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The Effect of Some Transition Metal Ions (Mn^{+2}, Co^{+2}, Fe^{+2}) on Paraoxonase Enzyme Activity in Bonito (*Sarda sarda*)

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Abstract

In this study, it was aimed to determine the effects of transition metal ions of manganese (Mn^{+2}), cobalt (Co^{+2}) and iron (Fe^{+2}) on paraoxonase (PON) enzyme in the muscle tissue of bonito. Research is located in the north of Turkey from the Black Sea obtained 25 pieces of bonito derived from fish muscle tissue is used. Solutions of Mn, Co and Fe transition metals as $MnCl_2$, $CoCl_2$ and $FeCl_2$ were prepared to be used in analysis. PON enzyme activity was determined by taking different volumes of these solutions. As a result of the obtained data, the enzyme activities of Mn^{+2}, Co^{+2} and Fe^{+2} transition metal ions were calculated and percentage activity graphs were drawn. As a result of the research, it was determined that transition metal ions Mn^{+2}, Co^{+2} and Fe^{+2} decreased PON enzyme activity in bonito fish. In addition, One Way Analysis of Variance (One-Way Anova) was performed to statistically demonstrate the effect of different concentrations of $MnCl_2$, $CoCl_2$ and $FeCl_2$ solutions on the PON enzyme activity. As a result of the analysis, it was determined that the effects of different concentrations of each solution on PON enzyme activity did not show a statistically significant difference.

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INTRODUCTION

Paraoxonase (PON), which has a glycoprotein structure, is a calcium (Ca^{+2}) dependent ester hydrolase with both arylesterase and paraoxonase activity (Durrington, 2001). Paraoxon, which is the result of the catabolic product of the parathion compound, inhibits acetylcholine esterase and some enzymes, which play an important role in the transmission of nerve impulses. However, the organism has formed defense systems against many of these effects. One of them is the PON enzyme (Costa et al., 1999; La Du et al., 1999). There are three forms of PON enzyme: PON1, PON2 and PON3. PON proteins differ from each other according to their expression and distribution in tissues (Carey et al., 2005). PON1 is the most studied isoenzyme, whose structure and functions are best illuminated according to PON2 and PON3 (Clendenning, 1996), determined to the q21.3-q22.1 region on the long arm of chromosome 7. PON2 is the second member of the PON gene family located on chromosome 7 q21.3-22.1 (Roest, 2008). PON2 is not found in serum; artery macrophage cells are found in various tissues and cells (Shiner et al., 2006; Fuhrman et al. 2008). PON3 is a protein with a molecular mass of about 40 kDa located on chromosome 7, between PON1 and PON2. PON3 is an enzyme with antioxidant properties like other isoenzymes and inhibits monocyte chemotaxis caused by oxidized LDL (Janka et al., 2002; Mackness et al., 2004). The PON enzyme hydrolyzes paraoxane and eliminates its harmful effects (Costa et al., 1999; La Du et al., 1999). PON enzyme shows an antioxidant feature by limiting lipid oxidation in LDLs thanks to HDL, which is bound to its structure. For this reason, the paraoxonase enzyme protects cells against oxidative stress and acts as a cellular antioxidant (Elena et al., 2006). The enzyme in question; It has been investigated in organisms such as fish, frogs, dogs, rats, sheep and mice and the structural and kinetic properties of the enzyme have been determined (Furlong et al., 1991; Chemnitus et al., 1983).

Environmental problems are one of the dangers that threaten human and animal health (Köse & Uysal, 2008). Heavy metals are among the most important inorganic factors that are among industrial wastes and some pesticides and pollute water. Heavy metals, which are in equilibrium at certain concentrations in aquatic environments under normal conditions, accumulate in the sediment, especially in urban and industrial areas, and are absorbed by biota (Wildi et al., 2004). Sediments are contaminated with heavy metals, threatening the health of aquatic ecosystems and causing a major source of stress. These metals that accumulate constitute a major risk factor for aquatic organisms located in and on the sediment (Delvalls et al., 1998).

Fish also absorb these heavy metals found in downstream creatures through the food chain (Aksun, 1986). Heavy metal pollution negatively affects not only aquatic organisms but also human health. Metals such as iron, copper, zinc and manganese,

which are among the essential elements, play an important role for living things. In addition, when essential elements are taken in excessive amounts, they can create toxic effects in living things (Türkmen and Ciminli, 2007). In this study, the effect of manganese (Mn^{+2}), cobalt (Co^{+2}) and iron (Fe^{+2}) transition metal ions on PON enzyme activity on the muscle tissue of bonito fish was investigated and in order to determine the antioxidant level of the enzyme. Total oxidant and total antioxidant capacity levels on muscle tissue were determined.

MATERIALS AND METHODS

The average weight of the material obtained from the Black Sea in the north of Turkey Studies 600-800 g from 40 to 45 cm long with 25 bonito form of fish. Muscle tissue of bonito fish taken from the sea in October, which is the seasonal season, and brought to the laboratory environment by cold chain was used in the study. Tissue samples taken from bonito were weighed 0.3 g and placed in dry centrifuge tubes, then 1.5 mL Tris-HCl buffer was added to them and homogenized. The homogenized tissues were centrifuged in a cooled centrifuge at +4 ºC and 3000 rmp speed for 30 minutes and supernants were separated. The separated supernatants were used on the same day. PON enzyme activity determination method recommended by Gülcü and Gürsu (2003) was used for PON enzyme activity determination.

The Effect of Manganese II Chloride ($MnCl_2$) on PON Enzyme Activity: In PON enzyme activity determination, 50 µL Tris-HCl buffer, 50 µL calcium chloride + paraoxone and 50 µL enzyme solution are added to the cuvette and the value at 37 ºC and 405 nm absorbance in ELISA It was read in 30 seconds. Then, after adding different volumes (10 µL, 20 µL, 30 µL, 40 µL and 50 µL) 0.001 M $MnCl_2$ solution to the measured cuvette, the absorbance at 405 nm was measured.

The Effect of Cobalt II Chloride ($CoCl_2$) on PON Enzyme Activity: In PON enzyme activity determination, 50 µL Tris-HCl buffer, 50 µL calcium chloride + paraoxone and 50 µL enzyme solution are added to the cuvette and its value at 37 ºC and 405 nm absorbance in ELISA It was read in 30 seconds. Then, after adding different volumes (10 µL, 20 µL, 30 µL, 40 µL and 50 µL) 0.001 M $CoCl_2$ solution to the measured cuvette, the absorbances at 405 nm were measured.

The Effect of Iron II Chloride ($FeCl_2$) Compound on PON Enzyme Activity: In PON enzyme activity determination, 50 µL Tris-HCl buffer, 50 µL calcium chloride + paraoxone and 50 µL enzyme solution are added to the cuvette and the value at 37 ºC and 405 nm absorbance in ELISA It was read in 30 seconds. Then, after adding different volumes (10 µL, 20 µL, 30 µL, 40 µL and 50 µL) 0.001 M $FeCl_2$ solution to the measured cuvette, the absorbances at 405 nm were measured.

While calculating the effects of transition metal ions of Mn^{+2}, Co^{+2} and Fe^{+2} on PON enzyme activities, enzyme activities determined in an environment without inhibitor were used as %100 enzyme activity.

In this study, the distribution of the data obtained was examined to determine the effect of $MnCl_2$, $CoCl_2$ and $FeCl_2$ solutions on PON enzyme activity corresponding to different concentrations (10 µL, 20 µL, 30 µL, 40 µL and 50 µL). Since the data showed normal distribution, One-Way Anova analysis was performed using the SPSS statistical package program (Çelik, 2012).

RESULTS

Effect of Transition Metals on PON Enzyme Activity

For the PON enzyme activity, the enzyme solution and prepared solutions ($MnCl_2$, $CoCl_2$ and $FeCl_2$) were added to the reaction medium, and the changes in the enzyme activity in the last case were measured. $MnCl_2$, $CoCl_2$ and $FeCl_2$ solutions were taken in different volumes and the measurements were repeated, the average of the results found was calculated and the enzyme activity determination chart and graph were drawn.

PON enzyme percent activity values obtained by using $MnCl_2$ solutions at different concentrations (10 µL, 20 µL, 30 µL, 40 µL and 50 µL) are given in Table 1.

Table 1. Percent activity values of paraoxonase enzyme determined in the medium with $MnCl_2$

Heavy Metal	Tris-HCl Buffer (µL)	Enzyme Solution Volume (µL)	Substrate Solution Volume (µL)	Metal Solution Volume (µL)	Metal Solution Concentration ($1x10^{-4}$M)	ΔOD (405nm)	Activity (U / mL min)	% Activity
						1.1549	18.9435	100.00
				10	0.6	1.2040	19.7497	104.25
				20	1.1	1.1955	19.6089	103.51
Mn	50	50	50	30	1.6	1.2005	19.6918	103.95
				40	2.1	1.1826	19.3975	102.40
				50	2.5	1.1651	19.1110	100.88

When figure 1 is examined, it is seen that after adding 10 µL of $MnCl_2$ solution to the reaction medium, the enzyme activity is determined as 104.25%, while the enzyme activity decreases as a result of increasing $MnCl_2$ concentrations and reaches 100.88% at 50 µL. As a result of the measurements, it was determined that the transition metal ion Mn^{+2} reduces the PON enzyme activity.

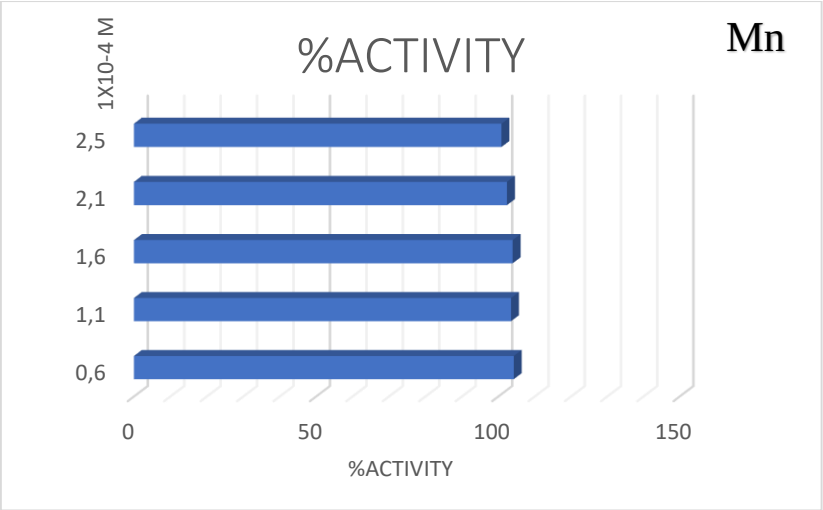

Figure 1. Activity determination figure for paraoxonase enzyme in $MnCl_2$ environment

Data for One-Way Anova (One-Way Anova) analysis to determine whether there is a statistical difference between different concentrations of $MnCl_2$ solution (10 µL, 20 µL, 30 µL, 40 µL and 50 µL) are shown in Table 2.

Table 2. One-way Anova analysis results of different concentrations of $MnCl_2$ solution

Total squares	**Total squares**	**SD**	**Mean square**	**f**	**p**
Between groups	506.7070	4	126.677	0.421	0.793
In-group	28590.277	95	300.950		
Total	29096.984	99			

As seen in Table 2, it was determined that there was no statistically significant difference between the different concentrations used, but there was a decrease in the PON enzyme activity depending on the increasing concentrations ($p > 0.05$).

PON enzyme percent activity values obtained by using $CoCl_2$ solution in different concentrations (10 µL, 20 µL, 30 µL, 40 µL and 50 µL) are given in Table 3.

Table 3. Percent activity values of paraoxonase enzyme determined in the medium with $CoCl_2$

Heavy Metal	**Tris-HCl Buffer (µL)**	**Enzyme Solution Volume (µL)**	**Substrate Solution Volume (µL)**	**Metal Solution Volume (µL)**	**Metal Solution Concentration ($1x10^{-4}$M)**	**ΔOD (405nm)**	**Activity (U/mL min)**	**% Activity**
						1.3206	21.6610	100,00
				10	0.6	1.5230	24.9809	115.33
				20	1.1	1.5201	24.9332	115.11
Co	50	50	50	30	1.6	1.4521	23.8175	109.95
				40	2.1	1.4506	23.7933	109.84
				50	2.5	1.4239	23.3562	107.83

Inhibitor concentrations and percent activity figure for PON enzyme indicated in Table 3 in $CoCl_2$ environment are given in Figurehen figure 2 is examined, it is seen that after adding 10 µL of $CoCl_2$ solution to the reaction medium, the enzyme activity is determined as 115.33%, while the enzyme activity decreases as a result of increasing $CoCl_2$ concentrations and reaches 107.83% at 50 µL. As a result of the measurements, it was determined that the Co^{+2} transition metal ion decreased the PON enzyme activity.

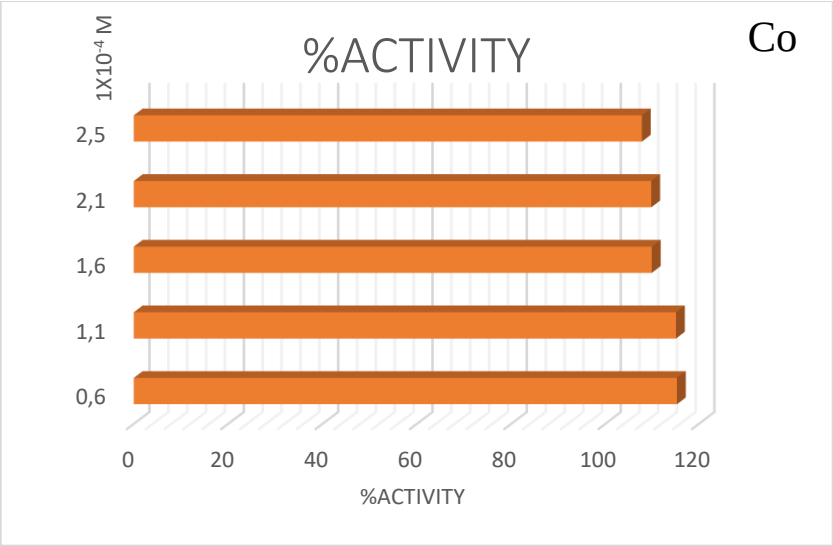

Figure 2. Activity determination figure for paraoxonase enzyme in environment with $CoCl_2$

Data for One-Way Anova (One-Way Anova) analysis to determine whether there is a statistical difference between different concentrations of $CoCl_2$ solution (10 µL, 20 µL, 30 µL, 40 µL and 50 µL) are shown in Table 4.

Table 4. One-way ANOVA analysis results of different concentrations of $CoCl_2$ solution

Total squares	**Total squares**	**SD**	**Mean square**	**f**	**p**
Between groups	1406.7360	4	351.684	0.449	0.773
In-group	74334.052	95			
Total	75740.788	99			

As seen in Table 4, it is seen that there is no statistically significant difference between the different concentrations of $CoCl_2$ solution used ($p > 0.05$).

PON enzyme percent activity values obtained by using $FeCl_2$ solutions in different concentrations (10 µL, 20 µL, 30 µL, 40 µL and 50 µL) are given in Table 5

Table 5. Percent activity values of paraoxonase enzyme determined in the medium with $FeCl_2$.

Heavy Metal	**Tris-HCl Buffer (µL)**	**Enzyme Solution Volume (µL)**	**Substrate Solution Volume (µL)**	**Metal Solution Volume (µL)**	**Metal Solution Concentration ($1x10^{-4}$M)**	**ΔOD (405nm)**	**Activity (U/mL min)**	**% Activity**
						0.9949	16.3186	100.00
				10	0.6	1.1542	18.9319	116.01
				20	1.1	1.1361	18.6350	114.20
Fe	50	50	50	30	1.6	1.1322	18.5714	113.81
				40	2.1	1.1338	18.5969	113.96
				50	2.5	1.1211	18.3882	112.68

Inhibitor concentrations indicated in Table 5 and percent activity graph for PON enzyme in $FeCl_2$ environment are given in Figure 3.

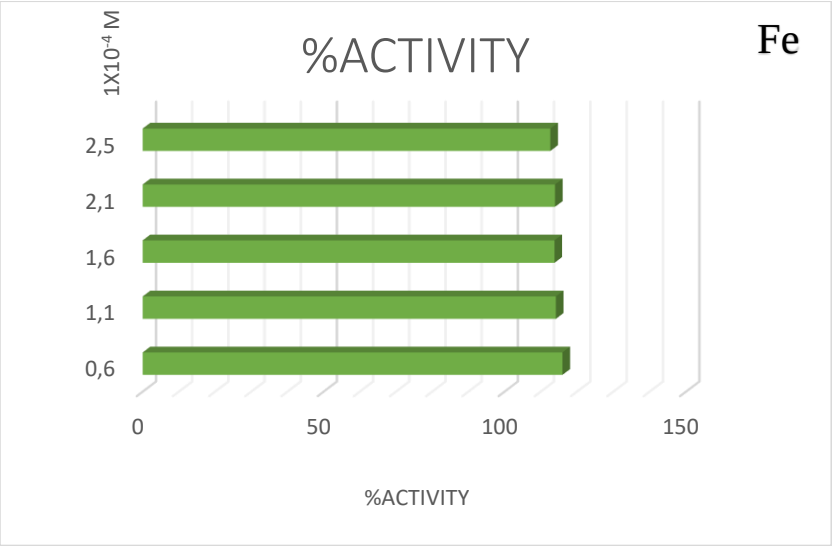

Figure 3. Activity determination figure for paraoxonase enzyme in $FeCl_2$ environment

When figure 3 is examined, it is seen that after adding 10 µL of $FeCl_2$ solution to the reaction medium, enzyme activity is determined as 116.01%, as a result of increasing $FeCl_2$ concentrations the enzyme activity decreases and reaches 112.68% at 50 µL. As a result of the measurements, it was determined that the transition metal ion Fe^{+2} caused a decrease in PON enzyme activity ($p > 0.05$).

Data for One-Way Anova (One-Way Anova) analysis to determine whether there is a statistical difference between different concentrations of $FeCl_2$ solution (10 µL, 20 µL, 30 µL, 40 µL and 50 µL) are shown in Table 6.

Table 6. One-way ANOVA analysis results of different concentrations of $FeCl_2$ solution

Total squares	Total squares	SD	Mean square	f	p
Between groups	46338.3940	4	11584.599	0.596	0.666
In-group	1846411.61	95	19435.912		
Total	1892750.00	99			

As seen in Table 6, it was determined that there was no statistically significant difference between the different concentrations used, but there was a decrease in the PON enzyme activity due to the increasing concentrations ($p > 0.05$).

DISCUSSION

In this study, in the hydrolysis of organophosphate compounds, which have widespread use as insecticide and nerve gas, on the PON enzyme found in the muscle tissue of bonito fish, which has antioxidant and antibacterial activity, manganese (Mn^{+2}), cobalt (Co^{+2}) and iron (Fe^{+2}) the effect of transition metal ions was investigated. When the effect of transition metal ions Mn^{+2}, Co^{+2} and Fe^{+2} on PON enzyme activity was examined, it was concluded that increasing concentrations of these ions decreased the PON enzyme activity. However, it was determined that this increase was not statistically significant.

In studies conducted in fish, it has been determined that various substances cause inhibition on serum and liver PON enzyme (Ganzalvo et al., 1997; Debord et al., 2003; Çiftçi et al., 2000; Debord et al., 1998). In a study conducted on fish of Scyliorhinus canicula, the inhibitory effect of Cu^{+2}, Ni^{+2}, Cd^{+2} and Hg^{+2} metal ions on PON enzyme activity was investigated in vitro. As a result, it was determined that Hg^{+2}, Ni^{+2}, Cd^{+2} and Cu^{+2} metal ions all exhibited inhibitory effect on PON enzyme activity, while Cu^{+2} metal ions had the strongest inhibitory effect (Sayın, 2012). Samra et al., (2010), examined the in vitro inhibitor effects of some metal ions, at 1.0 mM concentration on human PON1 enzyme activity. It was determined that Mg^{+2} and Mn^{+2} ions did not show any effect on human PON1 enzyme activity, Pb^{+2}, Co^{+2}, and Zn^{+2} ions decreased the activity, while Ni^{+2}, Cd^{+2}, and Cu^{+2} ions inhibited the PON1 enzyme activity (Samra et al., 2010).

In a study conducted on PON enzyme activity purified from rat liver, the inhibition types of Mn^{+2}, Co^{+2}, Cu^{+2} and Hg^{+2} metal ions were determined and Hg heavy metal was found to be the strongest inhibitor for PON. For purified PON, the inhibition power was listed as $Hg^{+2}>Co^{+2}>Mn^{+2}>Cu^{+2}$ from strong to weak (Pla et al., 2007). In another study on serum PON enzyme activity in Merino and curly breed sheep, it was determined that the commonly used heavy metals Mn^{+2}, Co^{+2}, Hg^{+2}, Cd^{+2}, Cu^{+2} and Ni^{+2} affected in vitro (Erol, 2012). In the study conducted on PON enzyme purified from human liver, the inhibition effect of EDTA compound, metals such as Mg^{+2}, Co^{+2}, La^{+3}, Zn^{+2}, Cu^{+2}, Ba^{+2}, Hg^{+2} and p-hydroxyliciva benzoate were investigated. It has been reported that EDTA, copper, barium, lanthanum and p-hydroxyliciva benzoate compounds cause competitive inhibition and Zn metal exhibits a non-competitive inhibition effect (Pellin et al., 1990). Erol et al., (2013), in another study, examined the effect of some metal ions on PON1 enzyme activity purified from blood samples taken from Merino and Kivircik sheep breeds. It was determined that Mn^{+2}, Hg^{+2}, Co^{+2}, Cd^{+2}, Ni^{+2}, and Cu^{+2} metal ions showed different levels of inhibition effect on PON

enzyme activity and Cu^{+2} heavy metal ion caused strongest inhibitor effect for PON (Erol et al., 2013). With this study, it is seen that the inhibitory effect of Mn^{+2} and Co^{+2} transition metal ions on PON enzyme activity in bonito fish is similar to the inhibitory effect on PON enzyme activity studied in merino and curly sheep breeds.

Dedeoğlu et al., (2014) observed in their study that changes occurred in the PON1 enzyme activity purified from bull semen in the presence of heavy metal ions Cu^{+2}, Mn^{+2}, Cd^{+2}, Zn^{+2}, Ni^{+2} and Pb^{+2} at different concentrations. They stated that while Cd^{+2} ion increases PON1 activity, other heavy metal ions inhibit PON1 enzyme at micromolar levels. Similarly, it was determined that the Mn^{+2} transition metal ion used in this study caused a decrease in PON enzyme activity purified from acorn. It is seen that the inhibitory effect of Mn^{+2} transition metal ion on PON enzyme activity studied in bonito fish is similar to the inhibitory effect on PON1 enzyme activity purified from bull semen.

CONCLUSION AND RECOMMENDATIONS

In a study conducted by purifying PON enzyme in carp fish, it was determined that Fe metal inhibits PON enzyme activity (Beyaztaş et al., 2007). In a study conducted on bonito fish, the effect of some heavy metals on glutathione transferase enzyme, which has detoxification and antioxidant properties such as PON enzyme, was examined and Pb^{+2}, Cr^{+2}, Fe^{+3}, Ag^{+}, Cu^{+2}, Cd^{+2} and Zn^{+2} metal ions inhibited enzyme activity (Güller et al., 2014). As a result of this research, it was determined that the Fe^{+2} transition metal ion caused a decrease in PON enzyme activity purified from bonito fish at low levels. It is seen that the inhibitory effect of Fe^{+2} transition metal ion on PON enzyme activity in bonito fish is similar to the inhibitory effect of PON enzyme purified from carp fish and glutathione transferase enzyme in bonito fish.

In the literature reviews, it is seen that there are limited studies on the effect of transition metal ions on antioxidant enzymes found in bonito fish and other fish species. As a matter of fact, there are quite a limited number of studies in which the effects of Mn^{+2}, Co^{+2} and Fe^{+2} transition metal ions used in this study on different fish species and different enzyme activities are determined. In this context, this research, in which the effect of transition metal ions Mn^{+2}, Co^{+2} and Fe^{+2} on PON enzyme activity in bonito is determined, is thought to provide depth and contribution to the field.

COMPLIANCE WITH ETHICAL STANDARDS

a) Author contributions:

1.ÇK, Prof.Dr.DÇ: She designed the study and interpreted the data.
2.Prof.Dr.DÇ: Audit, Consulted.
3.ÇK: Made Data Collection or Processing.
4.ÇK: She did the laboratory work.
5.ÇK: She carried out the laboratory work and prepared the article.
6.ÇK, Prof.Dr.DÇ: Critical Review done.

b) Conflict of Interest:

There is no conflict of interest.

c) Statement on the Welfare of Animals

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d) Declaration of Human Rights

This study does not include human participants.

REFERENCES

Aksun, F.Y. (1986). Heavy metal accumulation in pike fish (Esox lucius, 1758) living in Karamık Lake. VIII. National Biology Congress, İzmir 2: 454-461. Doi.org/10.29048/makufebed.411888

Beyaztaş, S., Türker, D., Sinan, S., & Arslan, O. (2007). Investigation of the inhibition effect of Cyprinus carpio paraoxonase enzyme with some heavy metals, 21st National Chemistry Congress, Malatya, 23-27. https://dergipark.org.tr/tr/pub/fufbd/issue/39198/461290

Carey, J., Diana, M., Shih, S., et al. (2005). The paraoxonase gene family and atherosclerosis. Free Radic Biol Med, 38(2):153–163. Doi.10.1016/j.freeradbiomed.2004.09.035.

Costa, L.G., Li, W.F., Richter, R.J., et al. (1999). Shih DM, Lusis A, Furlong CE. The role of paraoxonase (PON 1) in the detoxication of organophosphates and its human polymorphism. Chem Biol Int, 119-120:429. Doi: 10.1016/s0009-2797(99)00055-1.

Chemnitus, J.M., Losch, H., Losch, K., et al. (1983). Organophasphate detoxicating hydrolases in different vertebrate species. Com Biochem Physiol, 6C:85. doi: 10.1016/0742-8413(83)90048-8.

Clendenning, J.B., Humbert, R., Green, E.D., et al. (1996). Structural organization of the human PON1 gene. Genom, 35:586. Doi: 10.1006/geno.1996.0401 .

Çelik, N. (2012). Anova models of robust statistical inference and applications using skew distributions, Ankara University, Graduate School of Science, Ankara, Master Thesis.

Çiftçi, M., Küfrevioğlu, Ö.İ., Gündoğdu, M., Özmen, İ. (2000). Effects of some antibiotics on enzyme activity of glucose-6-phosphate dehydrogenase from human erythrocyte. Pharmacol Res, 41:109. https://www.agriculturejournals.cz/publicFiles/61658.pdf

Debord, J., Dantoine, T., Bollinger, J.C., Abraham, M.H., et al. (1998). Inhibition of aryesterase by aliphatic alcohols. Chem Biol Int, 113:105-115. Doi: 10.1007/s12041-017-0741-7.

Debord, J., Bollinger, J.C., Merle, L., Dantoine, T. (2003). Inhibition of human serumarylesterase by metal chlorides. J Inorg Biochem, 94:1-4. Doi: 10.1016/s0162-0134(02)00627-x.

Del Valls TA, Blasco J, Sarasquete M.C et al. (1998). Forja JM ve Gomez-Parra A. Evaluation of heavy metal sediment toxicity in littoral ecosystems using juveniles of the fish sparus aurata. Ecotoxicol Environ Saf, 41:157-167.

Dedeoğlu, N., Arslan, M., Erzengin, M. (2014). Purification of Holstein Bull Semen Paraoxonase 1 (PON1) by Hydrophobic Interaction Chromatography and Investigation of Its Inhibition Kinetics by Heavy Metals. Biol Trace Elem Res, 158:29–35 . Doi: 10.1007/s12011-014-9916-8

Deveci, H.A., Kaya, İ., Yılmaz, M and Karapehlivan, M. (2015). Effect of zinc sulphate on the levels of plasma paraoxonase activity, total oxidant and high density lipoprotein of transcaucasian barb (*Capoeta capoeta Guldenstaedt, 1773*). Fresen Environ Bull, 24(9):2732-2735.

Durrington, P.N., Mackness, B., Mackness, M.I. (2001). Paraoxonase and atherosclerosis. Arterioscler Thromb Vasc Biol, 21:473-80. Doi.org/10.1161/01.ATV.21.4.473

Elana, T., Elana, M., Magdalena, G., Isabel, L., Ana, M.P. (2006). Effects of caloric restriction and gender on rat serum paraoxonase1 activity. J Exerc Nutr Biochem, 17:197-203. Doi: 10.1016/j.jnutbio.2005.07.004

Erol, K., Gençer, N., Arslan, M., Arslan, O. (2012). Purification, characterization, and investigation of in vitro inhibition by metals of paraoxonase from different sheep breeds. Artificial Cells, Nanomedicine, and Biotechnology, 41(2):1255-130. Doi: 10.3109/10731199.2012.696065.

Erol K., Gençer, N., Arslan, M. & Arslan, O. (2013). Purification, characterization, and investigation of in vitro inhibition by metals of paraoxonase from different sheep breeds. Artifical Cells, Nanomedic,and Biotechnology, 41(2), 125-130. Doi: 10.3109/10731199.2012.696065

Fuhrman, B., Khateeb, J., Nitzan, O., et al. (2008). Urokinase plasminogen activator upregulates paraoxonase2 expression in macrophages via an NADPH oxidase-dependent mechanism. Arterioscler Thromb Vasc Biol, 1361-1367.

Furlong, C.E., Richter, R.J., Chapline, C., et al.(1991). Purification of rabbit and human serum paraoxonase. Biochem, 30:10133. Doi: 10.1021/bi00106a009.

Gonzalvo, M.C., Gil, F., Hernandez, F., et al.(1997). Inhibition of paraoxonase activity in human liver microsomes by exposura to EDTA, metals and mercurials. Chem Biol Interact , 105:169-179. Doi: 10.1016/s0009-2797(97)00046-x.

Gülcü, F., Gürsu, M.F. (2003). Standardization of paraoxonase and arylesterase activity measurements. J Biochem, 28 (2): 45-49.

Güller, U., Taşer, P., Çiftci, M., Küfrevioğlu, Ö.İ. (2014). Purification of Glutathione S-Transferase From Bonito (*Sarda Sarda*) Liver And Investigation of Metal Ions Effects on Enzyme Activity. Hacettepe J Biol Chem, 42(3):435-442

Janka, Z., Juhász, A., Rimanóczy, A.A., et al. (2002). Codon 311 polymorphism of paraoxonase2 gene is associated with apolipoprotein E4 allele in both alzheimer's and vascular dementias. Mol Psychiatry, 7(1):110-2. Doi: 10.1038/sj.mp.4000916.

Köse, E., Uysal K. (2008). Comparison of heavy metal accumulation rates in the muscles, skin and gills of the scaled carp (Cyprinus carpio L., 1758), which has not reached maturity. Dumlupınar University Institute of Science, Kütahya, Master Thesis.

La, Du, B.N., Aviram, M., Billecke., S et al. (1999). Navab M, Primo-Parmo S, Sorenson RC, Standiford TJ. On the physioogical role(s) of the paraoxonases. Chem Biol Int, 379:119-120.

Mackness, B., Mackness, M. (2004). Paraoxonase 1: biochemistry and contribution to atherosclerosis. University International Congress Series, 1262:91– 94. Doi:10.1016/S0531-5131(03)01736-9

Pla, L., Rodrigo, A.F., Hernandez, F., et al. (2007). Effect of metal ions and calcium on purified PON1 and PON3 from rat liver. Chem Biol Interact, 167 63–70. Doi: 10.1016/j.cbi.2007.01.006.

Pellin, M.C., Moretto, A., Lotti, M., et al. (1990). Distribution and biochemical properties of rat paraoxonase activity. Neurotoxicol Teratol, 12,611614. Doi: 10.1016/0892-0362(90)90071-j.

Roest, M. (2008). PON1 Genotypes and Coronary Heart Disease. Laboratory for Clinical Chemistry and Haematology. In: Mackness B, Mackness M, Aviram M, Paragh G (Eds.). The Paraoxonases: their role in disease development and xenobiotic metabolism. Netherlands: Springer, 139-147. Doi:10.1007/978-1-4020-6561-3_15.

Sayın, D., Türker Çakır, D., Gençer, N., Arslan, O.(2012). Effects of some metals on paraoxonase activity from shark (Scyliorhinuscanicula). J Enzyme Inhib Med Chem, 27(4):595-598. Doi: 10.3109/14756366.2011.604320.

Samra, Z.Q., Shabir, S., Rehmat, Z., Zaman, M., Nazir, A., Dar, N. & Athar, M.A. (2010). Synthesis of cholesterol-conjugated magnetic nanoparticles for purification of human paraoxonase 1. Applied Biochemistry. Biotechnology, 162, 671-686. Doi: 10.1007/s12010-009-8840-4.

Shiner, M., Fuhrman, B., Aviram, M. (2006). A biphasic U-shape effect of cellular oxidative stress on the macrophage antioxidant paraoxonase2 (PON2) enzymatic activity. Biochem Biophys Res Communat, 349:1094-1099. Doi: 10.1016/j.bbrc.2006.08.150.

Türkmen, M., Ciminli, C. (2007). Determination of metals in fish and mussel species by inductively coupled plasma-atomic emission spectrometry. Food Chem, 103:670-675.

Wildi, W., Domink, J., Thomas, RL., et al. (2004). River, reservoir and lake sediment contamination by heavy metals downstream from urban areas of switzerland. Lakes & Reservoirs: Res Manag, 9:75-87. Doi.org/10.1111/j.1440-1770.2004.00236.x