INTERNATİONAL JOURNAL OF AGRİCULTURE & BİOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18–0378/2019/21–3–583–589
DOI: 10.17957/IJAB/15.0932
http://www.fspublishers.org

Full Length Article

Effects of Different Concentrations of Diazinon on 8-Hydroxy-2-Deoxyguanosine and Histopathology, Antioxidant Enzyme, Acetylcholinesterase Activity and Plasma Metabolites in Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract

The aim of this study was to determine the all of effects on the liver of diazinon. Here, we were to investigation the effects of different concentrations of diazinon on 8-hydroxy-2-deoxyguanosine (8-OHdG) and histopathology, antioxidant enzymes, the plasma metabolites of rainbow trout (*Onchorhynchus mykiss*). During the six days, 15 rainbow trout were used for each group and then were subjected to two different concentration (0.15 mg/L and 0.30 mg/L) of Diazinon, respectively (LC_{50}=1.65 mg/L). A 0.15 mg/L level of diazinon did not effect aspartate aminotransferase (AST) and alanine aminotransferase (ALT). But, 0.30 mg/L concentration caused a significant increase in both parameters. 0.15 mg/L of diazinon caused a significant decrease in the lactate dehydrogenase (LDH) level. 0.15 and 0.30 mg/L of diazinon significantly increased antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR)). But, the total antioxidant capacity (TA) capacity, acetylcholinesterase activity (AChE), albumin (AL) and total protein (TP) levels significantly decreased at both concentration levels when compared to the control group ($P < 0.05$). There were significant changes in 8-OHdG levels and histopathological damage in liver tissues. As a results; diazinon caused changes in the histopathological and 8-OHdG and antioxidant enzymes as well as plasma AChE activity and metabolites of rainbow trout.

Keywords: Rainbow trout; Diazinon; Antioxidant enzymes; Histopathology; 8-OHdG; Plasma metabolites

Introduction

Contamination of chemical substances such as peticides and insects used in agriculture and industry into water environment causes toxic effects on aquatic organisms (Ibrahim and Harabawy, 2014). Over the centuries, pesticides like pyrethroids, organophosphate, organochlorine, carbamates, herbicides and organophosphorus compounds are used to control unwanted insects and diseases, as well as to increase food production in agriculture (Amin and Hashem, 2012). Pyrethroids like permethrin, cypermethrin, cyfluthrin, and deltamethrin have ability to be photostable, low toxicity and easy fragmentation at low concentration in animals including birds and mammals (Bradbury and Coats, 1989; Amin and Hashem, 2012). However, organophosphorus (OPs) pesticides such as diazinon, malathion, and chlorpyrifos can high toxicity on vertebrate and invertebrate living things and the rapid degradation of these pesticides in the environment can create a potential risk for water, fish and ecosystems (Gaffar *et al.*, 2015; Ayanda *et al.*, 2018).

Diazinon used intensively to increase agricultural production is a moderately persistent organophosphate insecticide. Diazinon is extrication an active oxon metabolite, diazoxon which inhibits acetylcholinesterase (AChE) activity in organisms and AChE depends on the inactivation of the neurotransmitter acetylcholine as well as cholinesterase activity in the nervous system of living organisms exposed to pollutants (Banaee *et al.*, 2011). AChE inactivation, which leads to synaptic blockage and disruption of signal transduction, occurs as a result of accumulation of neurotransmitter acetylcholine in the area of cholinergic synapse (Cong *et al.*, 2008). Nutritional, stress and reproductive conditions can affect AChE levels (Banaee *et al.*, 2011). Therefore, inhibition of AChE is considered as biomarker for living organisms exposed to organophosphorous pesticides. It is known that there are some mechanism in determining the harmful effects of

To cite this paper: Karataş, T., S. Yildirim and H. Arslan, 2019. Effects of different concentrations of diazinon on 8-hydroxy-2-deoxyguanosine and histopathology, antioxidant enzyme, acetylcholinesterase activity and plasma metabolites in rainbow trout (*Oncorhynchus mykiss*). *Intl. J. Agric. Biol*., 21: 583–589

pesticide poisoning in living organisms; there is a close relationship between oxidative stress and pesticide toxicity due to excessive free radicals formation. Pesticide toxicity has been determined to be the result of accumulation of reactive oxygen species (ROS), during the pesticide detoxification (Banaee *et al.,* 2013). The defense mechanisms of fish are important in terms of the prevention of harmful effects of ROS (Tejada *et al.,* 2007). In fish, the first cellular defense against ROS is carried out by antioxidant enzymes such as (SOD), (CAT), (GPx) and (GR) (Ekinci Akdemir *et al.,* 2016, 2017). Tissue and cell damage in fish exposed to organophosphorus compounds such as diazinon occur as a consequence of cell death. If the fish's defense system cannot prevent the excessive increase of ROS, it causes oxidative damage to tissues and cells (Uner *et al.,* 2006; Isik and Celik, 2008). Banaee *et al.* (2013) reported that oxidative stress caused by various xenobiotics has an important role in fish toxicity, including insecticides, herbicides, fungicides, heavy metals and pharmaceutical compounds. Monitoring of changes in antioxidant activity in fish toxicology may be helpful in determining of effects in hepatocytes of fish and the oxidative stress level (Sureda *et al.,* 2006; Li *et al.,* 2010).

Histopathological changes in fish tissues or organs are considered to be one of the biological indicators used to assess the general health status of fish as well as assessing the adverse effects of pollution, malnutrition, stress and environmental changes. These effects are important in determining the changes that occur on specific organs such as the gills, kidneys and liver which are responsible for the vital functions of fish (Gernhofer *et al.,* 2001; Marina *et al.,* 2007). Moreover, the identification of changes occurring in these organs is easier than it is functionally determined (Fanta *et al.,* 2003). It has been determined by studies that fish tissues are particularly sensitive to the evaluation of histological investigations of the liver (Amin and Hashem, 2012).

We think that the diazinon used for agricultural purposes will cause significant damage to the liver of the rainbow trout. This study was designed to assess the effect of two different concentrations of diazinon on 8-hydroxy-2-deoxyguanosine (8-OHdG) and histopatological investigations of rainbow trout (*Onchorhynchus mykiss*). In addition, we aimed to investigate the effects of diazinon on plasma metabolites such as AST, ALT, LDH, ALP, TP, AL and AChE activity and antioxidant enzymes (SOD, CAT, GPx and GR).

Materials and Methods

Experimental Animals

Rainbow trout (*Onchorhynchus mykiss*) were obtained from the Fisheries Faculty at Atatürk University in Erzurum province. 90 rainbow trout were used in this study and the average weight of the fish was 90±5 g. Fish divided into 3 groups including control and fifteen rainbow trout were used for each group (two repetition). The fish were fed at the ratio of 2% of body weight with commercial trout feed. This study was carried out at Department of Fisheries Application and Research Center Toxicology Experiment. The trial process was as follows: temperature of water 10.5 ± 0.5°C, dissolved oxygen 9.5 mg/L and pH 7.6. Water was given to the tanks with a flow of about 0.5 L/min to fish (Karataş *et al.,* 2014).

Acute Toxicity

Diazinon was obtained from Sigma-Aldrich (Germany). Diazinon was dissolved in acetone used as stock solution 40% of $C_{12}H_{21}N_2O_3PS$ (CAS Number 333-41-5 ≥ 60% purity). Fish were subjected to diazinon concentrations of 0.0 mg/L (control group), 0.15 mg/L (approximately 13%) and 0.30 mg/L (approximately 26%) for six days. LC50 value of diazinon toxicity testing for 4 days (96 h) was determined as 0.9–1.65 ppm by the Environmental Protection Agency of America (USEPA 2005) and 1.65 ppm by Karataş and Albayrak (2018).

Determination of Blood Plasma Levels

Blood was drawn from the caudal veins of the fish. Subsequently, blood samples were transferred to non-anticoagulant tubes. Levels of plasma AST, ALT, LDH, ALP, TP, AL were made according to the method defined by Banaee *et al.* (2011). Shortly, levels of total protein (TP) (Catalogue no: 3183734190) and albumin (AL) (Catalogue no: 3183688122) in the plasma were measured by procedures in biochemistry laboratories using biochemical kits (Roche Diagnostics, Mannheim, Almanya). AST, ALT and LDH were determined according to the change in absorbance values at 340 nm. ALP level was determined at 405 nm as a result of the conversion of p-nitrophenol phosphate to nitrophenol in alkaline buffer.

Determination of Plasma AChE Enzyme Activity

AChE activity was measured by mixing a sufficient amount of sample to 0.1 *M* phosphate, 0.015 *M* acetylcholine iodide and 0.01 *M* dithiobis nitrobenzoic acid mixture (Banaee *et al.,* 2011). Plasma AChE activity was measured at 405 nm for 3 min by using spectrophotometer (UV1800, Shimadzu, Japan) in the laboratory of Central Research and Application Center, Agri Ibrahim Cecen University.

Determination of Total Antioxidant Capacity

Samples taken from the liver were homogenized in a cold phosphate buffer with a plastic homogenizer for 2 min and then centrifuged at 15000 g for 14 min at 4°C. Total antioxidant capacity was measured by using the supernatants.

Determination of Antioxidant Enzymes

SOD (Item no: 706002), GPx (Item no:703102) and GR (Item no:703202) activities were measured by means of kits purchased from a commercial operation (Cayman, USA) and measured at 25°C. The antioxidante enzymes were made according to the method described by Banaee *et al.* (2013). Briefly, the GR activity was measured depending on the reduction of oxidized glutathione (GSSG) in the presence of NADPH and was determined by absorbance reduction at 340 nm. The cumene hydroperoxide was used as a substrate for the determination of GP_X activity. The presence of both glutathione reductase and NADPH, GSSG was directly turned into a decreased form by an accompanying oxidation of NADPH to NADP. SOD activity was observed at 505 nm on the basis of xanthine and xanthine oxidase to produce superoxide radicals reacting with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. SOD activity is measured based on the inhibition rate of this reaction. A unit SOD is a unit that shows a 50% inhibition of the level of decrease in INT according to the experimental conditions. CAT (Item no:707002) (Cayman, USA) activity was measured by determining the absorbance reduction at 240 nm according to the method described by Aebi (1984). CAT activity was measured with the help of the extinction coefficient for H_2O_2 (cm^{-1} M^{-1} a = 40). Bovine serum albumin by the Biuret method was used as standard for antioxidant enzymes and these enzymes were expressed as mg protein in liver tissue.

Histopathological Examination

Liver tissues cut from fish were kept in 10% formalin solution until analysis. They were routinely followed by an automated tissue monitoring device (Shormo citadel 2000, Thermo). Then, 4 μm thick sections were cut from these tissues which were blocked with paraffin. All of the sections were stained with Hematoxylin-Eosin (HE). Sections showed as no $^{(-)}$, mild $^{(+)}$, moderate $^{(++)}$ and severe $^{(+++)}$ according to the level of damage in the liver on light microscope (Bar:20 μm) (Birincioğlu *et al.,* 2011).

Immunohistochemical Examination

All sections taken for adherent (poly-L-Lysine) slides for immunoperoxidase assay were deparaffinized and dehydrated by passing through xylol and alcohol series. Then, it was washed in distilled water for 5 min. The sections were exposed to heat in microwave oven 4 times for 5 min in antigen retrieval (citrate buffer, pH 6.1) solution to prevent masking of the antigen in the nucleus. Then, they allowed cooling at room temperature for 30 min. At the end of this work, it was washed with distilled water, dried sections drawn with special glass pen. Endogenous peroxidase was inactivated by washing with phosphate buffer solution (PBS, pH 7.2) for 5 min and by holding in 3% H_2O_2 for 10 min. After washing in PBS for 5–10 min, protein block, compatible with all primer and secondary antibody, was allowed to incubate for 5 min to prevent nonspecific background staining. At the end of the incubation, the primary antibody (OHdG and PBS in the control group) was instilled without washing after the excess of the block solution remaining on the tissue sections was poured. The primer antibody was incubated one night at +4°C. After washing for 5 min with PBS 2 times, the biotinylated secondary antibody was incubated at room temperature for 10–30 min. Sections washed again with PBS, then were held with streptavidin-peroxidase for 10–30 min and washed again with PBS. After washing, 3-3' Diaminobenzidine (DAB) was added as chromogen and was held for 5–10 min according to the receipt of the chromogen. For floor coating, Mayer's hematoxylin was kept for 1–2 min and then washed in tap water. After this process, the slides were incubated for 3 min in 80% ethanol, 96% ethanol, 100% ethanol and xylenol (respectively) and the lamellae were closed. Light microscope (Leica DM 1000) were examined. The sections were evaluated as no $^{(-)}$, mild $^{(+)}$, moderate $^{(++)}$ and severe $^{(+++)}$ according to their immunity positivity (Kaygusuzoglu *et al.,* 2018).

Statistical Analysis

The Kruskal-Wallis for nonparametric tests test and the Mann-Whitney U test for the comparison of two groups for the analysis of differences between groups of semi-quantitatively obtained histopathologic data (SPSS 20.0 for Windows) were used. Data obtained metabolites and antioxidant enzymes were analyzed by (SPSS 20.0 for Windows) using one-way analyses of variance (ANOVA). Distinctions between groups were analyzed by using LSD tests. The significance level was described as $P<0.05$ (Abdelkader *et al.,* 2012).

Results

Alterations in total protein, albumin and plasma AChE levels (Table 1) of fish exposed to different concentrations of diazinon were significantly lower than the control group ($P<0.05$). But, the increase of dose did not affect total protein, albumin and plasma AChE levels ($P>0.05$). The changes in enzyme activities such as AST, ALT, LDH and ALP are given in Table 1. According to these results, while plasma AST and ALT levels at dose 0.15 mg/L of diazinon does not change, 0.30 mg/L of diazinon caused a significant increase in AST and ALT levels when compared to control group ($P<0.05$). Plasma LDH level of fish exposed to 0.15 mg/L were significantly lower than that control group and dose 0.30 mg/L of diazinon ($P<0.05$). There was no significant difference between plasma ALP levels of fish exposed to diazinon and control group. While 0.15 and 0.30 mg/L of diazinon caused a significant decrease in TA

Table 1: Changes in the plazma metabolites and plasma AChE activity of rainbow trout exposed to different concentration of diazinon

Metabolites	Control	Diazinon 0.15 mg/L	Diazinon 0.30 mg/L
ALT(IU/L)	15.6±0.47 [a]	12.6±0.64 [a]	20.1±0.13 [b]
AST(IU/L)	394.4±49 [a]	369.2±63 [a]	783.1±116 [b]
LDH (IU/L)	180.3±19.3 [a]	155.6±14.2 [b]	186.3±19.7 [a]
ALP (IU/L)	65.4±5.7 [a]	68.1±7.4 [a]	69.4±7.5 [a]
TP(mg/dL)	4.87±0.14 [a]	3.95±0.98 [b]	4.02±0.21 [b]
AL (mg/dL)	2.90±0.19 [a]	2.09±0.23 [b]	1.95±0.16 [b]
AChE (U/I)	7600.00±241 [a]	3700.00±128 [b]	3400.00±136 [b]

The results were given as mean and standard deviation. Different letters indicate differences between groups

Table 2: Alterations in the activities of TA and SOD, CAT, GPx and GR in liver tissues of rainbow trout exposed to different concentrations of diazinon

Antioxidant enzymes	Control	Diazinon 0.15 mg/L	Diazinon 0.30 mg/L
TA (µmol/mg)	5.8±0.35 [a]	3.7±0.63 [b]	3.2±0.24 [b]
SOD (U/mg)	0.89±0.06 [a]	1.32±0.18 [b]	1.65±0.29 [b]
CAT (K/mg)	0.41±0.03 [a]	0.50±0.04 [b]	0.53±0.03 [b]
GPx (U/mg)	0.15±0.02 [a]	0.19±0.01 [b]	0.21±0.04 [b]
GR (mU/mg)	13.6±1.96 [a]	29.4±4.8 [b]	28.7±3.2 [b]

The results were given as mean and standard deviation. Different letters indicate differences between groups

Table 3: Histopathological and immunohistochemical evaluation of liver tissues of rainbow trout exposed to different concentrations of diazinon

Treatment	Control group	Diazinon (0.15 mg/L dose)	Diazinon (0.30 mg/L dose)
Steatosis	-	++	+++
Degeneration	-	+	+++
Necrosis	-	+	++
Hyperemia	-	+++	+++
8-Hydroxy-2-Deoxyguanosine	-	++	+++

capacity compared to control group (P<0.05), it caused a significant increase in SOD, CAT, GPx and GR levels (P<0.05) (Table 2). The increase of dose did not affect TA capacity and SOD, CAT, GPx and GR levels.

The liver tissues of fish were compared histopathologically with the control group, while dose 0.15 mg/L of diazinon caused steatosis, degeneration, necrosis and hyperemia, dose 0.30 mg/L of diazinon was detected severe fatty vacuoles and hydropic degeneration, coagulation necrosis and hyperemia in vessels (Table 3 and Fig. 1). The liver tissues of fish subjected to different concentrations of diazinon are evaluated immunohistochemically. However, 8-OHdG expressions at dose 0.15 mg/L of diazinon was moderate level in hepatocytes, 8-Ohdg expressions at dose 0.30 mg/L of diazinon was found to be severe level in hepatocytes (Table 3; Fig. 2).

Discussion

The inhibition of AChE activites caused increase in acetylcholine levels directly affects the feeding, swimming

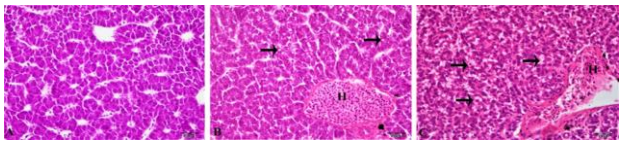

Fig. 1: (A) Control group; Normal histological structure of liver, (B) Diazinon (0.15 mg/L dose) group; necrosis, hydropic degeneration, fatty vacuoles (arrows) in the liver hepatocytes (C) Diazinon (0.30 mg/L dose) group; severe fatty vacuoles (arrows) and hydropic degeneration in the liver hepatocytes, coagulation necrosis in hepatocytes and hyperemia in vessels (arrowhead). Scale 20 μm

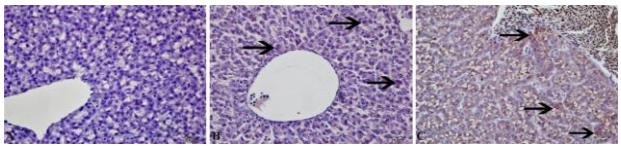

Fig. 2: (A) Control group, liver, 8 OHdG negative, IP, Bar: 20 μm. (B) Diazinon (0.15 mg/L dose) group, 8 OHdG expression at the moderate level in hepatocytes of liver, IP, Bar:20 μm. (C) Diazinon (0.30 mg/L dose) group, 8 OHdG expression at the severe level in hepatocytes of liver, IP, Bar:20 μm

activity, avoid of predators (Banaee *et al.,* 2011). Therefore, the accumulation of acetylcholine has an important influence on the behavior and physiology of fish. In this study, there were a significant (P<0.05) decrease in plasma AChE activities 0.15 and 0.30 mg/L of diazinon according to control group (Table 1). But, insignificant change in plasma AChE activities between both 0.15 and 0.30 mg/L diazinon concentrations were detected (Table 1). Seguchi and Asaka (1981) reported that plasma AChE activity of the sub-lethal concentration of diazinon rapidly increased and reached the maximum level of plasma AChE activity of the fish exposed to diazinon for 3 days. Banaee *et al.* (2011) reported that plasma AChE activity of diazinon decreased. This is probably due to diazinon metabolizes as an active oxone derivative that inhibits AChE activity. Diazoxon levels were found to be higher in fish exposed to diazinon (Fujii and Asaka, 1982). Our results show that diazoxone production in different doses of diazinon may be at an equal level.

Total protein (TP), which has an important role in physiology of living organisms, is very important for cells and tissues (Banaee *et al.,* 2016). Our results show that the TP levels in both concentrations of diazinon were significantly (p <0.05) decreased level (Table 1). This reduction in plasma protein is probably due to changes in metabolism of protein or free amino acids or liver synthesis. Furthermore, the decrease in total protein may be due to severe liver damage as well as tissue destruction and hepatocyte apoptosis. Albumin, which has many functions in blood plasma, is known as single chain protein (Banaee *et al.,* 2016). In this study, significant decreases were observed in albumin levels of fish exposed to diazinon according to

the control group. The reduced total protein level may be the reason for the decrease in albumin levels. Decrease in albumin levels may be due to the effect of diazinon on the biosynthesis of albumin in the liver, impaired liver function (Banaee, 2013). It has been determined that TP and AL levels may also be reduced in fish exposed to various pollutants, pesticides including liver damage (Velisek *et al.*, 2008; Banaee *et al.*, 2011).

The liver is responsible for important functions of basic metabolism and it is the main organ of the accumulation, biotransformation and elimination of pollutants in fish including degradation and bioinactivation of pesticides (Muthukumarave *et al.*, 2013; Topal *et al.*, 2015). For this reason, the degree of damage to the tissues and organs of the living organism is determined by the increase in AST and ALT levels (Srivastava *et al.*, 2004; Rao, 2006). In addition, AST and ALT involved in the metabolism of amino acids are known as liver enzymes and they are highly sensitive in hepatotoxicity and histopathologic changes (Stoyanova *et al.*, 2016). In present study, 0.15 mg/L and 30 mg/L of diazinon caused a significant (P<0.05) increase in ALT and AST levels compared to control group (Table 1). This may possibly be related to the release into the plasma by breaking down hepatocytes of diazinon (Yousef *et al.*, 2006). It was determined that there is a positive correlation between cell damage and enzymes such as AST and ALT (El-Demerdash *et al.*, 2004). An increase in AST and ALT levels may be related to an increase in levels of intracellular of reactive oxygen species (ROS). In other words, increased permeability of the liver cell membrane due to lipid peroxidation may lead to increase of AST and ALT levels. Similar results were observed in cammon carp (Banaee *et al.*, 2008) and rainbow trout (Banaee *et al.*, 2011) and in Channa punctatus (Agrahari *et al.*, 2007).

Lactate dehydrogenase (LDH) is an important enzyme in determination of tissue diseases and tissue damage (El-Demerdash *et al.*, 2004; Hasnain, 2005). In present study, 0.30 mg/L of diazinon did not affect plasma LDH level. But, 0.15 mg/L of diazinon caused a significant (P<0.05) decrease in this enzyme compared to control group and 0.30 mg/L of diazinon (Table 1). Probably, this may be associated with the inhibition of LDH. Hernández *et al.* (2006) found that some pesticides inhibit LDH activity. Rao (2006) and Agrahari *et al.* (2007) reported that pesticides were significantly reduced in LDH levels in different fish species such as O. mossambicus and C. punctatus.

Alkaline phosphatase (ALP), produced by cells surrounding the bile ducts in the liver, is found in different tissues of the body (Agrahari and Gopal, 2009). Although there was an increase in ALP levels in our study, this increase was not statistically significant (Table 1). The diazinon doses used in the present study were not sufficient to demonstrate ALP changes. Similar results were reported in tilapia (Rao, 2006), in sintendir (Banaee *et al.*, 2008) and in rainbow trout (Banaee *et al.*, 2011). But, increased ALP level may cause tissue damage and dysfunction due to pesticide toxicity (Sharma, 1990; Banaee, 2013).

The SOD and CAT levels of fish treated with both concentrations of diazinon were significantly higher than in the control group (Table 2). While SOD activity known as enzyme which prevents the conversion of superoxide to hydrogen peroxide and oxygen, CAT activity inhibits decomposing of hydrogen peroxide to water and oxygen (Banaee *et al.*, 2013). The increases in SOD and CAT activities in hepatocytes of fish exposed to different concentrations of diazinon may be a results of biochemical reactions which occurs against superoxide radicals and H_2O_2 production in hepatocytes. CAT activity, connected with the production of H_2O_2, can be correlated with changes occurring in the process of xenobiotic detoxification. İn this regard, Monteiro *et al.* (2006) have determined that methyl-parathion caused a significant increase in CAT activity in Brycon cephalatus, a freshwater fish. Similar changes were reported in Carassius auratus treated with 2-cholrophenol (Luo *et al.*, 2006) and in rainbow trout treated with diazinon (Banaee *et al.*, 2013).

It is known that GR activity is a very important effect in the conversion of GSSG to reduced glutathione (GSH) (Jos *et al.*, 2005; Sureda *et al.*, 2009). GR activity has quite a significant impact on diazinon detoxification since diazinon is associated with direct conjugation with GSH for excretion from the fish body (Banaee, 2013). GSH has an important role in neutralizing free radicals (Jos *et al.*, 2005; Sureda *et al.*, 2009). In our study, GR activity increased significantly (P<0.05) in both groups exposed to diazinon (Table 2).

The GPx activity in hepatocytes of both groups treated with both concentrations of diazinon increased significantly (P<0.05) compared to the control value (Table 2). The increase in GPx activity is required to reduce the amount of H_2O_2 and lipid hydroperoxide produced in hepatocytes of fish exposed to diazinon. Increased GPx level is important in terms of accelerate the conversion of GSH to GSSG. The increase in GSSG, which indicates the level of oxidation, may be due to the reduction in the TA capacity of the liver cells of fish exposed to diazinon. Increased GPx level in the liver of rainbow trout treated with diazinon for 7 days was determined (Banaee *et al.*, 2013).

The TA capacities of both groups treated with both concentrations of diazinon were significantly (P<0.05) reduced (Table 2). Over production of free radicals caused by pesticides may be the cause of reduced TA capacity (Monteiro *et al.*, 2006). Another reason for this may be related to the deterioration of enzymatic and non-enzymatic antioxidant synthesis. In other words, the decrease in TA capacity causes to be more sensitive against oxidative stress of the fish.

Different histopathologic changes in the liver tissues of rainbow trout treated with both concentrations of diazinon (0.15 and 0.30 mg/L) were observed. While 0.15 mg/L dose of diazinon led to steatosis, degeneration, necrosis and hyperemia, 0.30 mg/L dose of diazinon was

caused severe fatty vacuoles and hydropic degeneration, coagulation necrosis and hyperemia in vessels (Table 3 and Fig. 1). Excessive blood flow to the tissue due to the expansion of arterioles and increased inflammation can be the cause of hyperemia. It has been reported that the vacuoles and hydropic degeneration may be associated with deterioration of lipid transport rather than lipid biosynthesis (Colakoglu and Donmez, 2012). Salim *et al.* (2011) reported that toxic substances are effective in enlarged sinusoids in the formation of hepatocyte necrosis in tissues. Histopathologic changes in the liver tissues of brown trout exposed to different concentrations of deltamethrin (1.0 and 2.0 μg/L) were similar to the results of our previous study (Unpublished results). Cell necrosis and vacuolization were also detected in wistar albino rats exposed to heavy metals such as lead toxicity (Jarrar and Taib, 2012).

The 8-OHdG is commonly used to determine the DNA damage caused by ROS (Hintsala *et al.*, 2016). Moderate levels of 8-OHdG expression of diazinon (0.15 mg/L) were found in hepatocytes, high levels of diazinon (0.30 mg/L) were found in hepatocytes (Table 3 and Fig. 2). There was a statistically significant (P<0.05) difference between the groups. This may be a consequence of increased 8 OHdG activity in liver tissues versus oxidative stress. It has been reported that studies done on different pesticides such as imidacloprid and deltamethrin cause an increase in the 8OHdG levels of the pesticide doses (Arslan *et al.*, 2017; Özdemir *et al.*, 2018). Onouchi *et al.* (2012) determined that the production of superoxide anion (O_2) may directly lead to an increase in the level of 8-OHdG, as there is a positive correlation between 8-OHdG levels and superoxide anion. The levels of 8-OHdG in the liver tissues of brown trout treated with both concentrations of deltamethrin (1.0 and 2.0 μg/L) were similar to the results of our previous study (Unpublished results).

Conclusion

Subletal concentrations of diazinon lead to disorder in plasma metabolit, liver histopathology, 8-OHdG activity and total antioxidant capacity of rainbow trout while leading to an increase in antioxidant enzyme activities. Even subletal dose of diazinon may create toxic effects for rainbow trouts. More research is clearly needed to determine the effects of acute and chronic levels of diazinon for different time and doses.

Acknowledgements

The authors thank to Ataturk University, Faculty of Aquaculture, Inland Water Fish Research and Application Center, Ağrı Ibrahim Çeçen University, Central Research and Application Center and Dr. Naim UZUN and Dr. Ahmet Gökhan AĞGUL from Department of Biochemistry, Faculty of Pharmacy and Dr. Fazile Nur Ekinci Akdemir from Department of Nutrition and Dietetics, Health High School, Ağrı İbrahim Çeçen University, for their contributions in this study. This work was presented at the I. International Agricultural Science Congress.

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(Received 12 March 2018; Accepted 13 October 2018)