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RESEARCH ARTICLE

EFFECTS OF CYANIDE ON SOME ENZYME ACTIVITIES AND LIPID PEROXIDATION IN SOME TISSUES OF CARP (*Cyprinus carpio*)

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ABSTRACT

In this study, the changes in the catalase, superoxide dismutase, carbonic anhydrase activities, and the levels of malondialdehyde in the muscle, liver, gill, skin, brain, and intestinal tissues of carps (*Cyprinus carpio*), in which 0.1 mg/L and 0.2 mg/L concentrations cyanide were added, were investigated. It was determined that the catalase, superoxide dismutase, and carbonic anhydrase activities of fish exposed to cyanide were inhibited in some tissues and increased in some tissues. Especially in 15-day experiments statistically significant decreases were observed in enzyme activities. It was observed that malondialdehyde levels, which are one of the important markers of cell damage of tissues generally increased with cyanide exposure. In this study, malondialdehyde levels increased statistically significant in the liver and intestinal tissues in the 3-day experiment and the muscle tissue in the 15-day experiment.

Keywords: *Cyprinus carpio, Cyanide, Enzyme, Lipid Peroxidation.*

1. INTRODUCTION

Cyanide is a molecule formed by the triple bond of carbon and nitrogen atom. Cyanide and its compounds are used in various areas of industry like metal coating, rubber production, paint industry, and drug production. Cyanide is produced commercially to use in industry and it is also produced by some plant, insect, and bacteria species naturally. For instance, almond contains 297 mg/kg, wild cherry contains 140-370 mg/100g, and cassava contains 104 mg/100g cyanide [1]. Cyanide is released into the environment by natural means as well as industrial sources. After cyanide is used in industry, it is either chemically decomposed or stored in waste ponds. The discharge of cyanide as waste is also increasing day by day. Since cyanide is generally used with metal compounds, it also causes heavy metal pollution in the waters where it is discharged. In addition, high-bonding cyanide tends to form toxic complexes again [2].

Cyanide can enter the water sources from a variety of sources, such as wastewater from the metal plating and iron and steel industries, mining, and pesticides [3]. Efforts are made to reduce these rates by passing the wastewater through various processes. Although it is reduced, due to the highly toxic nature of cyanide, even low concentrations pose a danger to aquatic life in natural waters. Fish are the most sensitive group of aquatic organisms to pollutants. Therefore, even at low concentrations, fish are adversely affected by cyanide toxicity, causing permanent problems even if death does not occur. It has been reported in the literature that concentrations of 0.01 and 0.1 mg/L may be toxic to fish [4]. Wild et al. [5] reported that cyanide is in the range of 0.0001 - 0.05 mg/L in natural waters. EPA has also set the cyanide limit for freshwater environments as 0.052 mg/L [6].

Fish are the living species that are maximum affected by cyanide pollution in the aquatic environment. Being in the last link of the biological chain makes them so vulnerable. Cyanide causes various problems in fish, especially the lethal effect. When fish are exposed to 5-7.2 pg free CN^-/L cyanide, their swimming and reproductive systems are damaged, while they are at risk of death when exposed to 20-76 pg free CN⁻/L cyanide [7]. David and Kartheek [8] found decreases in catalase, superoxide dismutase, glutathione peroxidase, and glutathione S-transferase activities in carp fish to which they applied sodium cyanide at a concentration of 0.1 mg/L for 10 and 20 days compared to the control group. For another freshwater fish species, *Labeo rohita*, it was reported that ATPase enzyme was inhibited in lethal (0,32 mg/L) and sublethal (0,064 mg/L) concentrations [9]; glycogen and pyruvate amounts decreased at sublethal (0,106 ve 0,064 mg/L) concentrations [10]; the levels of structural and soluble protein decreased at sublethal (0,2 mg/L) concentration [11]. Bonanno et al., found that blood thiocyanate levels in *Amphiprion clarkii*, to which they applied 50 ppm cyanide for 20 and 45 seconds, reached levels of 301 - 468 ppb [12]. In a previous study, in which we tried to reveal the acute toxic effect of 0.5 mg/L cyanide, it was observed that cyanide at this concentration caused cellular damage and affected some antioxidant enzyme activities in carp [13].

In this study, it was aimed to determine the toxic potential of cyanide in aquatic environments and aquatic organisms, taking into account its dangerous properties. For this purpose, catalase, superoxide dismutase, and carbonic anhydrase activities and changes in malondialdehyde levels in muscle, liver, gill, brain, skin, and intestinal tissues of carp were investigated in the presence of cyanide.

2. SUBJECTS AND METHODS

2.1. Test Environment, Fish Nutrition, and Anesthesia

In this study, sump systems consisting of 30x40x60 cm aquariums were used. Each system consisted of 5 aquariums, 4 aquariums, and 1 cleaning tank (Figure 1).

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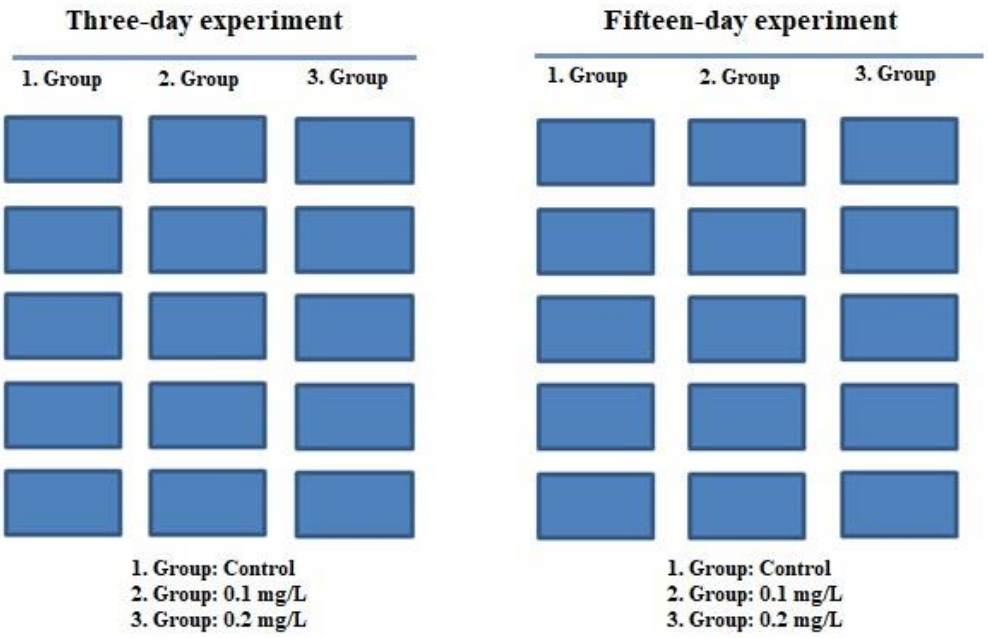

Figure 1. Experiment aquarium model (Each blue box means an aquarium).

The aquarium at the bottom of the system was determined as a cleaning tank and no fish were placed in this aquarium. A motor was added to the bottom aquarium and the circulation of the water in the system was ensured. Thus, aquariums with homogeneous water quality were obtained. Organic wastes were kept by the fiber material placed in the bottom aquarium. At the same time, an ultraviolet lamp was operated for 2 hours a day to clean the microorganisms in the water. The temperature of the waters in the aquariums is set at 22°C. To ensure the oxygenation of the water, an air stone was attached to each aquarium and the oxygen content of the aquarium water was not reduced below 6 mg/L. Aquarium maintenance was followed regularly. The water quality in the aquariums is kept at a level that does not adversely affect the health of the fish [14]. No fish died during the experiment.

Fish, whose height and weight were measured, were randomly placed in each aquarium as 4 fish. Since each system consisted of 4 aquariums, 16 fish were used in each group. Fish were fed with commercial feed at the rate of 1% of live weight. Sodium cyanide (NaCN) was used as the cyanide source (0.1 mg/L and 0.2 mg/L). The experiment continued for 3 days and 15 days.

At the end of the experiment, the fish were taken from the aquariums and transferred to the anesthesia pool. Clove oil (600 mg/L), which is both healthier and more effective than other chemical anesthetics, is used as an anesthetic agent [15, 16].

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2.2. Biochemical Analysis

Fish taken from anesthesia were dissected and muscle, liver, gill, skin, brain, and intestinal tissues were removed. They were washed with physiological water and kept at the temperature of -80°C until the analysis are made. Before the biochemical analysis, the tissues were homogenized within 50 µM, pH = 7.4 cooled phosphate buffer at 8000 rpm for two minutes. The supernatant obtained after the homogenates were centrifuged at 9500 rpm at +4°C for 30 minutes was used for the biochemical analysis.

In the experiments, catalase (CAT) activity analysis was carried out according to the method determined by Aebi [17]. According to this method, the decrease of hydrogen peroxide in the presence of CAT was monitored spectrophotometrically at a wavelength of 240 nm. CAT analysis was carried out following the order specified in Table 1.

Table 1. CAT activity measurement procedure.

	Blind	Experiment
Phosphate buffer	0.01 ml	-
H_2O_2 solution	3.00ml	3.00ml
Sample	-	0.01 ml

The specific catal activity calculation is as follows:

$$\frac{nkat}{mg\ protein} = \left[\left(\frac{\Delta A_C}{t}\right)\left(\frac{1}{\varepsilon H_2O_2}\right)\left(\frac{1}{\varepsilon}\right)\left(\frac{V_T}{V_S}\right)\left(\frac{1000\mu L}{10\mu L}\right)\right]\frac{DC}{Protein\ concentration} \quad (1)$$

(ΔA_C: Absorbance change, ε H_2O_2 (molar absorption coefficient of H_2O_2) = 40,98 $M^{-1}cm^{-1}$, V_T: Total volume, V_S : Sample volume, DC : Dilution coefficient)

Measurement of superoxide dismutase (SOD) activity is based on spectrophotometric monitoring of the effect of SOD enzyme on the autoxidation of 6-hydroxy dopamine at wavelengths of 490 nm. [18, 19, 20, 21]. SOD analysis was carried out following the order specified in Table 2.

Table 2. SOD activity measurement procedure.

	Blind	Experiment
0.05 M phosphate buffer (pH 7.4)	740 µl	690 µl
Sample	-	50 µl
0.01 M 6-OHDA solution	10 µl	10 µl

The specific SOD activity calculation is as follows:

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$$\frac{EU}{mg\ protein} = \frac{\left[\left(\frac{\frac{A_B - A_S}{A_K}}{2}\right) DC(V_T - V_S)\left(\frac{3\ mL}{V_S}\right)\right]}{Protein\ concentration} \quad (2)$$

(EU: Enzyme Unit, A_B: Blind absorbance, A_S: Sample absorbance, DC: Dilution coefficient, V_T: Total volume, V_S: Sample volume)

Carbonic anhydrase (CA) activity depends on the principle of having the ability to hydrolyze ester components by this enzyme. CA, p-nitrophenyl acetate, hydrolyzes to p-nitrophenol or p-nitrophenolate. This product indicates absorbance spectrophotometrically at 348 nm wavelength [22, 23]. CA analysis was carried out following the order specified in Table 3.

Table 3. CA activity measurement procedure.

	Blind	Experiment
0.05 M TRIS buffer (pH 7.4)	1.3 mL	1.3 mL
Sample	-	0.1 mL
Substrate	1 mL	1 mL
Pure water	0.7 mL	0.6 mL

The specific CA activity calculation is as follows:

$$\frac{EU}{mg\ protein} = \left(\frac{A_{last} - A_{first} - A_{blind}}{5}\right)\left(\frac{DC}{Protein\ concentration}\right) \quad (3)$$

A_{last}: Last absorbance, A_{first}: First absorbance, A_{blind}: Blind absorbance, DC: Dilution coefficient

The molar absorption coefficient of p-nitrophenol and p-nitrophenolate formed as a result of the activity of the CA enzyme (ε): $5.4 \times 10^{-3} M^{-1} cm^{-1}$, the molar absorption coefficient of p-nitrophenyl acetate used as a substrate (ε): $0.4 \times 10^{-3} M^{-1} cm^{-1}$. Dividing the absorption difference at 348 nm wavelength used in the formula by 5 gives the ion concentrations of p-nitrophenol and p nitrophenolate.

Malondialdehyde (MDA), is a product of lipid peroxidation and shows a colorful reaction with thiobarbituric acid (TBA). The reaction of MDA with TBA is read on a spectrophotometer at 532 nm wavelength [24]. MDA analysis was carried out following the order specified in Table 4.

Table 4. MDA activity measurement procedure.

	Blind	Experiment
TCA (10%)	2.5 ml	2.5 ml
Plasma	-	0.5 ml
Distilled water	0.5 ml	-
It was incubated in a 90°C water bath for 15 minutes. It was kept on ice for 15 minutes.		

Supernatant	2 ml	2 ml
TBA (0.675%)	1 ml	1 ml
It was incubated in a 90°C water bath for 15 minutes. It was kept on ice for 15 minutes.		

According to the results obtained, the concentration-absorbance graph was obtained. The concentration values of the samples were calculated by considering this graph. Protein determination was carried out using the Bradford method which is based on reading the complex at 595 nm wavelength [25]. In protein determination, firstly, a standard graph was obtained by using bovine serum albumin, which contains 1 mg of protein in 1 mL. In the calculation, the concentrations of the samples were determined from the standard graph. For the determination of protein in the tissue, the procedures were applied in the order indicated in the table below, and the absorbance was measured at 595 nm wavelength after a 10-minute waiting period.

Table 5. Protein amount measurement procedure.

	Blind	Experiment
Protein dye	5 mL	5 mL
Sample	-	0.1 mL
Pure water	0.1 mL	-

2.3. Statistical Analysis

In this study, SPSS 22 program was used for statistical analysis. Tables were created by calculating the mean and standard error values. The statistical difference between the groups which contain different concentrations of cyanide (0.1 mg/L and 0.2 mg/L) was determined by the One-Way ANOVA test. The statistical difference between the groups in which cyanide was applied at different times was determined by using Student's t-test. The results were analyzed at $p<0.05$ significance level.

3. RESULTS AND DISCUSSION

3.1. Catalase Activities

Cyanide is a molecule that forms intracellular reactive oxygen species and leads to the accumulation of hydrogen peroxide and superoxide anion [26, 27]. CAT is a crucial enzyme that provides the defence of cells against oxidative stress. It converts the hydrogen peroxide molecule, which occurs in metabolic events such as phagocytosis and mitochondrial electron transport, into water and oxygen gas. Also, it tries to eliminate the loss of function that occurs because of various pollutants in the metabolism. In such circumstances, CAT activity could be inhibited. In this study, it was observed that the CAT activities of *C. carpio* exposed to cyanide at concentrations of 0.1 and 0.2 mg/L for three and fifteen days decreased compared to the control group (Table 6).

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Table 6. CAT activities of fish used in the study.

	Catalase (nkat/mg protein)					
	3 Days			15 Days		
	C	0.1 mg/L	0.2 mg/L	C	0.1 mg/L	0.2 mg/L
Muscle	0.015±0.00	0.009±0.00	0.011±0.00	0.018±0.00^{a}	0.011±0.00^{b}	0.011±0.00^{b}
Liver	1.303±0.09	0.932±0.11	1.053±0.10	1.354±0.12	1.081±0.07	1.096±0.05
Gill	0.042±0.00	0.028±0.00	0.036±0.00	0.095±0.03	0.028±0.00	0.033±0.01
Skin	0.015±0.00	0.010±0.00	0.016±0.00	0.036±0.00	0.140±0.00	0.120±0.00
Brain	0.009±0.00	0.001±0.00	0.002±0.00	0.002±0.00	0.001±0.00	0.001±0.00
Intestine	0.192±0.03	0.080±0.01	0.062±0.02	0.092±0.03	0.122±0.04	0.175±0.05

(C: Control; Values shown with different letters contain statistical significance)

Only fish exposed to cyanide at concentrations of 0.1 and 0.2 mg/L for fifteen days increased their CAT activities in intestinal tissues compared to the control group. When the literature is examined, the studies are seen that CAT activities decrease depending on the applied chemical. David et al. [28] reported that NaCN decreased CAT activities in various tissues of fish in their study they carried out with fingerlings of common carp. When lethal and sublethal cyanide exposure, it was reported that the most significant decrease was seen in liver tissues, and then in gill, muscle, and brain tissues [28]. In another study, it was reported that exposure to 0.1 mg/L cyanide concentration for ten and twenty days decreased CAT activities in the liver tissues of carp fish compared to the control group [8]. When the related results in the literature are examined, it is understood that the findings of this study are coherent with the findings in the literature. When cyanide enters the living body, it forms a complex with the cytochrome c oxidase enzyme and prevents the functioning of enzymes such as CAT by blocking the antioxidant enzyme system. If this condition occurs at the acute level in high concentration, it is also reported to cause rapid death [28, 29].

3.2. Superoxide Dismutase Activities

SOD is another antioxidant enzyme that operates collectively with CAT. SOD catalyzes the free oxygen radical that formed in the cell to gaseous oxygen and hydrogen peroxide. In this study, while SOD enzyme was inhibited in muscle and skin tissues of *C. carpio* exposed to cyanide for three days, SOD activity increased in the liver, brain, gill, and intestinal tissues. SOD activities of muscle, liver, and intestinal tissues of *C. carpio* exposed to cyanide at concentrations of 0.1 mg/L and 0.2 mg/L for fifteen days decreased, and SOD activities of brain and gill tissues increased (Table 7).

Table 7. SOD activities of fish used in the study.

	Superoxide dismutase (EU/mg protein)					
	3 Days			15 Days		
	C	0.1 mg/L	0.2 mg/L	C	0.1 mg/L	0.2 mg/L
Muscle	48.92±1.84	45.27±1.18	44.03±1.5	46.84±1.06^{a}	46.17±1.02^{ab}	41.95±1.48^{b}
Liver	67.28±2.06	76.14±3.46	72.89±4.65	94.36±15.35	72.71±2.62	74.26±3.87
Gill	12.38±6.15	32.27±7.3	39.43±5.83	24.67±6.23^{a}	40.72±5.32^{ab}	50.3±3.68^{b}
Skin	79.66±2.66	69.28±5.38	71.26±2.37	89.61±1.30^{a}	108.96±3.95^{b}	79.11±4.06^{a}

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Brain	85.57±3.71	91.89±1.84	95.43±1.66	75.13±1.04	80.65±0.91	84.36±6.14
Intestine	55.22±1.14	66.72±5.05	55.78±8.42	71.31±4.46[a]	51.14±2.25[b]	56.47±3.34[b]

(C: Control; Values shown with different letters contain statistical significance)

Generally, SOD is inhibited by the joining of pollutants in the body. When the findings obtained in the study were examined, it was observed that the SOD enzyme was inhibited in only two tissues in a three-day exposure to cyanide, and more tissues in a fifteen-day exposure to cyanide. Accordingly, it can be said that the increase of cyanide exposure time mostly inhibits the SOD activity of *C. carpio* tissues. However, it was also determined that the SOD activities of some tissues of *C. carpio* increased with cyanide exposure. It may well be argued that the tissues that increase their activity do this as a reaction to cyanide exposure. David and Kartheek [8] reported that SOD activities were inhibited in the liver tissues of the *C. carpio* exposed to 0,1 mg/L cyanide. They related this condition to the deformation caused by reactive oxygen types in tissues after cyanide enters metabolism. In a previous study conducted by us, it was concluded that the SOD enzyme was inhibited in the liver tissue of *C. carpio* exposed to cyanide at a concentration of 0.5 mg/L for three days and increased its activity in the brain, muscle and gill tissue [13]. Responses of living things to pollutants vary according to many parameters such as exposure duration, temperature, pH, and metabolic condition. As a result, when we considered results derived from fish that were exposed to cyanide for 15 days, it could be seen that SOD activities were inhibited in more tissues. This condition shows that SOD inhibition can increase with higher concentrations and longer duration.

3.3. Carbonic Anhydrase Activities

CA is a metalloenzyme that contains zinc in its active site and catalyzes the hydrolysis of carbon dioxide and water. CA has particular importance for fish that have gill respiration. CA is responsible for the excretion of ammonia, ion regulation, osmoregulation, and acid-base balance in fish gills [30]. Besides, it is also known that the CA enzyme has the mission of destructing free oxygen radicals and preventing oxidative stress [31]. In this study, it was determined that the CA activities of *C. carpio* exposed to cyanide for three days increased in muscle, intestine, and skin tissues and decreased in liver and brain tissue (Table 8).

Table 8. CA activities of fish used in the study.

	Carbonic anhydrase (EU/mg protein)					
	3 Days			15 Days		
	C	0.1 mg/L	0.2 mg/L	C	0.1 mg/L	0.2 mg/L
Muscle	0.068±0.00	0.104±0.00	0.102±0.00	0.135±0.02[a]	0.087±0.01[a]	0.075±0.00[b]
Liver	2.352±0.50	1.145±0.34	1.136±0.11	1.191±0.11	2.778±0.75	2.418±0.35
Gill	0.244±0.01	0.236±0.02	0.369±0.01	0.233±0.02	0.215±0.01	0.232±0.01
Skin	0.371±0.03	0.553±0.13	0.453±0.07	0.348±0.03	0.450±0.08	0.419±0.04
Brain	0.229±0.03	0.228±0.03	0.200±0.02	0.292±0.03	0.279±0.02	0.309±0.04
Intestine	1.078±0.17	1.088±0.16	1.119±0.29	1.079±0.19[a]	0.716±0.24[ab]	0.475±0.08[b]

(C: Control; Values shown with different letters contain statistical significance)

In addition, it was determined that the CA activities of the gill tissues of fish exposed to 0.1 mg/L cyanide concentration decreased, while the CA activities of gill tissues of the fish exposed to 0.2 mg/L cyanide concentration increased. The results derived from the muscle, liver and intestine tissues of fish exposed to cyanide for 15 days are opposite of the ones exposed to cyanide for three days. The inhibition of CA activities in the muscle and intestine tissues is found to be statistically significant ($p<0,05$) for the fish exposed to cyanide of 0.2 mg/L for 15 days. Alım et al. [32] purified the CA enzyme found in the gills of tuna (*Thunnus thynnus*) and analyzed the inhibition effect of various metals for the CA enzyme in vitro. As a result of the study, they determined that the inhibition effects of metals were in the form of $Ag^{+}> Cu^{2+}> Pb^{2+}> Zn^{2+}> Cd^{2+}> Co^{2+}$ and that Ag^{+} metal was a strong CA inhibitor. They also mentioned that CA activity can be a good toxicology biomarker. Ceyhun et al. [33] investigated the effects of 0.25, 1.0 and 2.5 µg/L concentrations of deltamethrin, a pesticide, on CA activity in rainbow trout gill tissue and reported that it significantly inhibited CA activities within 24 to 48 hours.

While freshwater fish release ions such as H^{+}, NH_4^{+} and HCO_3 by diffusion, they absorb Na^{+} and Cl^{-} ions that form salt. This condition is known as osmoregulation and CA activity is vital for the regular occurence. Literature findings and data obtained from this study show that various chemicals inhibit CA activity in metabolism. This condition can even result in the death of living things. However, in the findings obtained in this study, it was observed that the CA enzyme increased its activity in some tissues with the presence of cyanide. It could be said that the increase of CA enzyme in various tissues is for the reason of tolerating stress conditions resulted from cyanide.

3.4. Malondialdehyde Levels

Lipid peroxidation can be briefly defined as the degradation of fats as a result of oxidation. This event occurs in unsaturated fatty acids of cell membrane phospholipids [34]. Lipid peroxidation is one of the most significant indicators of cell injury. MDA that occurs during the lipid peroxidation process is frequently used today as a good biomarker of oxidative damage [35, 36]. Oxidative stress caused by cyanide in metabolism is primarily responsible for the formation of lipid peroxidation in the cell. In this study, *C. carpio* were exposed to cyanide of 0.1 mg/L and 0.2 mg/L concentrations. When the findings were examined, it was observed that MDA levels increased in all tissues except brain and gill tissues after exposure to cyanide for three days (Table 9).

Table 9. MDA amounts of fish used in the study.

	Malondialdehyde (nmol/mL)					
	3 Days			15 Days		
	C	0.1 mg/L	0.2 mg/L	C	0.1 mg/L	0.2 mg/L
Muscle	168.22±33.63	257.23±31.34	251.38±33.34	152.18±29.64^{a}	254.25±28.47^{b}	290.52±28.69^{b}
Liver	114.20±16.22^{a}	196.55±14.05^{b}	162.24±25.59^{ab}	83.29±13.39	124.00±4.19	148.47±9.44
Gill	176.20±9.45	173.56±17.10	168.87±21.12	135.72±15.59	157.24±11.62	167.24±12.03
Skin	58.94±21.46	94.18±7.42	75.76±17.12	38.00±19.36	70.72±15.78	76.53±16.03
Brain	99.80±19.34	99.65±21.03	86.12±10.34	100.28±14.32	102.09±16.27	111.86±18.09
Intestine	101.65±8.24^{a}	145.77±10.37^{ab}	166.35±18.23^{b}	134.82±14.78	179.53±35.75	167.29±19.83

(C: Control; Values shown with different letters contain statistical significance)

Especially in the liver, which is an organ with a high metabolic rate, MDA levels increased statistically significantly. Hermenean et al. [37] determined that the MDA amounts in the liver and kidney tissues of *Leuciscus cephalus* they caught from the parts of the Tur River rich in metals such as iron, zinc, copper, cadmium and lead in Romania were quite high. It was determined that MDA levels increased significantly in the liver and gill tissues of carp exposed to permethrin as a different toxic substance [38]. David and Kartheek [8] reported that 0.1 mg/L NaCN concentration significantly increased the amount of lipid peroxidation in carps. Fish contain polyunsaturated fatty acids, which have a very important role in the maintenance of cell membrane-bound functions. The high MDA ratio and lipid peroxidation obtained in this study may be due to the high content of polyunsaturated fatty acids in fish.

4. CONCLUSIONS

When the results obtained from this study were examined, it was seen that the degree of effect of cyanide in different tissues of *C. carpio* was not the same. It was determined that the liver, gill and skin tissues of *C. carpio* exposed to cyanide at concentrations of 0.1 mg/L and 0.2 mg/L were the most adversely affected. This condition indicates that even though cyanide does not show a vast amount of accumulation in metabolism, it generates tissue damage in the living things it enters.

The reaction of a tissue against a chemical agent may differ from organism to organism. In the same organism reaction of different tissues may also differ. In this study, it can be said that the different levels of enzyme activities in *C. carpio* exposed to cyanide at 0.1 mg/L and 0.2 mg/L concentrations for three and fifteen days may be due to the different biochemical effects of cyanide in different tissues.

Increased lipid peroxidation in the cell is one of the important markers of cell damage. In this study, a general inference can be drawn as cyanide can cause cell injury since the MDA level, which is a product of lipid peroxidation, is on the rise as a result of cyanide exposure.

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REFERENCES

[1] Kuyucak, N., and Akçıl, A. (2013). Cyanide and removal options from effluents in gold mining and metallurgical processes, Minerals Engineering 50-51, 13-29.

[2] Patil, Y.B., and Paknikar, K.M. (2000). Biodetoxification of silver-cyanide from electro-plating industry wastewater, Letters in Applied Microbiology 30, 33-37.

[3] Dash, R.R., Gaur, A., and Balomajumder, C. (2009). Cyanide in industrial wastewaters and its removal: A review on biotreatment, Journal of Hazardous Materials 163, 1-11.

[4] Prashant, M.S., and Neelagund, S.E. (2007). Free cyanide-induced biochemical changes in nitrogen metabolism of the indian major carp *Cirrhinus mrigala*, Journal of Basic & Clinical Physiology & Pharmacology 18(4), 277-287.

[5] Wild, S.R., Rudd, T., and Neller, A. (1994). Fate and effects of cyanide during wastewater treatment processes, Sci. Total Environ 156, 93-107.

[6] EPA (2002). National Recommended Water Quality Criteria: 2002.

[7] Koçan, F. (2021). Environmental effects of cyanide gold production from ore, International Journal of Engineering Research and Development, 13(1), 250-264.

[8] David, M., and Kartheek, R.M. (2016). In vivo studies on hepato-renal impairments in freshwater fish *Cyprinus carpio* following exposure to sublethal concentrations of sodium cyanide, Environ Sci Pollut Res, 23, 722-733.

[9] Dube, P.N., and Hosetti, B.B. (2011). Inhibition of ATPase activity in the freshwater fish *Labeo rohita* (Hamilton) exposed to sodium cyanide, Toxicology Mechanisms and Methods 21(8), 591-595.

[10] Dube, P.N., Shwetha, A., and Hosetti, B.B. (2013). Effect of exposure to sublethal concentrations of sodium cyanide on the carbohydrate metabolism of the Indian Major Carp *Labeo rohita* (Hamilton, 1822), Pesq. Vet. Bras. 33(7), 914-919.

[11] Manjunatha, B., Mohiddin, G.J., Ortiz, J., and Selvanayagam, M. (2014). Effect of exposure to sublethal concentrations of sodium cyanide on the biochemical aspects in the liver of the freshwater fish, *Labeo rohita*, International Journal of Pharmacology and Pharmaceutical Sciences 2(1), 7-15.

[12] Bonnano, J.A., Breen, N.E., Tlusty, M.F., Andrade, L., and Rhyne, A.L. (2021). The determination of thiocyanate in the blood plasma and holding water of *Amphiprion clarkii* after exposure to cyanide. PeerJ. 9:e12409.

[13] Kavasoğlu, M., Sarıoğlu, Y., Uysal, K., Dönmez, M., Altıkat, S., Yetek, İ., and Kuru, H. İ. (2015). Effect of sodium cyanide on antioxidant enzyme activities and lipid peroxidation in some tissues of Mirror Carp (*Cyprinus carpio*). Pakistan J. Zool. 47(6). 1777-1782.

[14] Lloyd, R. (1992). Pollution and Freshwater Fish, Fishing News Book, UK.

[15] Han, M. C., Sağlıyan, A., and Polat, E. (2016). Akvaryum balıklarında karanfil yağının anestezik etkilerinin araştırılması, Harran Üniversitesi Veteriner Fakültesi Dergisi, 5 (1), 12 – 17.

[16] Otay, T., Küçükgül, A., Pala, A., and Şeker, E. (2014). Sazan balıklarının anestezisinde karanfilin kullanımı, Bilim ve Gençlik Dergisi, 2, 43 – 50.

[17] Aebi, H. (1974). Catalase Methods of Enzymatic Analysis, B. HU. New York and London, Academic, Press Inc, 673-677.

[18] Aydemir, T., and Tarhan, L. (2001). Purification and partial characterization of superoxide dismutase from chicken erythrocytes, Turkish Journal of Chemistry 24, 451-459.

[19] Crosti, N., Servidei, T., Bajer, J., and Serra, A. (1987). Modification of 6- hydroxydopamine technique for the correct determination of superoxide dismutase, J.Clin. Chem. Clin. Biochem. 25, 265-266.

[20] Heikkila, R.E., and Cabbat, F. (1976). A sensitive assay for superoxide dismutase based on the autoxidation of 6-hydroxydopamine, Analytical Biochemistry 75, 356-362.

[21] Tarhan, L., and Tüzmen, M.N. (2000). Some properties of Cu, Zn-superoxide dismutase from sheep erythrocyte, Turkish Journal of Chemistry 24, 109-116.

[22] Armstrong, J., Mc, D., Myers, D.V., Verpoorte, J.A., and Edsall, J.T. (1966). Purification and properties of human erythrocyte carbonic anhydrase, J. Biol. Chem. 214, 5137.

[23] Kandel, M., Gonall, A.G., Wong, S., and Kondel, S.I.. (1970). Some characteristics of human, bovine and horse carbonic anhydrase as revealed by inactivation studies, J. Biol.Chem. 245, 2444.

[24] Draper, H.H., and Hadley, M. (1990). Malondialdehyde determination as index of lipid peroxidation, Method Enzymol 180, 421-431.

[25] Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72, 248.

[26] Daya, S.S., Walker, R.B., and Anoopkumar-Dukie, S.S. (2000). Cyanide-induced free radical production and lipid peroxidation in rat brain homogenate is reduced by aspirin, Metab. Brain Dis. 15, 203-210.

[27] Douglas, C.J., Krishnan, P., Li, L., Palur, G.G., Yan, S., Joseph, L.B., and Gary, E.I. (2003). Cyanide enhancement of dopamine-induced apoptosis in mesencephalic cells involves mitochondrial dysfunction and oxidative stress, Neurotoxicology 24, 333-342.

[28] David, M., Munaswamy, V., Halappa, R., and Marigoudar, S.R. (2008). Impact of sodium cyanide on catalase activity in the freshwater exotic carp, *Cyprinus carpio* (Linnaeus), Pesticide Biochemistry and Physiology 92, 15-18.

[29] Eisler, R. (1991). Cyanide Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review, Contaminant Hazard Reviews, Report 23.

[30] Lionetto, M.G., Giordano, M.E., Vilella, S., and Schettino, T. (2000). Inhibition of eel enzymatic activities by cadmium, Aquat. Toxicol. 48, 561-571.

[31] Raisanen, S.R., Lehenkari, P., Tasanen, M., Rahkila, P., Harkonen, P.L., and Vaananen, H.K. (1999). Carbonic anhydrase III protects cells from hydrogen peroxide-induced apoptosis. FASEB J. 13, 513-522.

[32] Alım, Z., Çamur, B., Beydemir, Ş., and Küfrevioğlu, Ö.İ. (2014). The correlation between some metal concentrations and carbonic anhydrase activity in Tuna (*Thunnus Thynnus* Linnaeus, 1758) Gill, Hacettepe J. Biol. & Chem. 42(2), 219-224.

[33] Ceyhun, S.B., Şentürk, M., Erdoğan, O., and Küfrevioğlu, Ö.İ. (2010). In vitro and in vivo effects of some pesticides on carbonic anhydrase enzyme from rainbow trout (*Oncorhynchus mykiss*) gills, Pesticide Biochemistry and Physiology 97, 177-181.

[34] Yarsan, E. (1998). Lipid peroksidasyon olayı ve önlenmesine yönelik uygulamalar, Y. Y. Ü. Vet. Fak. Derg. 9(1-2), 89-95.

[35] Hodgson, E. (2004). A textbook of modern toxicology, Wiley, Hoboken.

[36] Paskerova, H., Hilscherova, K., and Blaha, L. (2012). Oxidative stress and detoxification biomarker responses in aquatic freshwater vertebrates exposed to microcystins and cyanobacterial biomass, Environ Sci Pollut Res 19, 2024-2037.

[37] Hermenean, A., Damache, G., Albu, P., Ardelean, A., Ardelean, G., Ardelean, D.P., Horge, M., Nagy, T., Braun, M., Zsuga, M., Keki, S., Costache, M., and Dinischiotu, A. (2015). Histopathological alterations and oxidative stress in liver and kidney of *Leuciscus cephalus* following exposure to heavy metals in the Tur River, North Western Romania, Ecotoxicology and Environmental Safety 119, 198-205.

[38] Uğurer, O. (2020). Investigation of antioxidant changes in common carps fish (*Cyprinus carpio* L. 1758) exposed to permethrine. Master thesis, Aksaray University Science Institute, Aksaray.

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APPENDIX

T.C.
DUMLUPINAR ÜNİVERSİTESİ
HAYVAN DENEYLERİ YEREL ETİK KURULU
ARAŞTIRMA BAŞVURUSU ONAYI

BAŞVURU BİLGİLERİ	ARAŞTIRMANIN ADI	**Kütahya İli'ndeki bazı su kaynaklarında siyanür miktarlarının tespiti ve siyanür'ün sazan balığı (*Cyprinus carpio*) üzerine bazı etkilerinin araştırılması**
	ARAŞTIRMA YÜRÜTÜCÜSÜ KURUMU	Doç.Dr.Kazım UYSAL DPU Fen Edebiyat Fakültesi Zooloji Anabilim Dalı
	PROJE YÜRÜTÜCÜSÜ KURUMU	Doç.Dr.Kazım UYSAL DPU Fen Edebiyat Fakültesi Zooloji Anabilim Dalı
	YARDIMCI ARAŞTIRICILAR	Doç.Dr.Kazım UYSAL Biyolog İrfan YETEK Uzman Biyolog Mustafa KAVASOĞLU Biyolog Yıldız ÇOLAK Uzman Biyolog Cemali YILDIZ
	ARAŞTIRMANIN TAHMİNİ SÜRESİ	12 Ay
	KULLANILACAK HAYVAN TÜRÜ VE SAYISI	Balık(Pullu Sazan-*Cyprinus carpio*) – 120 adet
	DESTEKLEYİCİ KURULUŞ	TÜBİTAK

DEĞERLENDİRİLEN İLGİLİ BELGELER	**Belge Adı**	**Tarihi**
	ARAŞTIRMA BAŞVURU FORMU	**12.11.2014**

KARAR BİLGİLERİ	**Karar No : 2014.12.03** **Tarih : 19.11.2014**
	Yukarıda başvuru bilgileri verilen araştırma projesi gerekçe, amaç ve yöntemler dikkate alınarak görüşüldü ve ilgili belgeler incelendi. Projenin etik açıdan uygun olduğuna, çalışmanın aşağıdaki hususlar dikkate alınarak yürütülmesine ve sorumlu araştırıcıya iletilmesine **OY BİRLİĞİ** ile karar verildi. 1) Projede herhangi bir değişiklik gerektiğinde kurulumuzdan onay alınması, 2) Projede çalışacağı bildirilen araştırıcılarda değişiklik olduğunda kurulumuzdan onay alınması, 3) Deney hayvanları üzerinde yapılacak girişimin başlangıç ve bitiş tarihinin bildirilmesi, 4) Çalışma süresinde tamamlanamaz ise ek süre talebinde bulunulması, 5) Çalışma tamamlandığında sonuç raporunun gönderilmesi.

ETİK KURUL BİLGİLERİ

ÜYELER

Unvanı / Adı / Soyadı EK Üyeliği	**Uzmanlık Dalı**	**Kurumu**	**İlişki (*)**	**İmza**
Doç.Dr. Aynur GÜLCAN Başkan	Mikrobiyoloji ve Klinik Mikrobiyoloji Anabilim Dalı	Tıp Fakültesi	☐ E ■ H	
Yrd. Doç. Dr. Ahmet KOÇAK Üye	Histoloji ve Embriyoloji Anabilim Dalı	Tıp Fakültesi	☐ E ■ H	
Yrd. Doç. Dr. Sezer AKÇER Üye	Anatomi Anabilim Dalı	Tıp Fakültesi	☐ E ■ H	
Yrd. Doç. Dr. Ceylan AYADA Üye	Fizyoloji Anabilim Dalı	Tıp Fakültesi	☐ E ■ H	
Yrd.Doç. Dr. Hasan METİNEREN Üye	Ortopedi ve Travmatoloji Anabilim Dalı	Tıp Fakültesi	☐ E ■ H	
Doç.Dr. M.Kasım ÇAYCI Üye	Biyoloji Anabilim Dalı	Fen-Edebiyat Fakültesi	☐ E ■ H	
Yrd.Doç.Dr. Muhammed OYLUMLU Üye	Kardiyoloji Anabilim Dalı	Tıp Fakültesi	☐ E ■ H	
Yrd. Doç. Dr. Zülfü BAYHAN Üye	Genel Cerrahi Anabilim Dalı	Tıp Fakültesi	☐ E ■ H	
Vet. HEKİM Aydın AKCILAR Üye	Veteriner HEKİM	Tıp Fakültesi DEHYUAM	☐ E ■ H	

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Erkan ERKOL Üye			☐ E ■ H	

* Araştırma ile İlişkisi