



-RESEARCH ARTICLE-

Effects of Lead and Selenium Interaction on Acetylcholinesterase Activity in Brain and Accumulation of Metal in Tissues of *Oreochromis niloticus* (L., 1758)

Gülsemin Şen*, Sahire Karaytuğ

Mersin University, Faculty of Fisheries, Department of Basic Sciences, Yenişehir Campus, 33160, Mersin, Turkey

Abstract

The potential accumulation of lead in different tissues of *Oreochromis niloticus* and the effects of selenium in AChE inhibition caused by lead in brain were investigated. Juvenile *O. niloticus* samples were exposed to combination of 1 mg L⁻¹ and 2 mg L⁻¹ lead and 1mg L⁻¹ lead+2mg L⁻¹ selenium and 2mg L⁻¹ lead+4mg L⁻¹ selenium for 1, 7 and 15 days respectively. The accumulation of lead in gill, brain, liver and muscle tissues was analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) as well as brain acetylcholinesterase (AChE, E.C.3.1.1.7) enzyme activity was also analyzed by spectrophotometric method. No mortality was observed during lead exposure in relation to time period and exposed concentrations. Lead accumulation was occurred in all tissues in relation to time. Maximum lead accumulation occurred in brain tissue, followed by the liver, gills and muscle tissues in relation to time period. Selenium caused a decrease on accumulation of lead in tissues (all selenium mixtures in muscle tissue on the first day, 1mg L⁻¹ Pb+2mg L⁻¹ selenium in gill tissue on the seventh day, in liver tissue on the seventh day except 2mg L⁻¹ Pb+4mg L⁻¹ selenium mixtures) at the end of each of all three test periods. Inhibition of AChE activity was caused by the highest concentration and by the short-term effect of lead. Such effect of lead was eliminated by selenium mixture. Lead and selenium mixture were resulted an increase in activity on 15th day at the highest concentration. Selenium led to decrease in the accumulation of lead in the tissues and caused to improvement in the loss of AChE activity.

Keywords:

Acetylcholinesterase, Lead, *Oreochromis niloticus*, Selenium

Article history:

Received 11 May 2017, Accepted 05 June 2017, Available online 20 June 2017

* Corresponding author: gulsemensen@mersin.edu.tr

Introduction

Having learned to process copper in the Stone Age, man kept dealing with various metals over time; on the one hand he has been affected by them, and started to pollute his environment. Fast population growth, modern agricultural applications, increasing number of industrial businesses caused excess accumulation of heavy metals in aquatic environments (Kalay et al., 2003). Heavy metals are known for their serious potentials to pollute the environment due to their presence in the environment and their being accumulated by aquatic organisms. Recently the organisms are negatively affected by the increased level of heavy metals in aquatic habitats (Çoğun & Kargin, 2013).

Lead (Pb) has no metabolic effect but organisms are exposed to it in all environments. Interactions between lead and biological systems have negative health impacts on human beings. Presenting a chemical similarity to calcium, it is processed with calcium, then attached to bone tissue. In spite of this, tissues other than bones also are regions where lead (Pb) is accumulated in fishes (Van Oasten, 1957). First blocking the opening of calcium canals sensitive to voltage, lead prevents calcium from entering the cells undergone exposed to pre-synaptic inhibition, thus releasing stimulated transmitter. However, it has been shown that lead enters neuronal intracellular environment (Bressler & Goldstein, 1991).

Selenium is an important element which prevents the accumulation of metals in organisms and essential micronutrient for animals. Intake of selenium in varying amounts affects different functions of the organism. Small amounts are needed for growth and development; medium intake is stored in the organism, and enables to maintain homeostatic functions. But, high amounts cause toxic effects (Eisler, 2000). In case of Se presence in the environment, metal accumulation has been shown to decrease in invertebrate animals (Tran et al., 2007) and in fish (Kehrig et al., 2009).

Acetylcholine is synthesized in neurons soma by combining choline with acetyl (originating from acetyl-CoA) via choline acetyl transferase (ChAT). Synthesized acetylcholine is transported to the nerve ends via axonal transport and released to the synaptic space (Geula & Mesulam, 1999). Acetylcholinesterase is responsible for hydrolysis of acetylcholine and therefore important for cholinergic neuronal system (Nachmansohn & Wilson, 1951). The inhibition of acetylcholinesterase causes accumulation of acetylcholine in the neuromuscular synapses and nerve synapses creates abnormal results, the most important one is the over activity of muscle tissues (Roex et al., 2003). This over activity in fish leads to changes of behavior such as hyperactivity and anorexia as well as physiological effects such as asphyxia, potentially conducive to death (Beauvais et al., 2000).

This study aims to evaluate the impact of accumulation potential of lead in different tissues in *O. niloticus*, and the impact of selenium on the brain AChE enzyme inhibition caused by lead during a period of 1, 7 through 15 days.

Material and Method

O. niloticus used in this research have been taken from The State Hydraulic Works Fish Culture at the Fisheries Faculty of Cukurova University and their adaptation to laboratory conditions have been provided by keeping them in 7 glass aquariums in the size of 40X100X40 cm and containing 120L stabilized tap water for 60 days. Similar size of fish, $12,40 \pm 0,57$ cm in length and $29,59 \pm 2,68$ g in weight were used in the experiment. A total of sixty three fishes were used, 21 fishes for each trial (replicated 3 times for 1, 7 and 15 days) including the control group.

Experiments have been made in Basic Sciences Investigation Laboratory at the Faculty of Aquaculture in Mersin University where the temperature is stabilized as $25\pm 1^\circ\text{C}$ and 12 hours light and 12 hours dark photoperiod is applied. Fishes were fed with commercial pelleted diet of 2% of total biomass (Çamlı Fish Food Industry., İzmir). The physiochemical parameters of the stabilized tap water which was used in the investigation have been determined as; dissolved oxygen $8,54\pm 0,37\text{ mgL}^{-1}$, pH $8,32\pm 0,20$, temperature is $21,07\pm 0,66^\circ\text{C}$, total alkalinity is $325\pm 0,80\text{ mgL}^{-1}$, total hardness is $230\pm 6,36\text{ mgL}^{-1}\text{ CaCO}_3$.

Considering the literature knowledge of sublethal concentrations in the investigation (Oladimeji & Offem, 1989, Ranzani-Paiva et al., 2011), 1 and 2 mg/L have been determined for lead ($\text{Pb}(\text{NO}_3)_2$); 2 and 4 have been determined for selenium (Na_2SeO_3). Experiments have been carried out as 3 series and 7 glass aquariums in the size of $40\times 100\times 40\text{ cm}$ have been used in each serial. Fish have been exposed to effect of concentration of 1 mgL^{-1} and 2 mgL^{-1} Pb with 1 mgL^{-1} Pb + 2 mgL^{-1} Se and 2 mgL^{-1} Pb + 4 mgL^{-1} Se in the 3 times repetitive investigation with the duration of 1, 7 between 15 days. A control group as well as determined doses have been used. The water in the experiment and control aquarium have been refreshed once in two days because there could be some changes in the concentration of experiment solutions due to adsorption, precipitation and evaporation.

The samplings were made on 1, 7 and 15. days and fish were taken from aquarium and anesthetized with ice before each dissection. The fishes were killed by cutting their cerebrospinal connections and brain tissues were taken. The tissues were washed with 0,59% serum physiologic and kept in -80°C until the AChE analysis have been made. Being kept in incubator adjusted to 150°C for 72 hours, gill, brain, liver and muscle tissues have been brought to stable weighing for the analysis of metal accumulation, and transferred to experiment tubes, tissues, whose dry weight were determined, have been digested for 3 hours at the end of the 72 hours by adding 2 mL nitric acid (Merck, %65, d:1.40) and 1 mL perchloric acid (Merck, %60, d:1.53) (Kargin & Erdem, 1992). Digested samples have been transferred to covered test tubes and completing to 5 mL with the addition of pure water they have been made ready to metal analysis. The metal analysis in tissues and organs is determined with Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (Agilent 7500ce). After homogenizing the tissues taken from freezer at 10.000 rpm in ice for three minutes by adding 1/10 percent 0.05 M Na-K phosphate buffer (pH=7.4) in the aim of the determination of AChE enzyme activity, this activity has been determined via spectrophotometric methods (Lowry et al., 1951, Ellman et al., 1961) in supernatants which were attained via 30 minutes centrifuging at $+4^\circ\text{C}$ and 9.500g.

Statistical analysis of data have been made by using one-way analysis of variance (ANOVA), Duncan method of multiple comparisons tests and statistical program SPSS 16.0. The differences between groups have been considered as $P<0,05$.

Results

The effects of selenium on the accumulation of lead in gill, brain, liver and muscle tissues at the end of the first, seventh, fifteenth days in *O. niloticus* have been shown in order on Table 1.A-1.D. Lead has shown time dependent accumulation in all fish tissues which were exposed to it. Pb accumulation has been determined mostly in brain tissues in all attempted processes and it has been followed by liver, gill and muscle tissues.

Se has provided a decreasing in Pb accumulation at the end of each three experimentation process except from all Se concentrations in muscle tissues on 1. day, 1 mg L^{-1} Pb+ 2 mg L^{-1} Se concentration in gill tissue on 7. day and 2 mg L^{-1} Pb+ 4 mg L^{-1} Se concentration in liver tissue on

7. day.

Decreasing accumulation of lead in 1mg L⁻¹ Pb+2mg L⁻¹ Se and 2mg L⁻¹ Pb+4mg L⁻¹ Se concentrations in gill tissue has been occurred in order as 73 and 74% at the end of 1. day, 5 and 61% at the end of the 7. day and 28 and 39% at the end of the 15. day.

Decreasing accumulation of lead in 1mg L⁻¹ Pb+2mg L⁻¹ Se and 2mg L⁻¹ Pb+4mg L⁻¹ Se concentrations in brain tissue has been occurred in order as 14 and 54% at the end of the 1. day, 40 and 31% at the end of the 7. day, 60 and 70% at the end of the 15. day.

Decreasing accumulation of lead in 1mg L⁻¹ Pb+2mg L⁻¹ Se and 2mg L⁻¹ Pb+4mg L⁻¹ Se concentrations in liver tissue has been occurred in order as 18 and 42% at the end of the 1. day and 76 and 55% at the end of the 15. day. Although the concentration of 1mg L⁻¹ Pb+2mg L⁻¹ Se has reduced the Pb accumulation 52% at the end of the 7. day, the accumulation has been increased in 2mg L⁻¹ Pb+4mg L⁻¹ Se concentration 42%.

Decreasing accumulation of lead 1mg L⁻¹ Pb+2mg L⁻¹ Se and 2mg L⁻¹ Pb+4mg L⁻¹ Se concentrations in muscle tissue has been occurred in order as 33 and 158% at the end of the 1. day, 65 and 48% at the end of the 7. day and 44 and 19% at the end of the 15. day.

Table 1.A. The effect of different selenium concentrations on different concentrations of lead in gill tissue at *O. niloticus* on 1., 7. and 15. days ($\mu\text{g Pb/gk.a.}$)

CONCENTRATION	TIME (DAY)		
	1. Day	7. Day	15. Day
	- ± -	- ± -	- ± -
Control	ND ^a	ND ^a	ND ^a
1 mgL ⁻¹ Pb	7,84 ± 0,33 ^{bx}	44,59 ± 1,85 ^{by}	76,79 ± 2,08 ^{bz}
1 mgL ⁻¹ Pb+2 mgL ⁻¹ Se	2,16 ± 0,31 ^{cx}	42,28 ± 1,10 ^{by}	55,36 ± 0,47 ^{cz}
2 mgL ⁻¹ Pb	11,86 ± 0,75 ^{bx}	78,28 ± 1,76 ^{by}	110,33 ± 0,52 ^{bz}
2 mgL ⁻¹ Pb+4 mgL ⁻¹ Se	3,06 ± 0,52 ^{cx}	30,70 ± 0,04 ^{cy}	67,83 ± 0,29 ^{cz}

* The letters a, b and c are used to indicate the difference between the control of Se concentration and corresponding Pb group; the letters x, y ve z are used to indicate the difference between times. There is a statistical difference between data which are shown with different letters.(P<0.05, N = 3).

ND : Not detectable.

- ± - : Mean ± Standart error

Table 1.B. The effect of different selenium concentrations on different concentrations of lead in brain tissue at *O. niloticus* on 1., 7. and 15. days ($\mu\text{g Pb/g k.a.}$)

CONCENTRATION	TIME (DAY)		
	1. Day - \pm -	7. Day - \pm -	15. Day - \pm -
Control	ND	ND ^a	ND ^a
1 mgL ⁻¹ Pb	47,97 \pm 0,85 ^{bx}	173,70 \pm 3,23 ^{by}	251,23 \pm 0,86 ^{bz}
1 mgL ⁻¹ Pb+2 mgL ⁻¹ Se	41,13 \pm 2,62 ^{cx}	104,57 \pm 0,72 ^{cy}	100,57 \pm 0,20 ^{cy}
2 mgL ⁻¹ Pb	83,64 \pm 0,62 ^{bx}	233,97 \pm 0,06 ^{by}	363,30 \pm 2,97 ^{bz}
2 mgL ⁻¹ Pb+4 mgL ⁻¹ Se	38,19 \pm 1,85 ^{cx}	162,67 \pm 3,20 ^{cy}	109,53 \pm 0,44 ^{cz}

* The letters a, b and c are used to indicate the difference between the control of Se concentration and corresponding Pb group; the letters x, y and z are used to indicate the difference between times. There is a statistical difference between data which are shown with different letters. (P<0.05, N = 3).

ND : Not detectable.

- \pm - : Mean \pm Standard error

Table 1.C. The effect of different selenium concentrations on different concentrations of lead in liver tissue at *O. niloticus* on 1., 7. and 15. days ($\mu\text{g Pb/g k.a.}$)

CONCENTRATION	TIME (DAY)		
	1. Day - \pm -	7. Day - \pm -	15. Day - \pm -
Control	ND ^a	ND ^a	ND ^a
1 mgL ⁻¹ Pb	12,99 \pm 0,85 ^{bx}	112,13 \pm 1,40 ^{by}	139,94 \pm 2,76 ^{bz}
1 mgL ⁻¹ Pb+2 mgL ⁻¹ Se	10,72 \pm 0,37 ^{cx}	53,36 \pm 0,36 ^{cy}	33,83 \pm 1,91 ^{cz}
2 mgL ⁻¹ Pb	8,72 \pm 0,83 ^{bx}	98,20 \pm 1,10 ^{by}	146,00 \pm 3,46 ^{bz}
2 mgL ⁻¹ Pb+4 mgL ⁻¹ Se	5,04 \pm 0,44 ^{cx}	139,00 \pm 2,82 ^{cy}	66,26 \pm 1,25 ^{cz}

* The letters a, b and c are used to indicate the difference between the control of Se concentration and corresponding Pb group; the letters x, y and z are used to indicate the difference between times. There is a statistical difference between data which are shown with different letters. (P<0.05, N = 3).

ND : Not detectable.

- \pm - : Mean \pm Standard error

Table 1.D. The effect of different selenium concentrations on different concentrations of lead in muscle tissue at *O. niloticus* on 1., 7. and 15. days ($\mu\text{g Pb/g k.a.}$)

CONCENTRATION	TIME (DAY)		
	1. Day	7. Day	15. Day
	- ± -	- ± -	- ± -
Control	ND ^a	ND ^a	ND ^a
1 mgL ⁻¹ Pb	5,25 ± 0,50 ^{bx}	20,24 ± 0,64 ^{by}	42,16 ± 3,10 ^{bz}
1 mgL ⁻¹ Pb+2 mgL ⁻¹ Se	6,98 ± 0,10 ^{cx}	7,16 ± 0,23 ^{cx}	23,81 ± 2,43 ^{cy}
2 mgL ⁻¹ Pb	15,47 ± 0,59 ^{bx}	16,48 ± 1,25 ^{bx}	40,02 ± 1,66 ^{by}
2 mgL ⁻¹ Pb+4 mgL ⁻¹ Se	39,97 ± 1,15 ^{cx}	8,56 ± 0,29 ^{cy}	32,37 ± 0,58 ^{cz}

* The letters a, b and c are used to indicate the difference between the control of Se concentration and corresponding Pb group; the letters x, y ve z are used to indicate the difference between times. There is a statistical difference between data which are shown with different letters. ($P < 0.05$, $N = 3$).

ND : Not detectable.

- ± - : Mean ± Standart error

The effects of lead and selenium concentrations on AChE activities in brain tissue at *O. niloticus* at the end of 1., 7. and 15. days are shown in Table 2. Pb has decreased specific AChE activity 48% in brain tissue at its attempted highest concentration on 1. day (Figure 2.A.). 4mg L⁻¹ Se implementation has prevented AChE activity lost which is caused by 2mgL⁻¹ Pb. Activity in the concentration group has shown 77% increase though it has been close to control level in contrast to group which is given 2mg L⁻¹ Pb.

No change has been determined in AChE activities of all test groups at the end of the 7. day. AChE activity has shown in order 41 and 50% increase in 1mg L⁻¹ Pb+2mg L⁻¹ Se concentration group in contrast to control and 1mg L⁻¹ Pb group (Figure 2.B.). Pb has not created any change in AChE activity.

Table 2. The effects of different concentrations of lead and selenium on specific AChE activities ($\mu\text{M}/\text{min}/\text{mg protein}$) in brain tissue at *O. niloticus* on 1., 7. and 15. days

CONCENTRATION	Specific activity of AChE		
	1. Day	7. Day	15. Day
	- ± -	- ± -	- ± -
Control	0,24±0,01 ^a	0,23±0,03	0,17±0,01 ^a
1 mgL ⁻¹ Pb	0,19±0,00 ^{ab}	0,17±0,02	0,16±0,01 ^a
1 mgL ⁻¹ Pb+2 mgL ⁻¹ Se	0,28±0,03 ^{ab}	0,18±0,05	0,24±0,03 ^b
2 mgL ⁻¹ Pb	0,13±0,01 ^b	0,14±0,00	0,10±0,01 ^a
2 mgL ⁻¹ Pb+4 mgL ⁻¹ Se	0,23±0,02 ^{ab}	0,13±0,01	0,15±0,01 ^a

*: The letters a and b are used to show differentiation between concentrations. There is statistical differences between data which are shown with different letters. ($P < 0.05$, $N = 3$).

- ± - : Mean ± Standart error

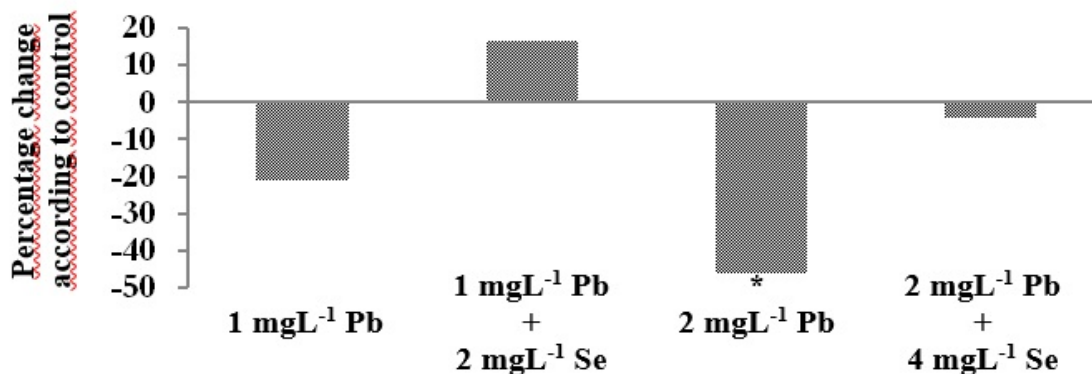


Figure 2.A. Percentage change of specific AChE activity in brain tissue according to control group in Pb and Se concentrations at *O. niloticus* on 1. day.

*:Shows statistical differentiation according to control group ($P < 0.05$).

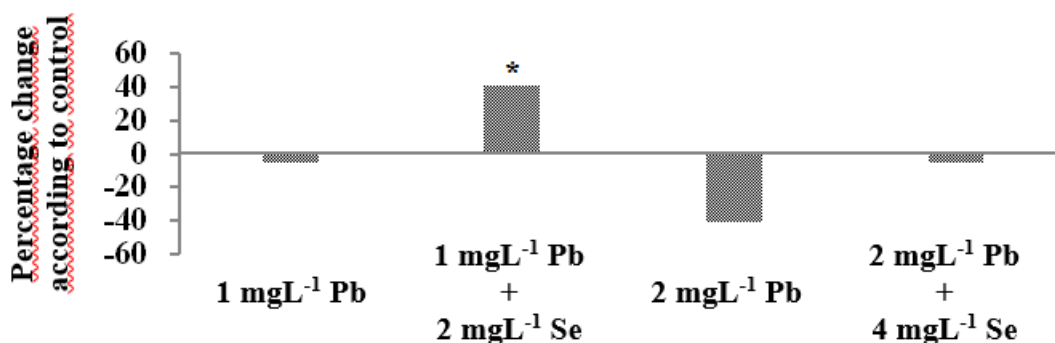


Figure 2.B. Percentage change of specific AChE activity in brain tissue according to control group in Pb and Se concentrations at *O. niloticus* on 15. day.

Discussion

In the case that excretion mechanisms in fish does not work equally to ingestion, toxic materials like heavy metals are stored in tissues and organs. The amount of metal accumulation increases dependently to effect time and the concentration of metal. Different heavy metals are stored in different tissues and organs of fish in different amounts (Flos et al., 1987) and it shows differences between species that in which tissue or organ a certain metal will be stored primarily (Kargin & Erdem, 1991). It is indicated that lead storage in tissues of *Procambarus clarkii* increases dependently to environment concentration of lead and time (Anderson et al., 1997). It is determined in an investigation about *Carassius auratus* that lead storage in tissues increases in a parallel way to increase of lead concentration in the environment (Tao et al. 1999).

It is determined that concentrations of heavy metals without lethal are stored mostly in metabolically active organs of fish such as liver (Kargin & Erdem, 1992). Because the liver tissue is one of the main place of synthesizing binding protein metals which work in transportation and detoxification of heavy metals, its function in lead detoxification is too much. It is determined in *Cyprinus carpio* that lead accumulation in tissues shows differences and lead accumulation reaches

high levels in organs like kidney and liver (Bervoets et al., 2009). While lead accumulation in liver tissue of *C. Carpio* increases dependent upon environment concentration and time of being under effect, it shows a constant decrease in muscle tissue dependent upon time after a certain concentration, and accumulation is more in liver tissue than in muscle tissue (Nevşat, 1995).

Lead in aquatic ecosystems can be taken by fish via gills and digestion. Lead can easily reach soft tissues make storage in tissues like kidney, brain, gill, bone and liver (Berman, 1980). The highest lead accumulation in concentrations of 0.1 mg L^{-1} Pb and 1.0 mg L^{-1} at the Nile tilapia (*Oreochromis niloticus*) takes place in kidney tissue and it is followed by gill, liver and muscle tissues (Çoğun & Şahin, 2012). It was indicated that gill is the first target organ of lead's toxic effect in *O. mykiss* according to other tissues and accumulation in this tissue reaches high levels (Roger et al., 2003).

Lead is accumulated also in brain and bone tissues at an important level beside the tissues like gill, liver and kidney (Varanasi & Markey, 1978, Demichele, 1984). It was determined that lead is accumulated mostly in brain tissue after kidney tissue in *Tilapia zillii* (Karataş & Kalay 2002). Muscle tissue in fish is not metabolically active tissue in terms of accumulation of heavy metals. It was determined that lead accumulation in muscle tissues of *C. carpio* and *O. niloticus* at each type of environment concentration and times that are exposed is less than other organs (Blevins & Pancorbo, 1986, Kargin, 1998). It was confirmed in this investigation that lead accumulation increases dependent upon time and concentration and accumulation in tissues takes place in order as brain, liver, gill and muscle tissues. It is thought that clearing the hurdle of blood and brain swiftly, lead which replaces with calcium ions (Ca^{+2}) centers in brain tissue and that's why accumulation in brain tissue is high. It was determined that high amount of lead is accumulated in brain and bone tissues of *Anabas testudineus* (Tulasi et al., 1992).

Heavy metal accumulation in fish changes according to interaction between metals as it changes according to tissue and organ (Pagenkopf, 1983). It was confirmed that arsenic and mercury show an antagonistic effect to selenium in the investigation on freshwater fish in Burkina Faso (Quedraogo & Amyot, 2013). It was also determined that accumulation of lead in kidney is less in the interaction of lead with cadmium and mercury than in the effect of only lead and it is more than in liver and muscle tissues in *O. aureus* (Allen, 1995). Metal accumulation in tissues and organs which was investigated under the effect of copper-zinc combination in *C. carpio* is less than the effects of copper and zinc separately (Cicik, 2003).

Complex of lead and selenium was defined as lead selenit (PbSe) and it was speculated that this complex structure decreases the free lead ions in body (Ganther et al., 1972, Frost, 1973). It was indicated that selenium creates complexes with heavy metals like cadmium, lead, silver and such complexes make these metals less harmful for cellular structures (Nehru et al., 1997). It was determined in this investigation that selenium decreases the accumulation of lead in tissues. This could be because of the chemical antagonistic effect of selenium on lead.

Increasing the accumulation of acetylcholine in brain, AChE inhibition causes overstimulation of cholinergic receptors. As a result of this, a general decrease emerges in control of nerve and muscle. In parallel with this, neurotoxin effects which seem under the effect of pollutants are associated with changes in normal behaviors (Pereira et al., 2012). It was determined in this investigation that lead created an inhibition in AChE activity only in its highest concentration which is applied and at the end of only first 24 hours. Heavy metals taken into the living body via many different ways are accumulated at very different levels in different tissues and organs. The ones which has physiologic importance are stored out of metals which can be thrown out after joining in some metabolic ways in living body. Stored metal, if it is toxic, can destroy the structures of enzymes (Yazkan et al., 2004). Being an important enzyme inhibitor, lead creates denaturation

and loss of activity especially in enzymes which include selenium and sulfur (Anon., 1992, Göker, 1996). It was determined that AChE activity is inhibited by metals like lead and zinc in the brain tissue of *Danio rerio* (Richetti et al., 2011). It is thought that the inhibition of AChE activity under the effect of lead is caused by binding of metals to the functional groups of proteins like imidazole, sulfhydryl and carboxyl (Najimi et al., 1997). Catalytic activity loss emerges because of this kind of changes in functional groups (Sant'Anna et al., 2011).

Glutathione-S-transferase (GST), glutathione peroxidases (GPOX) and superoxide dismutase (SOD) as antioxidant enzymes is very important in heavy metal detoxification mechanisms. Selenium (Se) is included in the structure of GPx enzyme which is antioxidant and has an important role in terms of preventing oxidative stress. Including in its every single unit one Se atom in the shape of selenosistein, GPx act in the reduction of hydrogen peroxide (H_2O_2) to water in cell (Büyükyüz et al., 2000). It was indicated that AChE inhibition in *Channa punctatus* showed a decrease with the addition of Se to the environment which includes high and low concentrations of As (Roy et al., 2006). It was confirmed that the inhibition of thioredoxin reductase activity of Se acts in preventing in vivo zinc toxicity (Branco et al., 2012). It is thought in this investigation that the protective effect of selenium against to AChE enzyme inhibition which is caused by lead is created by chemical antagonism that Se shows with lead. As it is known, a toxicant should be stored enough in its target first in order to be effective on its target (Casarett et al., 2007).

It is determined in this investigation that the highest concentration of lead and selenium combination on 15.day creates an increase in AChE activity. Inorganic selenium forms like sodium selenite cause a constant spasm in muscle by being attached to sulfhydryl groups of membrane proteins at chicken neuron muscle preparation, out of membrane and this effect decreases with the amount of extracellular Ca^{+2} (Lin-Shiau et al., 1990). It is determined that high dosage of sodium selenite causes irregularity in oscillation of presynaptic acetylcholine in *Caenorhabditis elegans* and damage in neurons (Annette et al., 2012). It was determined in brain tissues of rats which are under the effect of pesticide imidakloprid that increase in AChE activity can be caused by apoptic events which are induced by cellular ingestion of Ca^{+2} (Abou-Donia et al., 2008). It is also indicated that increase in AChE activity which provides a decrease in the amount of acetylcholine can be indicator of neurodegeneration as a result of function loss in acetylcholine receptors.

Conclusion

In its applied concentration, lead showed accumulation mostly in brain tissue being followed by liver, gill and muscle tissues. It is thought that selenium decreases the lead accumulation in tissues creating an chemical antagonistic effect on lead. Doing this, it is also determined that selenium shows beneficial effects when lead causes AChE inhibition in its short period effect at high concentration. It is thought that AChE activity increase in the fish which are under the effect of lead caused by selenium in long term and high concentration indicates neurodegeneration. The protective effect of selenium against lead can depend on concentration and time. That's why new studies are needed.

Acknowledgement

This study was supported by the Mersin University Research Fund (BAP)(Project No: MEÜ BAP-FBE TB (GŞ) 2012-2 YL).

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