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The Effects of Carbaryl and 2,4-Dichlorophenoxyacetic Acid on Oxidative Stress Index in *Capoeta capoeta* (Guldensteadt 1773)

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Authors' Contribution
İK designed the research and wrote manuscript and helped in experiments. MY performed experiments and helped to manuscript preparation. MMK helped to analysis and transportation of samples. AK analyzed the samples and MK edited analysis.

Key words
Fish, Carbaryl, Dichlorophenoxyacetic acid, Oxidative stress.

ABSTRACT

The aim of the present study is to investigate the oxidative stress index in *Capoeta capoeta* treated with carbaryl and 2,4-dichlorophenoxyacetic acid (2,4-D). Fifty *Capoeta capoeta* fish caught in Kars Creek were equally divided into five groups and acclimatized in separate tanks for 10 days. The fish were kept in tanks as follows: Group I (control) was kept in normal water. Group II, III, IV and V were kept in separate tanks containing 0.3 mg/L carbaryl, 0.6 mg/L carbaryl, 10 mg/L 2,4-D and 20 mg/L 2,4-D, respectively. The blood and liver samples were taken from the fish for biochemical studies at the end of the study period. Oxidative stress index (OSI) in plasma were evaluated as well as AST and ALT levels in the liver. Plasma OSI and liver AST levels in the groups with carbaryl and 2,4-D were compared with the control group, and they were found to be significantly higher. As a result, it was concluded that carbaryl and 2,4-D administered under of LC_{50} value caused liver damage by increasing the oxidative stress of *Capoeta capoeta*.

INTRODUCTION

Pesticides are produced to destroy harmful organisms and consists a potential hazard for other living organisms. They are transported by rainwater, drainage, surface flows and irrigation waters after the application of pesticides to the aquatic environments, either during application or in agriculture and forest areas, and they may affect other organisms in the environment (Stegeman and Lech, 1991; Onen *et al*., 2016). Pesticides are chemical or biological products that are used to regulate plant growth, remove and destroy harmful microorganisms and damaging nutrients during the production, consumption and storage of nutrients. They are used intensely and unconsciously due to their economical production and ease of use. It has a very important place in the agricultural area due to the fact that it can guarantee the yield and quality by protecting the agricultural product from the negative effects of diseases, insects, weeds and other harms (Ferrari *et al*., 2007; Parvez and Raisuddin, 2005; Deveci and Karapehlivan, 2017).

Carbaryl (1-naphthyl N-methylcarbamine) is from a carbamate group of pesticide with broad spectrum used in the control of citrus fruits, cotton, fruit, nuts, walnuts, ornamental plants, dark trees, grasses and crops as well as in poultry, livestock and pet animals (Vioque-Fernandez *et al*., 2009). 2,4-dichlorophenoxyacetic acid (2,4-D) is a herbicide widely used in weed control in the world and our country. This herbicide, which is abundant in soil, water and wastes of factories producing herbicides, causes mutations on the genetic materials of various organisms in the environment (Kumar *et al*., 1996). Carbaryl is held by the soil at different rates according to soil type. It is also mixes to rivers with rain and irrigation water and accumulates in lakes and seas. It has been reported that 2,4-D herbicide can be destroyed by anaerobic microorganisms living in

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bottom sediments of seas and lakes (Myers *et al*., 1994).

The reactive oxygen species (ROS) arising from environmental pollutants cause structural and functional changes in the cells of aquatic organisms and may also cause the changes in their biochemical parameters (Parvez and Raisuddin, 2005; Harma *et al*., 2003). The oxygen is a potentially toxic molecule, although it is necessary for aerobic organisms to survive. In the presence of oxidative stress, it is stated that fish tissue and cell membranes can easily be oxidized because of the high content of the polyunsaturated fatty acids in fish (Mendes *et al*., 2009). Some of the oxidants and the antioxidants in the blood are acting together and consist more oxidant and antioxidant effects than the individual ones (Erel, 2004; Bildik *et al*., 2004). Therefore, it is reported that total antioxidant statu (TAS) and total oxidant statu (TOS) analysis may be more useful than single measurement of oxidant and antioxidants. The molecules including mainly thiol groups with albumin, uric acid, ascorbic acid, vitamin E and bilirubin are the basic molecules contributing to TAS (Erel, 2004). Names such as total peroxide, serum oxidation activity, and reactive oxygen metabolites are found in TOS (Harma *et al*., 2003; Mendes *et al*., 2009). The TOS/TAS ratio is used to indicate the oxidative stress index (Harma *et al*., 2003).

The carbaryl and 2,4-D are frequently used in agricultural field. *Capoeta capoeta* belonging to the family *Cyprinidae* is a species of fish consumed by local people as a food source and like a pollution indicator of the stream. In this study, it was aimed to determine the effects of carbaryl and 2,4-dichlorophenoxyacetic acid, which are used intensively as pesticides, on blood and liver oxidative stress index in *C. capoeta* (Guldensteadt 1773).

MATERIALS AND METHODS

This work was supported by the Kafkas University Scientific Research Projects Commission (Project No: 2015-FM-23) with the permission of Kafkas University's Local Ethics Committee (KAÜ-HADYEK) dated 20.11.2014 with the code 2014/026. In this study, 50 *Capoeta capoeta* fish living in Kars Stream and weighing 120-150g were used. The pH of creek water collected from fish samples was measured 7.9-8.2, the dissolved oxygen amount was 5.1-9.2. mg/L and the temperature was 15.2-18.3°C. The fish caught from the Kars Stream were brought to the laboratory and placed in 300 L tanks and provided adaptation period for 10 days. The some physicochemical properties of the water in the tank are: pH 7.3±0.2, temperature 20±2°C, dissolved oxygen 7.8-9.1 mgL^{-1}, total hardness 159±8.4 $CaCo_3$ mgL^{-1}. It was consisted 5 groups including 10 fish in each group. The fish in first group were in the normal water environment (control group), the fish in group II, III, IV and V were kept in tanks containing 0.3 mg/L carbaryl, 0.6 mg/L carbaryl, 10 mg/L 2,4-D and 20 mg/L 2,4-D for 7 day, respectively. At the end of the study, the blood samples from caudal vein under light anesthesia (MS-222) were taken in heparinized tubes and centrifuged at 3000 rpm for 10 min and the plasma samples were obtained. By using commercial kits (Gaziantep, Turkey) for determination of plasma levels of TAS and TOS were performed according to the spectrophotometric method (Erel, 2004; Kıral *et al*., 2008). Oxidative stress index (OSI) was calculated by the TOS/TAS ratio (Harma *et al*., 2003).

$$OSI = [TOS\ (\mu mol\ H_2O_2\ Equivalent\ /L)\ /\ TAS\ (\mu mol\ Trolox\ Equivalent\ /L)] \times 100$$

The liver tissue samples taken for biochemical analysis were homogenized on ice for 2 min at 1200 rpm, diluted 5-fold with phosphate buffered saline (PBS). Homogenates were centrifuged at 1500 rpm for 10 min at 4ºC. The supernatant fractions were evaluated on the same day. The liver ALT and AST levels were measured in an autoanalyzer (HumaStar 600, Germany) using commercial assay kits (AST and ALT HumaStar kits, Cat No: 12021300 and 12022300, Germany).

Statistical analysis

The statistical analysis of the biochemical parameter results obtained from the experiment and control groups was performed using the statistical package program (SPSS 20.0 for Windows). The presence of significant differences between the groups was determined by one way analysis of variance (ANOVA). The results were expressed as mean ± standard deviation (X ± SD).

RESULTS

Macroscopic findings

There were some changes in fish movements was observed during experimental applications. In general, increase in swimming and opercular movements and reversal cases were observed. In the working period, the water and the materials in the tanks were renewed on average every 2 days. In the groups with 0.3 mg/L and 0.6 mg/L of carbaryl, the demand of the fish to feed was decreased according to the control group. The bleeding zones at the mouth and gill edges of 0.6 mg/L carbaryl-treated fish were observed. In the fish in the tanks applied 20 mg/L 2,4-D, acceleration and reversal movements were observed. At the end of 7th day, it was encountered the death of fish as 1 in the tank with 0.3 mg/L carbaryl, 3 in the tank with 0.6 mg/L carbaryl, 2 in the tank with 20 mg/L and 1 in the tank containing 10 mg/L 2,4-D.

Table I.- The levels of the plasma and liver parameters in *C. capoeta* received the carbaryl, 2,4-D and the control group.

	Control	Carbaryl		2,4-D	
		0.3 mg/L	0.6 mg/L	10 mg/L	20 mg/L
Plasma					
TAS (mmol Trolox eq/L)	0.32±0.06[a]	0.27±0.04[c]	0.24±0.07[b]	0.29±0.05[b]	0.27±0.06[b]
TOS (µmol Trolox eq/L)	7.51±0.62[a]	8.83±1.02[b]	9.05±0.98[b]	7.92±1.45[a]	9.27±0.84[b]
OSİ (arbitrary birim)	2.37±0.18[a]	3.19±0.52[b]	3.71±0.48[c]	2.79±0.48[ab]	3.52±0.65[b]
Liver					
AST (IU/L)	345.31 ± 17.48[a]	384.63 ± 15.02[b]	409.34 ± 20.55[b]	375.37 ± 16.81[b]	392.26 ± 19.06[b]
ALT (IU/L)	25.62 ± 4.24[a]	41.06 ± 6.79[c]	34.78 ± 5.21[b]	32.57 ± 3.49	35.15 ± 3.92

Mean ± standard deviation, X ± SD; [a, b, c], there is a significant difference between the averages with different letters in the same line (P <0.05).

Biochemical findings

Biochemical parameters determined in control, carbaryl and 2,4-D groups and statistical significance ratings are shown in Table I. Plasma OSI and liver AST levels in the groups with carbaryl and 2,4-D were compared with the control group, and they were found to be significantly higher. The levels of plasma OSİ in group II, III, and V were significantly higher compared to control group. On the other hand, compared to the control group, the levels of the liver aspartate amino tranfrase (AST) were statistically higher in fish received the pesticide. The liver AST levels were also higher than control group.

DISCUSSION

The free radicals are reactive radicals that contain an unpaired electron and occur in the body during normal metabolism or by external factors. The defense mechanisms of cells such as neutrophils and macrophages need free radical reactions, and overproduction of free radicals affects all important compounds such as lipids, proteins, carbohydrates and DNA in cells. Reactions of the ROS need to be controlled (Bildik *et al*., 2004; Gallardo *et al*., 2014). Free radical chain reactions usually begin by removing hydrogen from the molecules. Natural antioxidant defense systems have been developed in the body to improve the production of ROS and its damage. The antioxidant molecules with endogenous and exogenous origins, the damage caused by the oxidant substances are corrected by intracellular and extracellular defense mechanisms. The extracellular defense system contains various molecules such as bilirubin, transferrin, uric acid, albumin, ceruloplasmin. Intracellular free radical scavenging enzymes such as superoxide dismutase (SOD), glutathion-S-transferase (GST), glutathione peroxidase (GPx), catalase, glutathione reductase and cytochrome oxidase constitute the main antioxidant defense (Erel, 2005). In the living organism, the rate of formation of free radicals is in equilibrium with the rate of their elimination, which is called oxidative balance. As long as oxidative stability is achieved, the organism is resistant to the adverse effects of free radicals (Gallardo *et al*., 2014; Cenesiz *et al*., 2016; Erkılıç *et al*., 2017; Anusuya and Hemalatha, 2014). In the event of impairment of the oxidative balance, oxidative stress, which shows a serious imbalance between free radical formation and antioxidant defense mechanism, occurs and oxidative stress causes cell and tissue damage (Gallardo *et al*., 2014; Erkılıç *et al*., 2017). An important factor contributing to the oxidative stress that causes free radicals and trigger pathological reactions in living organisms is the use of pesticides with neurotoxic effects (Ferrari *et al*., 2007; Troudi *et al*., 2012; Anusuya and Hemalatha, 2014).

The doses of 1 and 3 mg/L carbaryl applied to rainbow trout (*Oncorhynchus mykiss*) were reported to decrease significantly in liver and kidneys, especially in liver reductase glutathione (GSH), GST and catalase activities after 48 and 96 h. Furthermore, it was expressed that reductions in GSH, GST and catalase activities in an experimental work for maximum 96 h reflect an indeterminate state of redox balance in the detoxification mechanism (Ferrari *et al*., 2007). The present study has suggested that reduction of plasma TAS levels of the carbaryl and 2,4-D groups and the increased OSI might be attributed to the pesticide stress and redox balance in fish received pesticide could not be provided. In one study, it was administered 100 and 200 mg/L 2,4-D to *Channa striatus* fishes for 1 week. It was reported that high levels of SOD and GPx and decreased catalase and GST activities were found in the obtained muscle tissue specimens (Anusuya and Hemalatha, 2014). In another study investigating the effects of 2,4-D, female wistar rats were divided to two

groups and administered by adding 600 ppm of drinking water from 14th day of gestation to 14th day after birth. Compared with the control group, it was concluded that in the 2,4-D group, the pups had a significant decrease in body weight. Although increases in malondialdehyde (MDA) levels were observed in the mother and the offspring, liver SOD, catalase and GPx activities were reduced. It has also been shown that plasma aminotransferases (ALT, AST), gamma glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), bilirubin and albumin levels are significantly increased. These findings suggest that 2,4-D causes hepatotoxicity in adult and lactating rats (Troudi *et al*., 2012). In the present study, the increases in plasma OSI and liver AST and ALT levels in both 2,4-D and carbaryl group showed that pesticides may be related to damage to the liver and the adverse effects on the antioxidant defense system components (Table I). It is conceivable that plasma GSH levels and GST or GPx activities in fish may be affected by 2,4-D and carbaryl due to that TAS levels reflect antioxidant molecules containing sulfhydryl groups (Erel, 2004). In the study, it was determined higher TOS values and lower TAS values than the control group in fish applied both carbaryl and 2,4-D. This causes a significant increase in the presence carbaryl and 2,4-D of OSI in the fish. For this reason, it can be used expression that amounts used of carbaryl and 2,4-D cuase to depletion of antioxidant capacity and confrontation with intense oxidant substances. In an applied research to determine the acute toxic effects of carbaryl on *Mystus vittatus*, LC_{50} values at the 72 h was determined as 17.5 ppm and the concentration of 32.5 ppm carbaryl caused 100% mortality within 24 h. In addition to these, it was reported that even sublethal doses accelerated the swimming activity and increased the intensity of opercular movements as indicators of possible stress (Arunachalam *et al*., 1980). In the present study, it was determined that the 0.3 and 0.6 mg/L carbaryl given to *Capoeta capoeta* for 7 days caused significant macroscopic and biochemical changes. Furthermore, there were increases in swimming, backstroke and opercular vibration movements in groups including pesticide during experimental aplications. It was clearly apparent that even low doses were factors of a stress environment in group fish given pesticides.

As a result, it was found that significant changes in plasma oxidative stress and liver enzyme activities occurred in fish exposed to carbaryl and 2,4-D from pesticides. Considering these toxic effects of carbaryl and 2,4-D on aquatic organisms such as fish in pesticide applications, it has been also concluded that these pesticides should be used with caution in agricultural use and pay attention to application doses.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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