CAT measurements showed that vitamin C could decrease and moderate the activity level of these two enzymes. In fact, the moderation of activity level of mentioned enzymes was due to the role of this vitamin in neutralizing superoxide anion free radical O2-, which resulted in decrease of activities of these two enzymes. In general, acid ascorbic has biologically acted in decreasing and balancing biochemical reactions during oxidative stresses. This vitamin can decrease the rate of reactive oxygen species (ROS) produced in various physiological functions (Buettner and Moseley, 1993). Based on the previous studies, vitamin C due to its electronegativity can act as reviver, inhibitor and scavenger of reactive oxygen species. Simply put, the ascorbatic form of vitamin C can neutralize and revive oxiradicals through electronegativity up to two levels. In this way, this compound has the moderating effect on enzymatic antioxidant defense activities (Deshpande et al., 1996).

In an experiment that was not using fishes, the role of vitamin C as an antioxidant to protect against oxidative stresses induced by imidoclopric pesticide in liver tissues of Swiss male albino mice was investigated. The results indicated that use of

vitamin C in the diet before and after dealing with this pesticide could cause a balance in activities of CAT and SOD in liver tissue (El-Gendy et al., 2010). Also in another work, the effect of vitamin E as an antioxidant in facing with chronic toxicity of Atrazine in the diet of female African catfish (*Clarias gariepinus*) was investigated, in which there was decrease in activity levels of SOD and CAT enzymes in liver tissues (Kadry et al., 2012). The results of this study were in line with some of the related studies (El-Gharieb et al., 2010; Singh et al., 2011).

Moreover using vitamin C as non-enzymatic antioxidant could increase total antioxidant capacity in diazinon treated fish. In sum, regarding the fact that the non-enzymatic antioxidant defense system of the cell especially vitamins play the main and fundamental role in TAC, the increase of this cellular defense part through using vitamin supplements - especially vitamin C supplements - can have essential role in increasing total antioxidant defense level of the cell.

It could be stated that total antioxidant is a series of antioxidants present in the organisms' cells which is an index to indicate total rate of enzymatic and non-enzymatic antioxidants (Prior and Cao, 1999). In other words, TAC is not dependent only to the activity of one or two enzymes, but includes a series of vitamins C, E, albunmin, Beta Carotene, etc. in addition to intra cellular enzymes (Miller et al., 1993). In a study conducted by Winston et al. (1998), it was revealed that glutathione, ascorbic acid (vitamin C), uric acid and vitamin E compose 70% of the total antioxidant. According to previous studies, the use of vitamin E and selenium can be enhance non-enzymatic antioxidant defense and adjust the levels of SOD and CAT to their normal values. Such an intervention can also increase TAC in fish (Ali et al., 2015).

Geetha et al. (2010) reported that using chlorella extract supplement as an antioxidant compound in diet of Grey mullet (*Mugil cephalus*) in breeding environment could increase the total antioxidant level (Geetha et al., 2010). Increasing the total antioxidant capacity will increase the rate of free radical removal (Karaoz et al., 2002). Also present study showed that using vitamin C could decrease the MPA index and in turn decrease the damages resulted from lipid peroxidation. This decrease could be explained through two main and fundamental characteristics of vitamin C in acting as scavenger or removing free radicals especially hydroxyl radical and also in recycling the vitamin E radicals called tocopheroxyl which has an important role in preventing lipids oxidation processes.

The effect of vitamin C in protecting proteins, lipids, carbohydrates and nucleic acids against attacks of free radicals has been proved in natural metabolisms of animals (Jakeman and Maxwell, 1993). To corroborate the results of this study, a similar study could be mentioned in which the effect of vitamin C against oxidative stress induced by Imidaclopride in livers of male mice was investigated. The results showed that using this vitamin in diet of the mice before and after facing with toxin decreased the MDA level (El-Gendy et al., 2010). Furthermore, using lycopene, a carotenoid and antioxidant, in diet of Cyprinus carpio could decrease the MDA effects of chloropyrifos organophosphorous toxin (Ural, 2013).

According to previous studies, vitamin C was introduced as an important water-soluble antioxidant which could recycle vitamin E from tocopheroxyl radicals and keep the scavenging capacity of free radicals in suitable condition (Benzi et al., 1999). In other words, vitamin E as an antioxidant is responsible for finalizing chain reactions in oxidation process of polyunsaturated fatty acids (Lu and Liu, 2002). To emphasize the abovementioned issues, in a study, the role of vitamin C and E in combination against diazinon toxicity was investigated in pancreatic tissues of wistar mice in which MDA level increased in groups without vitamin while its level decreased significantly in groups fed with vitamin C and E (Gokalp et al., 2005).

In conclusion, induction of subacute dose of diazinon (0.1 mg L 1) caused significant changes in the level of SOD and total antioxidant capacity. Also, exposure to diazinon increased the level of catalase activity and lipid peroxidation throughout the period the experiment. Application of dietary vitamin C with a dose of 1000 mg kg 1, as a factor enhancing the antioxidant capacity of the cell, modulated and adjusted SOD, CAT enzyme activity levels and increased the total antioxidant capacity of the cells. Furthermore, these levels of vitamin in the diet reduced lipid peroxidation. On the other hand, using a dose of 300 mg vitamin had no significant effect on the SOD and CTA enzymes. However this level of vitamin increased and decreased significantly TAC and MDA, respectively. Nevertheless, this level of vitamin C was less effective than a dose 1000 mg of vitamin C. With respect to studies that examined presence of agricultural contaminants and pesticides in domestic water sources and their destructive effects on fishes, application of suitable materials with antioxidant characteristics in diet of species of the fish in order to increase antioxidant defense seems necessary.

ACKNOWLEDGEMENT

The present study was conducted using facilities of the Faculty of Natural Resources of Tehran University, and Azar Laboratory, Tehran. We acknowledge all those who supported us in conducting this research.

REFERENCES

Abdou, H.M. & El-Mazoudy, R.H. (2007). Myotoxic and hyperlipidemic effects of diazinon in female rats. *Journal of Magnetic Resonance Imaging*, 28:292-298. doi: 10.1016/j.pestbp.2009.11.007

Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*, 105:121-126. doi: 10.1016/s0076-6879(84)05016-3

Akturk, O., Demirin, H., Sutcu, R., Yilmaz, N., Koylu, H. & Altuntas, I. (2006). The effects of diazinon on lipid peroxidation and antioxidant enzymes in rat heart and ameliorating role of vitamin E and vitamin C. *Cell Biology and Toxicology*, 22:455-461. doi: 10.1007/s10565-006-0138-5

Ali, M., Mirvaghefi, A. & Asadi, F. (2015). Effects of vitamin E, selenium and vitamin C on various biomarkers following oxidative stress caused by diazinon exposure in rainbow trout. *Ege Journal of Fisheries and Aquatic Sciences*, 32(3):151-158

Amirkabirian, N., Teimouri, F., Esmaily, H., Mohammadirad, A., Aliahmadi, A. & Abdollahi, M. (2007). Protection by pentoxifylline of diazinon-induced toxic stress in rat liver and muscle. *Toxicology Mechanisms and Methods*, 17:215-221. doi: 10.1080/15376510600943783

Arthur, JW., Zischke, J.A., Allen, K.N. & Hermanutz R.O. (1983). Effects of diazinon on macroinvertebrates and insect emergence in outdoor experimental channels. *Aquatic Toxicology*, 4:283-301. doi:10.1016/0166-445x(83)90023-1

Bailey, H.C., Deanovic, L., Reyes, E., Kimball, T., Larson, K., Cortright, K., Connor, V. & Hinton, D.E. (2000). Diazinon and chlorpyrifos in urban waterways in northern California, USA. *Environmental Toxicology & Chemistry*, 19:82-87. doi:10/1002/etc.5620190109

Banaee, M., Sureda, A., Mirvaghefi, A.R. & Ahmadi, K. (2011). Effects of diazinon on biochemical parameters of blood in rainbow trout (Oncorhynchus mykiss). *Pesticide Biochemistry and Physiology*, 99:1-6. doi: 10.1016/pestbp.2010.09.001

Benzi, I.F.F., Chung, W.Y. & Strain, J.J. (1999). Antioxidant (reducing) efficiency of ascorbate in plasma is not affected by concentration. *The Journal of Nutritional Biochemistry*,10:146-150. doi: 10.1016/s0955-2863(98)00084-9

Bigard, A.X. (2001). Lesions musculaires induites par l'exercice et surentrainement. *Science & Sports*,16:204-215. doi: 10.1016/s0765-1597(00)00037-x

Buettner, G.R. & Moseley, P.L. (1993). EPR spin trapping of free radicals produced by bleomycin and ascorbate. *Free radical research communications*, 19: S89-S93. doi:10.3109/10715769309056s89

Castillo, C., Hernandez, J., Valverde, I., Pereira, V., Sotillo, J., Alonso, M.L. & Benedito, J.L. (2006). Plasma malonaldehyde (MDA) and total antioxidant status (TAS) during lactation in dairy cows. *Research in Veterinary Science*, 80:133-139. doi: 10.1016/j.rvsc.2005.06.003

Das, K.C., Lewis-Molock, Y. & White, C.W. (1997). Elevation of manganese superoxide dismutase gene expression by thioredoxin. *American Journal of Respiratory Cell and Molecular Biology*, 17:713-726. doi: 10.1165.ajrcmb.17.6.2809

Deshpande, S.S., Deshpande, U.S. & Salunkhe, D.K. (1996). Nutritional and health aspects of food antioxidants. In: Madhavi DL, Deshpande SS, Salunkhe DK (EDs.) Food antioxidants, technological, toxicological, and health perspectives, pp 361-470. New York: Marcel Dekker.

De Zwart, L., Meerman, J., Commandeur, J. & Vermeulen, N. (1999). Biomarkers of free radical damage applications in experimental animals and in humans. *Free Radical Biology and Medicine*, 26: 202-226. doi: 10.1016/s0891-5849(98)00196-8

Doba, T., Burton, G.W. & Ingold, K.U. (1985). Antioxidant and co-antioxidant activity of vitamin C. The effect of vitamin C, either alone or in the presence of vitamin E or a water-soluble vitamin E analogue, upon the peroxidation of aqueous multilamellar phospholipid liposomes. *Biochimica et Biophysica Acta*, 9: 298-303. doi: 10.1016/0005-2760(85)90285-1

Durmaz, H., Sevgiler, Y., Uner, N. (2006). Tissue-specific antioxidative and neurotoxic responses to diazinon in *Oreochromis niloticus*. *Pesticide Biochemistry and Physiology*, 3:215-226. doi: 1016/j.pestbp.2005.07.004

Eisler, R. (1998). Diazinon hazards to fish, wildlife and invertebrates: a synoptic review, Biological report 85, U.S. Fish and Wildlife service, Washington, DC.

El-Gendy, K.S., Aly, N.M., Mahmoud, F.H., Kenawy, A. & El-Sebae, A.H. (2010). The role of vitamin C as antioxidant in protection of oxidative stress induced by imidacloprid. *Food and Chemical Toxicology*, 48:215-221. doi: 1016/j.fct.2009.10.003

El-Gharieb, M.A., El-Masry, T.A., Emara, A.M., Mohammed, A. & Hashem, M.A. (2010). Potential hepatoprotective effects of vitamin E and Nigella sativa oil on hepatotoxicity induced by chronic exposure to malathion in human and male albino rats. *Toxicological & Environmental Chemistry*, 92:391-407. doi:10.1080/02772240902955719

Fabiana, G.F., Pilarski EM, Onaka, F.R.D, Moraes, M.L. & Martins, M.L. (2007). Hematology of Piaractus mesopotamicus fed diets supplemented with vitamins C and E, challenged by Aeromonas hydrophila. *Aquaculture*, 271:39-46. doi: 10.1016/j.aquaculture.2007.06.021

Frei, B., Stocker, R. & Ames, B.N. (1988). Antioxidant defenses and lipid peroxidation in human blood plasma. *Proceedings of the National Academy of Sciences United States of America*, 85: 9748-9752. doi: 10.1073/pnas.85.24.9748

Fujii Y. & Asaka, S. (1982). Metabolism of diazinon and diazoxon in fish liver preparations. *The Bulletin of Environmental Contamination and Toxicology*, 29:455-460. doi: 10.1007/BF01605611

Geetha, B.V., Navasakthi, R. & Padmini, E. (2010). Investigation of antioxidant capacity and phytochemical composition of Sun Chlorella- an in vitro study. *Aquaculture Research & Development*, 1:104. doi: 10.4172/2155-9546.1000104

Gokalp, O., Buyukvanli, B., Cicek, E., Ozer, M.K., Koyu, A., Altuntas, I. & Koylu, H. (2005). The effects of diazinon on pancreatic damage and ameliorating role of vitamins E and C. *Pesticide Biochemistry and Physiology*, 81: 123-128. doi: 10.1016/j.pestbp.2004.11.001

Goth, L. (1991). A simple method for determination of serum catalase activity and revision of reference range. *Clinica Chimica Acta*, 196:143-152. doi: 10.1016/0009-8981(91)90067-M

Halliwell, B. & Gutteridge, J.M.C. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal*, J.219:1-14. doi: 10.1042/bj20140006

Hazarika, A., Sarkar, S.N, Hajare, S., Kataria, M. & Malik, J.K. (2003). Influence of malathion pretreatment on the toxicity of anilofos in male rats: a biochemical interaction study. Toxicology 185:1-8. doi: 10.1016/s0300-483x(02)00574-7

Isik, I. & Celik, I. (2008). Acute effects of methyl parathion and diazinon as inducers for oxidative stress on certain biomarkers in various tissues of rainbow trout (*Oncorhynchus mykiss*). *Pesticide Biochemistry and Physiology*, 92:38-42. doi: 10.1016/j.pestbp.2008.06.001

Jakeman, P. & Maxwell, S. (1993). Effect of antioxidant vitamin supplementation on muscle function after eccentric exercise. *European Journal of Applied Physiology and Occupational Physiology*, 67: 426-430. doi: 10.1007/bf00376459

Jarvinen, A.W. & Tanner, D.K. (1982). Toxicity of selected controlled release and corresponding unformulated technical grade pesticides to the fathead minnow (*Pimephales promelas*). *Environmental Pollution*, 27: 179-195. doi: 10.1016/01471(82)90024-1

Jialal, I., Vega, G.I. & Grundy, S.M. (1990). Physiological levels of ascorbate inhibit the active modification of low density lipoprotein. *Atherosclerosis*, 82: 185-191. doi: 10.1016/0021-9150(90)90039-L

Kadry, S.M., Marzouk, M.S, Amer, A.F., Hanna, M.I., Azmy, A.H. & Hamed, H.S. (2012). Vitamin E as antioxidant in female african catfish (*Clarias gariepinus*) exposed to chronic toxicity of atrazine. *Egyptian Journal of Aquatic Biology & Fisheries*,16:83-98. doi: 10.1016/s0013-9351 (02)0006-2

Karaoz, E, Gultekin, F., Akdogan, M., Oncu, M. & Gokcimen, A. (2002). Protective role of melatonin and a combination of vitamin C and E on lung toxicity induced by chlorpyrifos-ethyl in rats. *Experimental and Toxicologic Pathology*, 54: 97-108. doi: 10.1078/0940-2993-00236

Keizer, J., D'Agostino, G., Nagel, R., Volpe, T., Gnemi, P. & Vittozzi, L. (1995). Enzymological differences of AChE and diazinon hepatic metabolism: correlation of in vitro data with the selective toxicity of diazinon to fish species. *Science of the Total Environment*, 171: 213-220. doi: 10.1016/0049-9697(95)04687-0

Kohen, R., Vellaichamy, E. & Hrbac, J. (2000). Quantification of the overall reactive oxygen species scavenging capacity of biolog-ical fluids and tissues. Free Radical Biology and Medicine, 28, 871-879. doi:10.1016/s0891-5849(00)00191-X

Koprucu, S.S., Koprucu, K., Ural, M.S, Ispir, U. & Pala, M. (2006). Acute toxicity of organophosphorous pesticide diazinon and its effects on behavior and some hematological parameters of fingerling European catfish (*Silurus glanis* L.). *Pesticide Biochemistry and Physiology*, 86: 99-105. doi: 10.1016/j.pestbp.2006.02.001

Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S. & Cosic, V. (2001). Method for the measurement of antioxidant activity in human fluids. *Journal of Clinical Pathology*, 54:356-361. doi: 10.1136/jcp.54.5.356

Ledwozyw, A., Michalak, J., Stepien, A.K. & Adziolka, A. (1986). The relationship between plasma triglycerides, total lipids and lipid peroxidation products during huma atherosclerosis. *Clinica Chimica Acta*, 155: 275-284. doi: 10.1016/0009-8981(86)90247-0

Lu, C. & Liu, Y. (2002). Interaction of lipoic acid radical cations with vitamins C and E analogue and hydroxycinnamic acid derivatives. *Archives of Biochemistry and Biophysics*, 406: 78-84. doi: 10.1016/s0003-9861(02)00411-3

Mahfouz, R., Sharma, R., Sharma, D., Sabanegh, E. & Agarwal, A. (2009). Diagnostic value of the total antioxidant capacity (TAC) in human seminal plasma. *Fertility and Sterility*, 91:805-811. doi: 10.1016/j.fertnstert.2008.01.022

Marklund, S. & Marklund, G. (1974). Involvement of the superoxyde anion radical in the auto oxidation of pyrogallol and a convenient assay for superoxyde dismutase. *European Journal of Biochemistry*, 47:469-474. doi: 10.1111/j.1432-1033.1974.tb03714.x

McCord, J.M. & Fridovich, I. (1969). Superoxide dismutase: an enzymic function for erythrocuprein. *Journal of Biological Chemistry*, 244: 6049-6055. doi: 10.1074/jbc.244.22.6049

Miller, N.J., Rice-Evans, C., Davies, M.J., Gopinathan, V. & Milner, A. (1993). A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science*, 84:407-412. doi: 10.1042/cs0840407

Monterio, D.A., Almeida, J.A.D., Rantin, F.T. & Kalinin, A.L. (2006). Oxidative stress biomarkers in the freshwater characid fish, Brycon cephalus, exposed to organophosphorus insecticide Folisuper 600 (methyl parathion). *Comparative Biochemistry and Physiology*, 141:143-149. doi: 10/1016 j.cbpc.2006.01.004

Monterio, D.A., Rantin, F.T. & Kalinin, A.L. (2009). The effects of selenium on oxidative stress biomarkers in the freshwater characid fish matrinxa, brycon cephalus exposed to organophosphate insecticide folisuper 600 BR (methyl parathion). *Comparative Biochemistry and Physiology*, 149:40-49. doi: 10.1016/j.cbpc.2008.6.012

Moreau, R., Dabrowski, K., Czesny, S., Chila, F. (1999). Vitamin C-vitamin E interaction in juvenile lake sturgeon (*Acipenser fulvescens*), a fish able synthesize ascorbic acid. *Journal of Applied Ichthyology*, 15: 205-257. doi: 10.1111/j.1439-0426-1999/tb00245.x

Naqvi, S.M, Vaishnavi, C. (1993). Bioaccumulative potential and toxicity of endosulfan insecticide to non-target animals. *Comparative Biochemistry and Physiology*, 105:347-361. doi: 10/1016/0300-9629(93)90219-T

Oruc, E.O. (2010). Oxidative stress, steroid hormone concentrations and acetylcholinesterase activity in Oreochromis niloticus exposed to chlorpyrifos. *Pesticide Biochemistry and Physiology*, 96: 160-166. doi: 10.1016/j.pestbp.2009.11.005

Prior, R.L. & Cao, G. (1999). In vivo total antioxidant capacity: comparison of different analytical methods. *Free Radical Biology and Medicine*, 27:1173-1181. doi: 10.1016/s0891(99)00203-8

Schlenk, D. (2005). Pesticide biotransformation in fish. In: Mommsen TP, Moon TW (Eds) Biochemistry and molecular biology of fishes, 6th edn, pp 171-190. Amsterdam: Elsevier.

Singh, M., Sandhir, R. & Kiran, R. (2011). Effects on antioxidant status of liver following atrazine exposure and its attenuation by vitamin E. *Experimental and Toxicologic Pathology*, 63: 269-276. doi: 10.1016/j.ept.2010.01.005

Turkez, H. & Togar, B. (2011). Olive (Olea europaea L.) leaf extract counteracts genotoxicity and oxidative stress of permethrin in human lymphocytes. The Journal of Toxicological Sciences, 36: 531-537. doi: 10.2131/jts.36.531

Tsao, C.S. (1997). An overview of ascorbic acid chemistry and biochemistry In: Packer L, Fuchs J (eds) Vitamin C in health and disease, pp 25-58. New York: Marcel Dekker.

Ural, M.S. (2013). Chlorpyrifos-induced changes in oxidant/antioxidant status and haematological parameters of *Cyprinus carpio*: Ameliorative effect of lycopene. *Chemosphere*, 90:2059-2064. doi:10.1016/j. Chemosphere.2012.12.006

Uner, N., Oruc, E.O., Sevgiler, Y., Sahin, N., Durmaz, H. & Usta, D. (2006). Effects of diazinon on acetylcholinesterase activity and lipid peroxidation in the brain of *Oreochromis niloticus*. *Environmental Toxicology and Pharmacology*, 21:241-245. doi: 10.1016/j.etap.2005.08.007

Valavanidis, A., Vlahogianni, T., Dassenakis, M. & Scoullos, M. (2006). Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicology and Environmental Safety*, 64:178-189. doi: 10.1016/j.ecoenv/2005.03.013

Vale, J.A. (1998). Toxicokinetic and toxicodynamic aspects of organophosphorus OP insecticide poisoning. *Toxicology Letters*, 64:102-103. doi: 10.1016/s0378-4274(98)80136-7

Verlhac, V. & Gabaudan, J. (1997). The effect of vitamin C on Fish Health. Roche Technical Buletin, Hoffmann-La Roche Ltd, Basel, Switzerland, 30 pp.

Verlhac, V., Obach, A., Gabaudan, J., Schüep, W. & Hole, R. (1998). Immunomodulation by dietary vitamin C and glucan in rainbow trout (*Oncorhynchus mykiss*). *Fish and Shellfish Immunology*, 8:409-424. doi: 10.1006/fsim/1998.0148

Virtue, W.A. & Clayton, J.W. (1997). Sheep dip chemicals and water pollution. *Science of the Total Environment*, 194-195:207-217. doi: 10.1016/s0048-9697(96)05365-x

Winston, G.W, Regoli, F., Dugas, A.J, Fong, J.H. & Blanchard, K.A. (1998). A rapid gas chromatographic assay for determining oxyradical scavenging capacity of antioxidants and biological fluids. *Free Radical Biology and Medicine*, 24:480-493. doi: 10.1016/s0891-5849(97)00277-3

Xing, H., Li, S., Wong, Z., Gao, X., Xu, S. & Wang, X.M. (2012). Oxidative stress response and histopathological changes due to atrazine and chlorpyrifos exposure in common carp. *Pesticide Biochemistry & Physiology*,103: 74-80. doi: 10.1016/j.pestbp.2013.03.007

Zama, D., Meraihi, Z., Tebibel, S., Benayssa, W., Benayach, F., Benayache, S. & Vlitinck, A.J. (2007). Chlorpyrifos-induced oxidative stress and tissue damage in the liver, kidney, brain and fetus in pregnant rats: the protective role of the butanolic extract of *Paronychie argentea* L. *Indian Journal of Pharmacology*, 39:145-150. doi: 10.4103/0253-7613.33434