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Oxidative Damage in Some Tissues of Van Fish (*Alburnus tarichi* Güldenstädt, 1814) Having Abnormal Ovary

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Abstract: Van Lake is the largest soda lake of Turkey and there is only one fish species (Van Fish, *Alburnus tarichi* Güldenstädt, 1814) that can survive under the extreme conditions of the lake. There are several researches carried out on the issue of lake contamination recently. Gonad abnormalities observed among these fish are one of the most important issues. In the present study, the malondialdehyde (MDA) and reduced glutathione (GSH), and the antioxidant enzyme levels such as superoxide dismutase (SOD) and glutathione peroxidase (GSH – Px) are compared in gills, livers, and gonads of the Van Fish (the ovary abnormality of which are determined in Van Lake) and the normal fish. Compared to the normal fish, the MDA levels of the abnormal fish show a considerable amount of increase in all of the tissues ($P<0.05$). There is a significant decrease in the SOD activity in the gill and ovary tissue and in the GSH – Px activity in the livers of the abnormal individuals compared to the normal individuals ($P<0.05$). As for the GSH levels, there is no difference found when the normal and abnormal tissues are compared. Consequently, when the fish with ovary abnormalities which are thought to be exposed to contaminators in a certain time period is compared to the normal fish, it can be said that their antioxidant defense systems are affected.

Keywords: *Alburnus tarichi*, Lake Van, Ovary abnormalities, Oxidative enzymes, Van Fish

Anormal Gonadlı Van Balığının (*Alburnus tarichi* Güldenstädt, 1814) Bazı Dokularında Oksidatif Hasarın Belirlenmesi

Özet: Van Gölü dünyanın en büyük sodalı gölü olup gölde ekstrem şartlara uyum sağlayan tek bir balık türü olan Van balığı yaşamaktadır. Son yıllarda, gölün kirliliğine ilişkin pek çok çalışma yapılmıştır. Özellikle balıklar arasında gözlenen gonad anormallikleri bunlardan en önemlisidir. Bu çalışmada, Van gölünde ovaryum anormalliği belirlenen Van balığı ve normal balıklarda malondialdehit (MDA) seviyeleri ve redükte glutatyon (GSH) düzeyleri ile superoksid dismutaz (SOD) ve glutatyon peroksidaz (GSH-px) gibi antioksidan enzim seviyeleri solungaç, karaciğer ve gonadlarda karşılaştırıldı. MDA seviyeleri anormal bireylerde normal bireylere göre bütün dokularda önemli oranda artış gösterdi ($P<0.05$). Anormal bireylerin solungaç ve ovaryum dokularındaki SOD aktivitesi ve karaciğer dokusundaki GSH-Px aktivitesi normal bireylere göre azalma önemli bulundu ($P<0.05$). GSH seviyesinde ise normal ve anormal dokuların karşılaştırılmasında fark bulunmadı. Sonuç olarak belirli bir dönemde kirleticilere maruz kaldığı düşünülen anormal ovaryumlu balıkların normal balıklarla karşılaştırıldığında antioksidant savunma sistemlerinin etkilendiği söyleyenebilir.

Anahtar kelimeler: *Alburnus tarichi*, Oksidatif enzimler, Ovaryum anormallikleri, Van Balığı, Van Gölü

Introduction

Van Lake is the largest soda lake of Turkey. Even though Van Fish (*Alburnus tarichi* Güldenstädt, 1814) is the only species living in the lake, they have a great importance in the region. Van Fish is also called as Tarek and Pearl mullet. Due to the decrease in the population, it is added to the red list in 1996. The reason for this decrease is shown to be only due to overfishing (Freyhof 2014). However, gonadal abnormalities have recently been observed to a great extent on both male and female fish. These abnormalities are characterized by a decrease in gonad weight and gonadosomatic index values, collagen tissue accumulation in gonad and immature oocyte follicles (Unal et al. 2007). In the related studies, the reason of these abnormalities were stated to be caused by endocrine disrupting chemicals (Unal et al.

2007) and heavy metals (Oğuz and Yeltekin 2014). In parallel to these studies, some endocrine disrupting chemicals and heavy metals were detected in the lake Van (Bilgili et al. 1995; Oğuz and Kankaya 2013).

Free radicals damage the cellular elements such as lipid, protein, and DNA by affecting them. The antioxidant defense systems are developed in order to control the free radical formation and to prevent their harmful effects. However, this system sometimes cannot prevent the effect of the free radicals. A condition named oxidative stress emerges. Different studies report that contamination cause oxidative stress on fish (Bainy et al. 1996; Valavanidis et al. 2006).

Agricultural, industrial and domestic wastes are gathered in wetlands and the living animals in these wetlands are exposed to these chemicals. The chemicals called xenobiotics cause adaptive alterations or deteriorations on the biologic systems of the living organisims. Some bio-indicators are utilized in order to determine the contamination levels in fish. Antioxidants are one of the most important indicators (Lushchak 2011; Pandey et al. 2003; Valavanidis et al. 2006). In the laboratory and field studies, a correlation is observed among the contaminators and antioxidant enzyme levels (Gül et al. 2004; Sanchez et al. 2005; Xing et al. 2012).

In the present study, the malondialdehyde (MDA) levels and reduced glutathione (GSH) levels, and the activities of antioxidant enzyme levels such as superoxide dismutase (SOD) and glutathione peroxidase (GHS-Px) in gill, liver and ovary tissues of the Van Fish with normal and abnormal gonads were analyzed.

Material and Methods

Fish were caught from offshore of Van Yüzüncü Yıl University (Figure 1.) using casting nets. The fork length and weight of the caught female fish were measured and then dissected. In the dissection phase, the female and abnormal fish were morphologically determined (Figure 2.). Samples were taken from the gill, liver and gonad tissues and stored at -80 ºC until the analysis. By using the operculum samples of the fish, their ages were determined. All study procedures were carried out according to national animal care regulations.

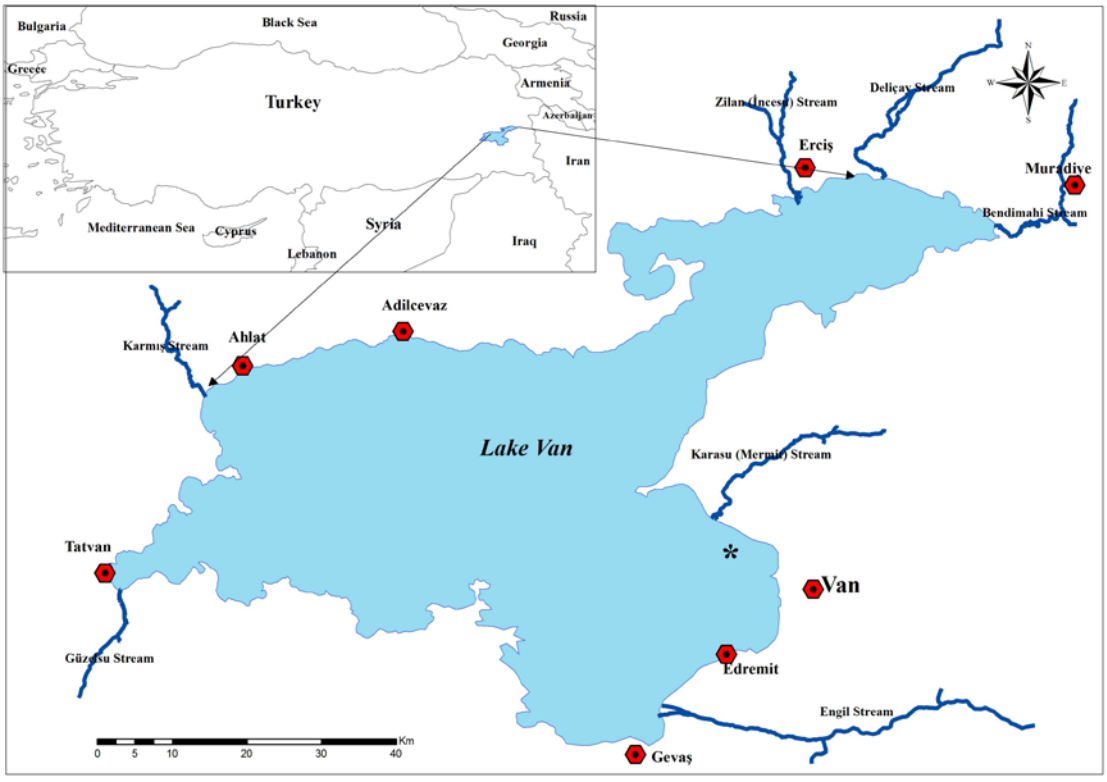

Figure 1. Localization of Lake Van (*Sampling area).

For biochemical analysis, the tissues were homogenized for 5 min in 50mM ice-cold KH_2PO_4 solution (1:5 w/v) using stainless steel probe homogenizer (ultrasonic frequency 20 KHz; Jencons Scientific, Leighton Buzzard, Beds, UK) for 5 min and then centrifuged at 7000 g for 15 min.

Figure 2. Normal (A) and abnormal (B) ovaries of female Lake Van Fish.

The concentration of MDA in tissues was determined using the method described by Jain et al. (1989) based on thiobarbituric acid reactivity. The concentration of GSH in tissues was measured using the method described by Beutler et al. (1963). GSH-Px activity was assayed as described by Paglia and Valentine (1967) based on that of GSH-Px catalyzes and the oxidation of GSH by cumene hydroperoxide. Superoxide dismutase activity was measured at 505 nm by calculating inhibition percentage of formazan dye formation (McCord and Fridovich 1969).

The data were expressed as the mean ± standard error of the mean (SEM). The data were analyzed using t test comparison to determine the differences between groups. Significance was accepted at $P < 0.05$.

Results and Discussion

A reference region which does not have contamination in the lake is not used in this study. The contaminators might mix in the wave circulation of the waters of the lake. Moreover, due to the fact that the fish migrate depending on the water temperature, they have a limited habitat, which increases the possibility of being exposed to the contaminators in the water during a certain living period. Therefore, instead of a reference region, the fish with abnormal ovary which is thought to be exposed to contaminators are compared with the fish with normal ovary in terms of their MDA, GSH, SOD and GSH-Px levels.

The fish used in the study have the reproductive maturity (III+), fork length and total weight were 15.2–20.8 cm and 42.5–111.9 g, respectively. Ovary abnormalities are significantly observed on the dissected fish (Figure 2.). As a result of the somatic indexes, significant differences were detected between normal and abnormal fish (data not shown).

As is known, free radicals cause lipid peroxidation on the organisms. The malondialdehyde (MDA) level in the tissue is a useful biomarker in order to indicate the lipid peroxidation (Ayala et al. 2014; Draper

and Hadley 1990). In this study, the MDA level shows increase in gills, livers and gonads of the fish with abnormal ovary. Gills are the organs which are directly exposed to the effect of the contaminators. Liver and gonad are closely related to each other for female fish. The yolk proteins released from the liver are carried with blood into the gonads and accumulate in oocytes. This process does not emerge on abnormal fish and instead of mature oocytes, there observed immature oocytes and great number of atretic follicles (Unal et al. 2007). Moreover, heavy metals accumulated in abnormal ovary and liver might be the cause of increase in the MDA levels (Oğuz and Yeltekin 2014).

In the study, the values of MDA, SOD, GSH and GSH-px in gill, liver and gonad tissues were displayed in Table 1., 2., and 3. As a result of this analysis, when the gills of the abnormal fish were compared to the gills of the normal fish, an increase was observed in the MDA and SOD levels (Table 1) ($P<0.05$). For the abnormal fish, the levels of MDA and GSH-Px values decrease in the liver (Table 2) and the MDA and SOD levels show a great amount of decrease in gonads (Table 3) ($P<0.05$). The GSH levels were found to be insignificant for all the tissues belonging to both groups.

Table 1. Antioxidant parameters in gill from fish having normal and abnormal gonads

	MDA	**GSH**	**SOD**	**GSH-px**
Normal fish	36.721±4.211	17.532±1.296	4550.984±62.441	7.064±0.740
Abnormal fish	84.970±9.054*	18.891±1.031	4861.607±32.130*	5.432±0.513

Data represent Mean±SEM (n:10). All activities are expressed in nmol/g. *: $P<0.05$

Table 2. Antioxidant parameters in liver from fish having normal and abnormal gonads.

	MDA	**GSH**	**SOD**	**GSH-px**
Normal fish	42.744±2.776	14.484±0.699	4444.832±177.759	101.784±6.796
Abnormal fish	55.697±1.451*	16.901±1.847	4333.186±137.853	22.914±2.528*

Data represent Mean±SEM (n:10). All activities are expressed in nmol/g. *: $P<0.05$

Table 3. Antioxidant parameters in gonads from fish having normal and abnormal gonads.

	MDA	**GSH**	**SOD**	**GSH-px**
Normal fish	48.638±3.806	20.760±2.0278	5021.791±32.316	4.491±0.248
Abnormal fish	83.286±2.664*	19.230±2.735	4854.233±48.423*	4.710±0.427

Data represent Mean±SEM (n:10). All activities are expressed in nmol/g. *: $P<0.05$

In this study, while the SOD levels of the fish with normal ovary increase; the SOD level of their gonads decreases (Table 3). The GSH-Px level of the liver decrease, yet, there is no alteration observed in the GSH-Px levels of the other tissues. Even though some heavy metals are important for the livings since they are used in metabolic events, they also have a high level of toxic effect (Ercal et al. 2001; Jomova and Valko 2011; Sanchez et al. 2005). The accumulations of the metals like Zn and Co on abnormal Van Fish livers and gonads (Oğuz and Yeltekin 2014) might lead to a decrease in GSH-Px and SOD levels successively on liver and gonad tissues. Moreover, the intensity, influence duration and type of the contaminators might cause an increase or a decrease in the level of antioxidant enzyme levels (Xing et al. 2012).

Even though the oxidative enzymes are used in order to monitor the wetland contamination, this kind of enzyme levels can change for different fish species. Therefore, the chemicals which cause the contamination have different effects on enzyme levels of the livings (Förlin et al. 1995). They even have different effects on different tissues of the same species (Ahmad et al. 2000). Such effects are also observed in this study.

Pathologic studies are a useful alternative method to oxidative stress biomarkers in determining the contamination of the wetlands (Bernet et al. 1999; Teh et al. 1997). The histopathology study of the fish with abnormal ovary is only carried out in ovary; there found no studies carried out in the liver, gill, kidney or other tissues of the fish. However, in the histologic studies carried out independently from gonad abnormalities, it is reported that some histopathological alterations are observed in gill and kidney tissues of Van Fish (Oğuz 2015a; Oğuz 2015b).

Even though it is observed that Van Fish with ovary abnormalities were sensitive to oxidative stress created by the contaminators, it is also necessary to carry out experimental studies in which heavy metals and endocrine disrupting chemical groups by which the fish are thought to be influences—are applied. Moreover, the tissues of the fish with ovary abnormalities other than gonad tissues should be comprehensively studied in the field of histopathology.

Conclusion

Van Fish can be used as bioindicators of pollutants (Endocrine Disrupting Chemicals and heavy metals) in the Lake Van by studying the induction of oxidative stress. However, gill, liver tissue pathologies is necessary to study histologically in addition to gonad tissues.

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