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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Some oxidative stress parameters in heart tissue of Zebrafish (*Danio rerio*) caused by mancozeb

Zebrabalığı (*Danio rerio*) kalp dokusunda mancozeb'in neden olduğu bazı oksidatif stres parametreleri

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Abstract: The potential toxic effects of mancozeb exposure on some antioxidant enzyme were investigated on heart tissue of zebrafish in this study. Zebrafish groups were exposed to different doses of mancozeb (Group A: 5 mgL^{-1} and Group B: 7.5 mgL^{-1}) for 120 hours. In this study, catalase (CAT) activity, malondialdehyde (MDA) level and total protein (TP) level were determined with spectrophotometer. Our results showed that CAT activity was found 2.541±0.771 mg L^{-1} in A group and 2.011±0.201 mg L^{-1} in B group in this study. CAT and MDA activity levels decreased in the experiment group according to control group. MDA levels were found 0.025±0.003 mg L^{-1} in A group and 0.025±0.003 mg L^{-1} in B group. TP levels were found 9.75±1.51 mg L^{-1} in A group and 10.18±0.32 mg L^{-1} in B group. TP levels increased in the all experiment groups according to control group. We observed that the changes in the CAT activity and MDA levels were time and as well as mancozeb dose dependent. As a result, mancozeb is a very toxic substance for zebrafish and other aquatic organisms.

Keywords: Mancozeb, oxidative stress, catalase, malondialdehyde, zebrafish

Öz: Bu çalışmada, zebra balığı kalp dokusunda mancozeb'in bazı antioksidan enzimler üzerindeki potansiyel toksik etkileri araştırılmıştır. Zebra balığı grupları, 120 saat boyunca farklı dozlarda mancozeb (Grup A: 5 mg L^{-1} ve Grup B: 7,5 mg L^{-1}) maruz bırakıldı. Bu çalışmada, katalaz (CAT) aktivitesi, malondialdehit (MDA) ve total protein (TP) seviyeleri spektrofotometre ile belirlenmiştir. Elde ettiğimiz sonuçlar CAT aktivitesinin ve MDA seviyesinin tüm deney gruplarında azaldığını gösterdi. Bu çalışmada CAT aktivitesi A grubunda 2,541±0,771 mg L^{-1}, B grubunda ise 2,011±0,201 mg L^{-1} olarak bulunmuştur. CAT ve MDA aktiviteleri tüm deney gruplarında kontrol grubuna göre azalmıştır. MDA seviyeleri A grubunda 0,025±0,003 mg L^{-1} ve B grubunda 0,025±0,003 mg L^{-1} olarak bulunmuştur. TP seviyeleri A grubunda 9,75±1,51 mg L^{-1}, B grubunda ise 10,18±0,32 mg L^{-1} olarak bulunmuştur. Deney grubunda TP düzeyi kontrol grubuna göre artmıştır. CAT aktivitesi ve MDA seviyelerindeki değişikliklerin deney süresine ve mancozeb dozuna bağlı olduğu belirlenmiştir. Sonuç olarak; mancozeb zebra balığı ve diğer sucul organizmalar için oldukça toksik bir maddedir.

Anahtar kelimeler: Mancozeb, oksidatif stres, katalaz, malondialdehit, zebra balığı

INTRODUCTION

Aquatic biota of existing agricultural areas are often exposed to broad spectrum of pesticides that reach water ecosystems through unintended direct application, sprey drift and runoff, thus posing a possible risk for nontarget aquatic organisms (Wagner et al., 2014; Faggio et al., 2016). Pesticides are chemicals used to control pests, their primary use is to increase agricultural yields and prevent the spread of vector-borne disease that have implications to health and the economy (Kumar et al., 2019). Mancozeb (Manganese ethylenebis/dithiocarbamate) is one of the most widely used fungicide in agriculture particularly in many fungal diseases in a various agriculture areas (EPA, 2005). Fish expose to various stressors in their environment such as heavy metals, pesticides, industrial wastes etc. In recent years many of their natural habitats are being altered by anthropogenic effects, including industrial waste and urban pollutants. Zebrafish (*Danio rerio*) is a significant vertebrate model organism. Recently the zebrafish has frequently been used for toxicological studies in various organs and tissues such as liver, gills, intestine, pancreas, brain and heart. Although the stuctural organization of zebrafish organ and tissues different from humans, the two species are similar in terms of function of metabolic pathways of antioxidant enzymes (Qiu et al., 2019). The use of zebrafish as a toxicity model for mammals and humans is well established (McCollum et. al., 2011; Ribas and Pifeffer, 2013). CAT, SOD and GPx enzymes constitute the first step of the antioxidant defence systems (Percin and Sogut, 2010). In this study different mancozeb levels present in the water were investigated in zebrafish under laboratory conditions to describe maximum concentrations allowed in the water and to determine concentrations of fish intake appropriate for human consumption (Andreu-Sanchez et al., 2012). Thus to better

understand the harmful effects of mancozeb and oxidative stress process induced by mancozeb, this study examined dose dependent oxidative stress in zebrafish heart tissue by determining the MDA and protein level and CAT activity.

MATERIAL AND METHODS

Experimental design

Mancozeb is the member of ethylene bisdithiocarbamates (EBDC) fungicides a broad spectrum protectant from fungi for the control of a wide range of diseases in agricultural areas (EPA 2005). First, a stock solution of mancozeb was prepared. The mean body lengths of zebrafish were body length =31.6 ± 1.17 mm (n=25). Zebrafish acclimatized for two week under standard laboratory conditions. Fish were fed twice a day on well-aerated tap water in glass aquariums and with a photoperiod consisting of 14h light/10h dark period during experiments. Healty zebrafish groups were divided into two experimental groups (A and B) and a control (C) group. Different doses of mancozeb were applied in the experimental groups 5 and 7.5 mgL^{-1} respectively. 120 hours exposure of mancozeb was accepted as an indicator of chronic effect during the tests. After 120 hours fish were dissected immediately with sterile surgical instruments. The heart tissues of fish were removed. The heart tissue samples added cold 0.1M phosphate buffer (pH 7.4) and homogenized by using glass beads with ice bath cooling. Then homogenates centrifugated at 10.000g for 30 min at 4^0C to obtain the supernatant for analysing the biochemical parameters the supernatants were stored at 4^0C and all applications were performed under ice-bath cooling to keep the enzyme activities stable. CAT, MDA and TP levels were detected using spectrophotometric methods.

Catalase (CAT) activity assay

CAT activity was determined according to the method by Aebi. The principle of assay is based on the determination of rate constant of hydrogen peroxide decomposition by the CAT. Briefly the activity was determined by measuring the decrease in absorbance at 240 nm of a reaction mixture consisting of H_2O_2, in phosphate buffer, pH7.0 and requisite volume of tissue sample (Aebi, 1984).

Malondialdehyde (MDA) estimation assay

MDA levels was determined according to the method by Ledwozyw. The MDA content was measured after incubation at 95^0C with thiobarbituric acid in aerobic conditions. The pink color produced by the reactions was measured with spectrophotometer at 532 nm. Specific activity was defined as the unit of activity per milligram protein (Ledwozyw, 1986).

Total protein (TP)

Soluble protein concentration was measured with the Coomassie Brilliant blue G-250 using bovine serum albümin as a standard at 595 nm (Bradford, 1976).

Statistical analysis

The SPSS 23.0 package program was used for analysis. Study data were given as arithmetic means and standard deviations. The one way analysis of variance and student t-test were used for the determination of the significance of the differences between the groups. A value of $p<0,05$ was considered statistically significant.

RESULTS

Our results show that mancozeb cause changes at a biochemical level in heart tissue of zebrafish. In this study, we determined low CAT activity in the heart tissue of samples after both of mancozeb treatment. Our results demostrated reduced levels of CAT and MDA in zebrafish heart tissue when compared to the control group. The decrease in CAT activity could be due to the excess production of superoxide radicals. MDA which is itself responsible for some of the damaging effect of free radicals on cell membranes whereas severe oxidative stress in the cells can cause cell injury and death of cell. The decreased MDA levels may be consequence of cellular oxidative damage due to pesticide exposure. Total results of this study including MDA and protein levels, CAT activity in heart tissue of zebrafish with or without exposed to mancozeb are in Table 1.

Table 1. Catalase (CAT) activity, malondialdehyde (MDA) and total protein (TP) level changes related to after different doses of mancozeb exposure (Values were expressed as mean± SD)

	Exposure concentration mg L^{-1}		
	A group (Mancozeb 5 mg L^{-1})	B group (Mancozeb 7,5 mg L^{-1})	C group (Control group)
CAT	2.541± 0.771	2.011 ± 0.201	3.230 ± 0.698
MDA	0.025± 0.003	0.025 ± 0.003	0.118 ± 0.012
TP	9.75 ± 1.51	10.18 ± 0.32	8.46 ± 1.49

DISCUSSION

All pesticides cause nondegredable residues on soil, water and living organisms. CAT plays a significant protective role in animal tissues against reactive oxygen species (ROS) attack. CAT is one of the primary enzyme which involves in peroxide detoxification and has a special importance for the clearance of H_2O_2. In this study we found low CAT activity in the heart tissue of zebrafish. Similar results have also been reported in various fish species. (Plhalova et al. 2017) investigated the subchronic effects of neem-azal T/S (it is a biopesticide and containing 1% of the active ingredient azadirachtin A) on the mortality, growth and histopathology of juvenile zebrafish. Also the researchers reported that influenced indices of oxidative stress on liver of zebrafish. They found that the results of their study indicate that these tested concentrations of neem-azal T/S affect fish growth and have a negative effects on the indices of oxidative stress in the juvenile stage of zebrafish, as well as cause mild

histopathological changes in liver tissue. Blahova et al.(2013) reported a considerable decline in CAT activity in all test groups of zebrafish exposed to atrazine (Blahova et al., 2013). Environmental toxicants such as pesticides cause oxidative damage by directly increasingly cellular concentration of ROS and also by reducing the cellular antioxidant capacity. In our study, catalase activity decreased in heart tissue of zebrafish after exposed to mancozeb for 120 hours. This situation may be as the consequence of cellular oxidative damage due to pesticide exposure at different concentrations during the experiment. So, we can infer that oxidative balance might play an important role in prevention of antioxidative enzyme activity due to mancozeb exposure. If the decrease of CAT levels because of high superoxide radicals, it can be cause to inhibit CAT activity. Zhu et al., (2019) reported that fenobucarb (2-sec-butylphenyl methylcarbamate) is a possible risk factor for cardiovascular and cerebrovascular systems in fish. They found that fenobucarb induced severe heart failure, reduced heart contractions and myocardial apoptosis in their study (Zhu et.al., 2019). Our results demonstrated that pesticides could induce organ failure in a dose dependent manner. Our results mean that pesticides would have potential to cause harmful effects in aquatic animals.

Lipid peroxidation (LPO) is one of the most important early events in cell degeneration leading to necrosis and occurs primarily in the cell membrane (Tabassum 2016). Environmental toxicants such as heavy metals, pesticides, can generally cause oxidative stress and LPO has been widely used as a marker of oxidative stress in living cells (San and Yonar 2017; Tsaboula et al., 2016). MDA is one of the most preferred indicator of LPO in all living organisms. MDA is the product formed as a result of LPO and is a parameter extensively used to show the oxidative damage on cells and tissues (Zengin, 2018). Alterates on the levels of main metabolites such as MDA and on the activity of some antioxidant enzymes such as CAT have been described as biomarkers of oxidative stress (Pascual et al., 2003; Morales et al., 2004. According to Clasen et. al., (2018) pesticides have severe adverse consequences in fish and their potential risk to human health due to their bioaccumulation in farmed fish too (Clasen et. al., 2018).

Although proteins are now used generally as a marker of ROS, they infrequently have been investigated in aquatic pollution. TP levels are one of the main targets for the explanation of effects of environmental toxicants such as pesticides or heavy metals in aquatic organisms. Miron et al. (2008) reported that exposure to clomazoneon *Leporinus obtusidens* for 8 days, resulted as increased protein levels in fish liver (Miron et al., 2008). Moraes et al. (2011) also reported similar results related with the total protein levels when carps were exposed to imizethapy+imizapic pesticides (Moraes et al., 2011). In addition, according to Karaca et. al. (2014) the antioxidant enzyme activities on fish may be considered sensitive markers of organochlorine pesticide exposure on their nature habitats (Karaca et.al., 2014). In this study, exposed to mancozeb, demonstrated with the increase in protein levels in heart tissue and caused oxidative stress. At the same time levels of TP had also significantly increased in the heart tissue of samples after mancozeb exposure. According to a study, high activity of antioxidant enzymes LPO have been found in the blood of the captive Northern Bluefin Tuna samples. Konyalıoğlu and Perçin (2017) have been found that high activity of antioxidant enzymes (LPO) in the blood of the captive Northern Bluefin Tuna (NBT) samples in their study. When compared GSH levels of blood have turned out to be low in comparison to the wild NBT samples. Their findings show that captive NBTs suffer from stress more than wild NBTs. This is important to know the differences in enzyme levels in wild fish species (Konyalıoğlu and Perçin, 2017). Vieira et. al., (2018) indicated that high concentrations of imidaclobrid (IMI) induced a marked increase in LPO in liver and kidney tissues of fish. According to researchers, pesticide exposure caused oxidative stress on various biological molecules, including lipid, proteins and antioxidant enzymes (Vieira et. al., 2018). Our results support these findings in this study. Maharajan et. al., (2018) reported that pyriproxyfen could cause harmful effect on early developmental stages such as egg and larvae of zebrafish at higher concentration. They showed that these damages that will occur during the development stage of organs and tissues such as liver, pancreas or heart will affect the whole life of the fish (Maharajan et. al., 2018).

In conclusion this study investigated the existance of oxidative stress biomarkers in zebrafish heart tissue under laboratory conditions and found that decreased level of MDA and CAT activity with an increased level of TP in relation to oxidative stress. The results are significant for reporting acute mancozeb toxicity in terms of biochemical changes: mancozeb is substantially toxic to fish. Special studies such as detecting biomarkers in polluted aquatic environments, recommended to ensure continue the sustainability of healthy environment.

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