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Abstract

Selenium (Se) is an essential element involved in many physiological processes and is critical for maintaining a dynamic endogenous antioxidant system. In this experiment, we investigated the oxidative stress, neurotoxicity and immunity of juvenile Sobaity seabream, *Sparidentex hasta*, to different concentrations of Se. Juvenile *S. hasta* (mean length, 14.6 ± 1.7 cm, and mean weight, 87.4 ± 5.6 g) were subjected to waterborne Se at concentrations of 0, 40, 80, 160, 320 and 400 µg L^{-1} for 2 and 4 weeks. The investigation of oxidative indicators showed a significant increase in the activity of liver and gill superoxide dismutase (SOD) and glutathione S-transferase (GST) after the period exposure. At the end of the test period, the highest Se exposure also resulted in a significant rise in glutathione (GSH) levels in the liver and gills. Following exposure to waterborne Se, catalase activity showed different patterns in fish organs: liver > brain > gills > kidney > muscle. In the investigation of neurotoxicity, waterborne Se exposure reduced AChE activity in the muscles and brain tissues. Additionally, waterborne Se exposure significantly elevated plasma and kidney lysozyme activity in non-specific immune responses. At high Se concentrations, peroxidase and anti-protease activities were reduced. These alterations in parameters can be considered as suitable indicators for evaluating Se toxicity in the aquatic ecosystem.

Keyboards: Selenium, *Sparidentex hasta*, GST, SOD GSH, AChE

Introduction

Selenium (Se) is an essential micronutrient for humans and animals, including fish. This metal is the main component of the glutathione peroxidase enzyme structure, which functions to protect cells against oxidative damage caused by free radicals (Watanabe et al. 1997). Selinium is derived from geomorphological processes although anthropogenic activities, such as sewage sludge, coal-fired power plants, metal ores, aquaculture drainwater, and sewage sludge all contribute to elevated Se concentrations in aquatic environments (Lemly 2002). Although moderate selenium levels in organisms may improve the immune system by protecting neutrophils from oxygen-derived radicals (Arthur et al., 2003), fish exposure to elevated Se concentrations can cause oxidative stress and superoxide (Spallholz & Hoffman 2002). ROS is recognized as an important concern in water toxicology that is strongly associated

to the toxicity of substances. Antioxidant activity against this biological disorder occurs in the intracellular space (Orun et al.; Talas et al. 2008; Ates et al. 2008).

Superoxide dismutase (SOD) is recognized as the primary defensive barrier against superoxide anion radicals and is considered as the first line of defense against oxidative stress. SOD accelerate the conversion of superoxide anions into molecular oxygen and hydrogen peroxide (McCord & Fridovich 1969; Das et al. 1997). Glutathione (GSH) is a biologically active peptide and the most abundant intracellular thiol-based antioxidant that is widely present in cells and acts as a sulfhydryl buffer. GSH can participate in free radical scavenging, detoxification through conjugation reactions catalyzed by glutathione S-transferases (GST), substance absorption, cell growth, cellular immunity, DNA biosynthesis, etc. (Nordberg & Arnér, E.S. 2001; Xue et al. 2022). Catalase, which is found in the cells of aerobic organisms, decomposes hydrogen peroxide into water and oxygen (Aebi 1984).

Despite the positive neuroprotective effects of selenium against exposure to neurotoxic chemical (Panter et al. 1996), this element, at high concentrations, is capable of damaging the nervous system as a neurotoxicant (Imam et al. 2001; Zafar et al. 2003). Acetylcholine, as one of the most important neurotransmitters, acts as a muscle activator in the peripheral nervous system and enhances sensory perceptions in the central nervous system. The main component of the fish cholinergic system is acetylcholinesterase (AChE), which controls the transmission of nerve impulses at cholinergic synapses. Due to the significant enzyme activity of AChE in the central nervous system of fish, a significant decrease in acetylcholinesterase activity is usually observed in fish that are exposed to various toxic substances such as organic phosphorus compounds, metals, and chemicals (Modesto & Martinez 2010). Therefore, the inhibition of acetylcholinesterase activity has the potential to be used as a biomarker to investigate neurotoxicity (Manzo et al. 1995).

Se in a concentration higher than the permissible limit affects the function of the immune system in conjunction with oxidative stress (Fairbrother & Fowles 1990; Fairbrother et al. 1994). Among non-specific immune responses, lysozyme plays a prominent role as a bacteriolytic agent against microbial invasion in fish (Yousif et al. 1994). The level of lysozyme in fish changes based on the state of health, stress, gender, temperature, and toxic substances in the water, especially water pollutants such as metals (Balfry & Iwama 2004; Saurabh & Sahoo 2008). Peroxidase activity is known as an indicator of leukocyte activation, which is considered an important immunological parameter (Tapia-Paniagua et al. 2011). Monitoring the level of antiproteases in fish, as one of the important elements of the non-specific immune system of vertebrates (Ellis 2001), is an appropriate indicator for measuring the possible impact of environmental hazards on the fish immune system (Saurabh & Sahoo 2008).

The hypothesis of the present study was that increasing the concentration of waterborne selenium would affect the physiological parameters and immune system of fish species *S. hasta*. Based on this, the effect of different concentrations of Se on oxidative stress, neurotoxicity, and non-specific immune responses were investigated over two periods of 2 and 4 weeks.

Materials and methods

Fish maintenance and conditions

Sobaity seabream juveniles (*Sparidentex hasta*) were purchased from a marine fish farm in Hormozgan Province, southern Iran. The fish were kept for 15 days under laboratory conditions for acclimatization,

acclimation before Se exposure. During the acclimation, juveniles were fed with a commercial diet (Biomar) twice daily. Fish were maintained under 12 h light and 12 h dark phase at ambient temperatures (Table 1). After acclimation period, 120 healthy specimens (body length, 14.6 ± 1.7 cm, body weight, 87.4 ± 5.6 g) were selected and divided into 20 L glass aquaria (each aquarium contained 6 fish)) at varying concentrations of selenium exposure. After preparing the sodium selenite solution (Sigma, St. Louis, MO, USA), selenium at different concentrations of 0, 40, 80, 160, 320, and 400 µg L^{-1} were added into the glass aquaria. Although the probability of the juvenile fish encountering concentrations of 320 and 400 µg L^{-1} in aquatic ecosystems is very low, and thus, the current experiment provided an opportunity to evaluate the toxicity of selenium in fish. Half of the water in the glass aquaria was removed daily and recharged with freshwater with the same concentration of selinium. Fish were anesthetized in buffered 3-aminobenzoic acid ethyl ester methanesulfonate at the end of weeks 2 and 4 (Sigma Chemical, St. Louis, MO).

Table 1. Water and environmental quality parameters in different experimental treatments.

Parameter	Value
Water temperature (°C)	28.2 ± 28.6
Air temperature (°C)	30.3 ± 31.4
pH	7.63-7.91
Salinity (ppt?)	~ 38
NO^-_2 (µg L^{-1})	0.9 ± 0.2
NO^-_3 (µg L^{-1})	9.21 ± 0.95
Ammonia (µg L^{-1})	13.22 ± 0.8
Chemical oxygen demand (mg L^{-1})	1.22 ± 0.21
Dissolved oxygen (mg L^{-1})	8.5-9.4

Antioxidant enzyme analysis

The prepared samples of liver and gill tissues of the euthanized juveniles were homogenized with 10 volumes of ice-cold homogenization buffer using a tissue homogenizer. The obtained homogenate was centrifuged at 10,000g for 30 minutes under refrigeration, and the supernatant was stored at -80°C for analysis. Superoxide dismutase (SOD) activity was measured with 50% inhibitor rate about the reduction reaction of WST-1 using SOD Assay kit (Colorimetric). One unit of SOD is defined as the amount of the enzyme in 20 µL of sample solution that inhibits the reduction reaction of WST-1 (WST-1 = 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disul fophenyl)-2H-tetrazolium, monosodium salt) with superoxide anion by 50%. SOD activity was expressed as unit mg $protein^{-1}$.

The concentration of glutathione-S-transferase (GST) was determined according to the modified method of Habig (1974). A combination of 0.2 M phosphate buffer (pH 6.5), 10 mM GSH (Sigma) and 10 mM 1-chloro-2,-dinitrobenzene, CDNB (Sigma, St. Louis, USA) was used as the reaction mixture. The enzyme activity was estimated according to the change in absorbance at 340 nm at 25°C as nmol min^{-1} mg $protein^{-1}$.

To investigate the reduction of glutathione activity, 0.2 ml of fresh supernatant was mixed with 1.8 ml of distilled water and then mixture was then mixed with three ml of precipitation solution (1.67 g of metaphosphoric acid, 0.2 g of EDTA and 30 g of NaCl in 100 ml of distilled water), and centrifuged at 10000 g for 5 minutes. 1.0 ml of the supernatant was then added to 4.0 ml of 0.3 M NaHPO4 solution after which time 0.5 ml of DTNB (5,50-dithiobis-2-nitrobenzoic acid) was added. The decrease in glutathione level was

expressed as the difference in the absorbance values of the samples in the presence and absence of DTNB at 412 nm. The level of GSH was measured as µmole mg protein^{-1} in the tissues.

The catalase (CAT) activity was assessed using the method described by Cohen et al. (1970). The measurement was based on monitoring the decomposition of hydrogen peroxide (H2O2) by observing the decrease in absorbance at a wavelength of 240 nm. CAT activity was expressed as units per milligram of protein (U mg^{-1} protein).

Inhibition of AChE activity

Brain and muscle tissues were homogenized in 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged 10000g for 30 min at 4 °C containing Triton-X 100. The supernatants were removed and used for estimating the AChE activity (Chhajlani et al. 1989; Rosenfeld et al. 2001). AChE activity was estimated according to the modified method of Ellman et al. (1961). AChE activity was normalized to protein content and expressed as nmol min 1 mg protein 1. Briefly, the activity on the homogenate was measured by determining the rate of hydrolysis of acetylthiocholine iodide (ACSCh, 0.88 mM) in final volume of 300 µL, with 33 µL of 0.1 M phosphate buffer, pH 7.5 and 2 mM DTNB. The reaction was initiated with the addition of the substrate acetylthiocholine and the hydrolysis of substrate was recorded with the formation of the thiolate dianion of DTNB in 412 nm for 2-3 min (in intervals of 30s) using a 96-well microplate reader. Protein concentration was determined using Bradford's method (1976), with a bovine plasma albumin (Sigma, St. Louis, USA) as standard.

Non-specific immune responses

The blood samples were separated to obtain plasma for analysis. Kidney tissues were excised and homogenized homogenized using a homogenizer (hand-held WT130) with 10 volumes of ice-cold homogenization buffer (0.004 M phosphate buffer, pH 6.6). The homogenate was centrifuged at 10 000g for 10 min under refrigeration and the obtained supernatant was stored at 70 °C (MDF-U53V, SANYO Electric Co. Ltd., Japan) for later analysis. Protein concentration was measured employing the Bio-Rad Protein Assay Kit (Bio-Rad Laboratories GmbH, Munich, Germany) according to the Bradford dye-binding procedure, using bovine serum albumin as standard.

Lysozyme activity

The concentration of kidney and plasma lysozyme based on the lysis of the Gram+ *Micrococcus lysodictycus* (Sigma, St. Louis, USA) as a substrate (0.2 mg/mL^{-1} 0.05 M phosphate buffer, pH 6.6 for the kidney sample and pH 7.4 for the plasma) was measured using a turbidimetric assay (Ellis 2001). Lyophilized egg white lysozyme (Sigma) was used to create the standard curve, and the change in turbidity was recorded at intervals of 0.5 minutes and 4.5 minutes at an OD of 530 nm. The obtained results were reported as 1 µg mL^{-1} and 1 µg g^{-1} equivalent to egg white lysozyme activity.

Peroxidase activity

The peroxidase activity, indicative of leukocyte activation (Quade MJ, Roth JA, 1997), was assessed using a colorimetric technique. After diluting 5 µL of plasma with 50 µL of HBSS (Hank's Balanced Salt Solution) in 96-well plates, plasma samples were mixed with peroxidase substrate (80 µM 3,30,5,50-tetramethylbenzidine hydrochloride (TMB; Sigma Aldrich) and 2.5 mM H2O2). The color-change reaction was stopped after 2 min by adding 50 µL of 2 M sulfuric acid and the absorbance (optical density: OD) was

read at 420 nm in a plate reader (FLUOstar Omega, Bmg). The standard sample without plasma was considered as blank. Peroxidase activity was determined by defining one peroxidase unit that results in an absorbance change of 1 OD.

Assay of anti-protease activity

The modified Ellis method (1990) was used to measure the anti-protease activity (Magnadóttir et al. 1999). 20 µL of plasma was incubated with an equal volume of standard-purity trypsin solution for 10 min at room temperature (approximately 24°C). Next, 200 µL of 0.1 M phosphate buffer, pH 7.0, and 250 microliters of 2% azocasein were added and incubated for 1 hour at 24°C. Then, 500 L of 10% trichloroacetic acid (TCA) was added and incubated for 30 minutes at room temperature (24°C). The final mixture obtained was centrifuged at 6000 g for 5 minutes. 100 µL of the supernatant was transferred to a 96-well non-absorbent microtray containing 100 µL well^{-1} of 1 N NaOH. The OD was read at 450 nm by using a UV-Visible spectrophotometer. The blank was phosphate buffer instead of plasma and trypsin, and the reference sample was phosphate buffer instead of plasma. Then the percentage inhibition of trypsin activity compared to the reference sample was calculated for each plasma sample. All the reagents and chemicals were from Sigma Aldrich.

Statistical analysis

This experiment was performed in two exposure periods of 2 weeks and 4 weeks and in three replications. SPSS statistical package (version 26.0 software) was used for statistical analysis. Significant differences between groups were using one-way ANOVA was employed to determine and Duncan's test for multiple comparisons or Student's *t*-test for two groups. The significance level was set at $P < 0.05$.

Results

Antioxidant enzymes activity

The effect of different concentrations of water-borne Se on antioxidant enzymes activity, AChE activity, non-specific immune responses in *S. hasta* was measured (Figure 1). The SOD, GST and GSH activity of *S. hasta* were significantly affected by Se, especially in high concentrations. The liver SOD activity of *S. hasta* exhibited a notable increase as the water-borne Se concentration reached 160 µg L^{-1}, starting from the end of the second week. Similarly, the liver SOD activity showed a significant rise with water-borne Se concentrations of 80 mg and over. Substantial increase was noted for the liver and gill GST activity when fish were exposed to Se concentrations of 80 mg and higher, reaching its peak levels at concentrations of 320 and 400 mg. In the case of liver GSH activity, a considerable increase was observed when fish exposed to concentrations over 80 µg L^{-1} water-borne Se at the end of the 4th week of exposure and for gills was detected the highest GST activity when *S. hasta* was exposed to concentrations higher than 160 µg L^{-1} of water-borne Se.

The catalase activity exhibited significant variations among different organs, with this difference being more pronounced with increasing Se concentrations (Table 2). The highest level of catalase activity was observed in the liver and brain, with a significant increase observed with the rise in selenium concentration and duration of exposure. In contrast, the lowest level of enzyme activity was observed in the muscle and kidney.

Brain and muscle AChE activity

The relationship between the water-borne Se concentration and the AChE activity in the brain and muscles showed a notable inverse correlation (Figure 2). As Se concentration escalated, there was a marked decline in AChE activity. Specifically, after four weeks of exposure to 80 mg of Se, brain AChE activity exhibited a statistically significant reduction when juxtaposed with the control group. This downward trend in AChE activity became more pronounced with the elevation in Se concentration. The most significant decline in AChE activity was observed at the end week 4 when exposed to 400 µg L^{-1} Se. there was an approximate 33% decrease AChE activity in comparison to the control group. Similarly, the reduction of muscle tissue AChE activity at the end of the 4th week of exposure to 80 µg L^{-1} Se showed a significant difference with the control group. Inhibition of AChE activity reached its peak at the end of week 4 with exposure to 320 and 400 µg L^{-1} Se, approximately 30% and 38%, respectively, compared to the control group.

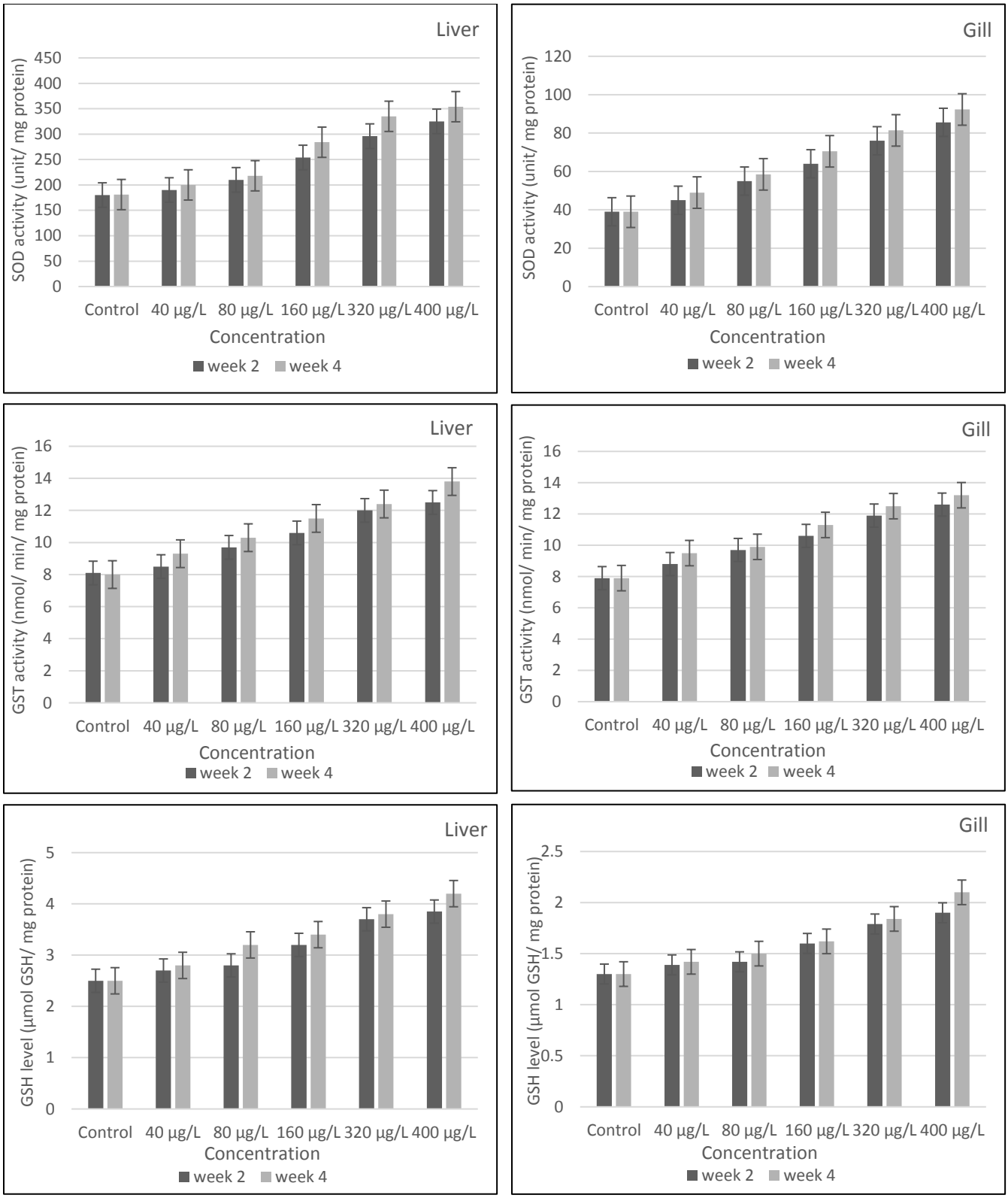

Figure 1. SOD, GST and GSH level in liver and gill of juvenile Sobaity seabream, *S.hasta* exposed to different Se. concentrations. Vertical bar denotes a standard error

Table 2. Changes of Catalase activity (U mg^{-1} protein) in body organs of juvenile Sobaity seabream, S.hasta, exposed to different Se concentrations. Data are expressed as mean ± SE.

Organ	period (week)	Selenium concentration (µg L^{-1})					
		Control	40	80	160	320	400
Liver	2	5.20 ± 0.31	5.32 ± 0.27	5.84 ± 0.36	6.42 ± 0.35	6.80 ± 0.35	6.95 ± 0.49
	4	4.96 ± 0.15	5.53 ± 0.29	5.91 ± 0.44	6.52 ± 0.42	6.77 ± 0.40	7.31 ± 0.56
Gill	2	3.31 ± 0.49	3.52 ± 0.56	3.61 ± 0.39	3.92 ± 0.50	3.68 ± 0.41	3.61 ± 0.66
	4	3.20 ± 0.32	3.39 ± 0.35	3.91 ± 0.15	4.15 ± 0.28	4.10 ± 0.18	4.38 ± 0.61
Kidney	2	2.23 ± 0.11	2.29 ± 0.20	2.51 ± 0.15	2.79 ± 0.28	2.76 ± 0.14	2.86 ± 0.19
	4	2.19 ± 0.13	2.39 ± 0.24	2.72 ± 0.29	2.92 ± 0.48	2.81 ± 0.37	3.15 ± 0.33
Muscle	2	1.10 ± 0.12	1.25 ± 0.17	1.52 ± 0.17	1.72 ± 0.31	1.83 ± 0.29	1.91 ± 0.49
	4	1.14 ± 0.19	1.31 ± 0.38	1.55 ± 0.26	1.65 ± 0.21	1.86 ± 0.34	1.97 ± 0.30
Brain	2	4.11 ± 0.15	4.23 ± 0.18	4.67 ± 0.20	4.76 ± 0.33	4.74 ± 0.26	4.89 ± 0.30
	4	4.19 ± 0.13	4.33 ± 0.16	4.71 ± 0.17	4.83 ± 0.31	4.90 ± 0.29	4.95 ± 0.51

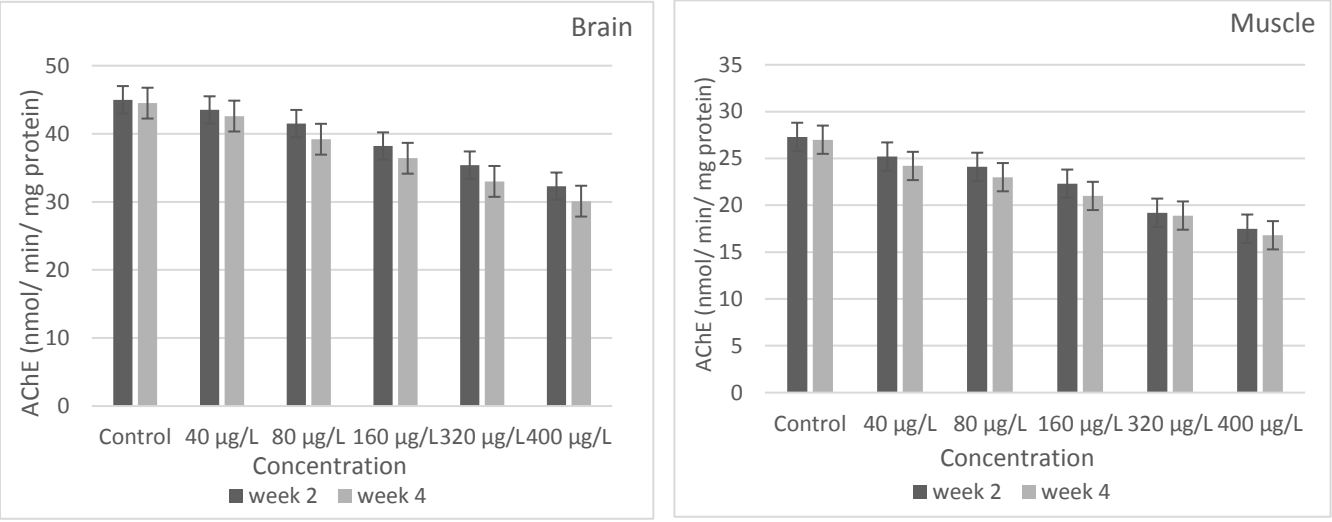

Figure 2. Changes of AChE activity in the Brain and Muscle of juvenile Sobaity seabream, *S.hasta*, exposed to different Se concentrations. Vertical bar denotes a standard error.

Plasma and kidney lysozyme levels showed a significant increase compared to the control group after exposure to 80 micrograms of Se in week 4 (Table 3). The highest level of plasma lysozyme increased with exposure to concentrations of 320 and 400 µt Se, 7.41 ± 0.59 and 7.93 ± 0.67, respectively, was observed at the end of week 4. The highest level of kidney lysozyme activity with exposure to concentrations of 320 and 400 µg Se, 93.7 ± 4.4 and 98.6 ± 5.7, respectively, was seen at the end of week 4.

Table 3. Changes in plasma and kidney lysozyme levels in *S. hasta* following treatment with different Se concentrations after exposer 4-week period. Data are expressed as mean ± SE.

Parameters	period (week)	Selenium concentration (µg L^{-1})					
		Control	40	80	160	320	400
Plasma (µg mL^{-1})	2	5.24 ± 0.47	5.6 ± 0.42	6.49 ± 0.66	6.81 ± 0.52	7.35 ± 0.49	7.95 ± 0.56
	4	5.31 ± 0.69	6.21 ± 0.73	6.72 ± 0.53	6.93 ± 0.31	7.41 ± 0.59	7.93 ± 0.67
Kidney (µg g^{-1})	2	62.5 ± 5.9	67.4 ± 4.8	73.4 ± 6.3	82.5 ± 6.2	89.7 ± 5.6	95.5 ± 6.2
	4	63.2 ± 4.7	70.5 ± 3.8	75.8 ± 6.1	85.8 ± 4.9	93.7 ± 4.4	98.6 ± 5.7

The peroxidase and anti-peroxidase activity levels were initially measured at their baseline values in the control group. However, as the selenium concentration increased, there was a gradual decrease in the activity of these two physiological parameters (Table 4). Notably, a significant change in peroxidase and anti-peroxidase activity levels was observed after exposure to a concentration of 160 µg/L. This decreasing trend persisted until the end of week 4, reaching a concentration of 400 µg/L.

Table 4. Changes in Peroxidase and Anti-protease activity in *S. hasta* following treatment with different Se concentrations after exposer 4-week period. Data are expressed as mean ± SE.

Parameters	period (week)	Selenium concentration (µg L^{-1})					
		Control	40	80	160	320	400
Peroxidase activity (U mL^{-1})	2	6.21 ± 0.41	6.11 ± 0.32	5.72 ± 0.44	5.10 ± 0.39	4.61 ± 0.52	4.26 ± 047
	4	6.23 ± 0.50	6.05 ± 0.43	5.41 ± 0.37	4.82 ± 0.66	4.37 ± 0.57	4.11 ± 0.67
Anti-protease activity (% inhibition)	2	38.5 ± 1.4	38.1 ± 1.8	37.6 ± 2.1	36.8 ± 1.9	35.4 ± 1.3	33.5 ± 1.5
	4	38.4 ± 1.6	37.9 ± 3.8	37.2 ± 1.8	36.0 ± 1.7	34.8 ± 1.8	33.1 ± 2.3

Discussion

The current study investigated the physiological response of juvenile Sobaity seabream, Sparidentex hasta, exposed to varying concentrations of the Se using a laboratory based experiment. The main findings of the study provides important baseline information on the potentially harmful effects of Se on the health of juvenile fish.

Oxygen is an essential component metabolic processes in aerobic organisms, such as aquatic animals, is oxygen. However, exposure to heavy metals such as selenium can cause ROS, redox reactions, and free radical production which subsequently may cause serious damage to cellular structures (Spallholz et al. 2004; Ahmad et al. 2004; Brucka-Jastrzębska 2010). Fish exhibit a wide range of antioxidant responses. The catalytic conversion of superoxide anions into hydrogen peroxide (H_2O_2) is facilitated by superoxide dismutase (Öztürk-Ürek et al. 2001; Kim & Kang 2015). This reaction can be considered as the initial defensive response against oxidative toxicity. Liver and gill tissues are usually used to investigate the antioxidant responses of oxidative stress (Kim & Kang 2016; Kim et al. 2017).

During the present study, SOD activity in both the liver and gill tissues of *S. hasta* significantly increased with increasing Se concentration. Several studies have indicated an increase in SOD activity following fish exposure to heavy metals (Farombi et al. 2007). Kim et al. (2017) reported significant increases in the SOD activity of black sea bream, *Acanthopagrus schlegelii*, when exposed to waterborne zinc. Kim & Kang (2015) also reported that SOD activity was increasing in juvenile red sea bream (*Pagrus pagrus*), when exposed to different waterborne Se concentrations. Similarly, an increase in SOD activity was observed in rainbow trout, *Oncorhynchus mykiss*, exposed to 50 and 100 µM of sodium selenite (Misra & Niyogi 2009). Following this, Pacini et al. (2013) found that the activity of SOD increased in both the liver and kidney tissue of Siberian sturgeon (*Acipenser baeri*) fish when exposed to Se. (Chaâbane et al. 2020). Gopi et al. (2021) also pointed out the increase SOD level in Mozambique tilapia (*Oreochromis mossambicus*) exposed to waterborne Se. Finally Lee et al. (2022) showed that exposure of Olive Flounder (*Paralichthys olivaceus*) to chromium (Cr) resulted in a significant increase in SOD activity in both liver and gill. It has been suggested that increased SOD activity is a defense mechanism to prevent tissue damage by removing excess ROS caused by exposure to pollutants such as metals.

Glutathione-S transferase (GST), which catalyzes the conjugation of glutathione (GSH) to various electrophiles, functions as a critical defense mechanism against ROS as well as playing an important role in the detoxification of deleterious electrophilic xenobiotics such as environmental toxicants (Keen & Jakoby 1978; White et al. 2003). Significant increase in GST in the liver and kidney tissues of tilapia (*O. mossambicus*) was observed after exposure to waterborne cadmium (Basha & Rani 2003). It has been suggested that the increase in of GST in response to oxidative stress can be considered as a defensive mechanism against oxidative stress or cellular damage (Marí & Cederbaum 2001). In the present study, we observed that exposure to different concentrations of water-borne Se, especially at high concentrations, significantly increased GST activity in *S. hasta*. Nile tilapia (*Orechromis niloticus*) that were exposed to copper showed considerable reductions in GST activity (Kanak et al. 2014). It is possible that the decrease in GST is compensate by the action of other enzymes in the antioxidant system, which help eliminate reactive oxygen species (ROS). In another study by Kim & Kang (2015), GST activity has been reported to increase after exposure *P. major* to waterborne Se dose-depended. The increase of GST activity in the liver and gills can be considered as a result of the detoxification process for organic xenobiotics. Kim et al. (2017) reported depleted GST activity in *A. schlegelii* exposed to waterborne Zn. Exposure of topmouth gudgeon (*Pseudorasbora parva*) to Se showed a greater than 50% reduction in GST activity in gills (Ma et al. 2018). The results of our study are in accord with on previous result by Gobi et al. (2018) reported that GST activity both in liver and gill of *O. mossambicus* increased with the waterborne Se in dose-dependent. Gopi et al. (2021) also reported an elevation in GST level by exposure *O. mossambicus* to Se pollution.

GSH, a thiol-containing peptide, plays a crucial role in protecting cells from the adverse effects of xenobiotics like metals and serves as a substrate for glutathione S-transferase (GST) activity (Lange et al. 2002). The reduction in GSH concentration, as part of the cellular response, chelates and detoxifies metals and subsequently protects cells against metal exposure (Sanchez et al. 2005). Selenite is converted into hydrogen selenide under the influence of GSH, which may generate ROS (Seko et al. 1989) through the reaction with oxygen and when not eliminated by antioxidants can lead to oxidative damage (Miller 2006; Misra & Niyogi 2009). Juvenile rainbow trout (*Oncorhynchus mykiss*) exposed to waterborne selenite exhibited decreased GSH levels (Miller et al. 2007). In a study conducted by Pandey et al. (2008), it was observed that the exposure of spotted snakehead (*Channa punctate*) to multiple trace metals resulted in a significant reduction in GSH levels. The exposure of *P. major* to increasing concentrations of selenium led to an increase in the levels of GSH in both the liver and gills (Kim & Kang 2015). Li et al. (2008) reported a significant increase of GSH in medaka (*Oryzias latipes*) exposed to waterborne Nano-Se of 100 µg Se/L. It has also been reported that after 28 days of exposure of *P. parva* to selenium, there was a significant decrease in GSH levels in the gills, by at least 35% (Ma et al. 2018). In line with our results in the current study, Li et al. (2018) demonstrated that exposing *O. mossambicus* to Se lead to increased GSH Activity in both liver and gill. Gopi et al. (2021) observed an elevation in GSH activity by exposure *O. mossambicus* to Se. These changes in GSH levels may contribute to oxidative damage caused by waterborne Se. These findings suggest that waterborne selenium exposure significantly impacts antioxidant enzymes and induces oxidative stress in fish.

Catalase is one of the antioxidant enzymes that plays a crucial role in the defense system against ROS and is used as a biomarker for assessing oxidative stress (McFarland et al. 1999). In the present study, catalase activity increased in response to both selenium concentration and duration of exposure. Nagaraju and Rathnamma (2014) exhibited a significant increase in catalase activity in various organs of the Grass carp (*Ctenopharyngodon idella*) exposed to the Chlorantraniliprole compared to the control group. Similarly, Kumari et al. (2014) observed a significant increase in catalase activity in fish *Labeo rohita* exposed to

chromium after 48 to 72 compared to the control group. Mechlaoui et al. (2019) investigated the effect of selenium supplementation in the diet on certain physiological parameters of gilthead seabream (*Sparus aurata*). They observed a significant increase in catalase activity in the liver of fish fed with the experimental diet after 63 days compared to the control group. Such an increase in catalase levels can be considered as a response to elevated levels of H2O2 and superoxide anions (John et al. 2001). On the other hand, the increase in catalase activity may serve as an adaptive mechanism to prevent the accumulation of toxic ROS (Regoli et al. 2006). Contrary to the results of other researches, catalase (CAT) activity was inhibited for both liver and gill tissues of *O. mossambicus* after 96 h exposure to selenium (Gobi et al. 2018).

The elevation of ROS triggers cellular apoptosis (Risso-De Faverney et al. 2001; Krumschnabel et al. 2005), potentially leading to neurodegenerative and immune disorders in humans (Franco et al. 2009). Acetylcholine is a crucial neurotransmitter in both the central and peripheral nervous systems, and the inhibition of acetylcholinesterase has been suggested as a biomarker for assessing neurotoxicity (Manzo et al. 1995). Fish exposed to toxic substances often display a noticeable decline in acetylcholinesterase activity (Modesto & Martinez 2010), and the acetylcholine (ACh) accumulation resulting from AChE inhibition could impact the fleeing and reproductive behaviors of fish (Bretaud et al. 2000). Kim and Kang (2015) reported significant AChE inhibition of *P. major* exposed to waterborne Se. The study conducted by (Gülsemin & Karaytuğ 2017)showed that high concentrations of lead (Pb) inhibited the AChE activity of *O. niloticus*, while the mixture of Pb and Se increased the activity of AChE and improved the activity of this enzyme. Gopi et al. (2021) also observed inhibition of AChE activity in brain tissue of fish exposed to Se concentrations. According to Ma et al. (2018) there was a notable increase in AChE activity in both the muscle and brain of *P. parva* following a 28-day exposure to selenium. In our study, a significant inhibition of AChE activity was observed in the brain and muscle of *S. hasta* exposed to waterborne Se. Similar to previous research results, higher brain AChE activity was also observed compared to muscle. The exposure of fish to selenium led to the observed neurotoxic effects, as evidenced by the inhibition of cholinesterase activity (Gopi et al. 2021).

During the current investigation, plasma and kidney lysozyme levels in *S.* hasta increased when exposed to elevated Se concentrations. The immune system has the potential to provide biomarkers that can effectively evaluate environmental pollution (Bainy & Marques 2003). Lysozyme is one of the important parameters in non-specific immunity that plays a significant role in immune function. Monitoring the lysozyme levels in fish is a valuable indicator for assessing the potential impact of environmental hazards on the innate immunity of fish species (Saurabh & Sahoo 2008). Exposure to pollutants such as heavy metals alters lysozyme levels and causes changes in immune regulatory functions (Bols et al. 2001). The bactericidal action of lysozyme has clearly shown the role of its defense system in fish (Grinde et al. 1988). Kim & Kang (2015) reported significant increase of plasma and kidney lysozyme levels exposed to waterborne Se in *P. major*. In a study conducted by Li et al. (2020) is reported that the levels of lysozyme was significantly increased in serum of *C. argus* following exposure to waterborne Se. This significant increase in lysozyme activity could be due to an immunological response following exposure of the *S. hasta* to waterborne Se.

Peroxidase is a key enzyme that utilizes oxidative radicals to produce hypochlorous acid for the purpose of eradicating pathogens. According to Kim & Kang (2015), exposure to elevated concentration of Se resulted in a decrease in peroxidase activity in *P. major*. Peroxidase activity in in a variety of body tissues (brain > liver > gill > kidney > heart > muscle) of *Catla catla* increased after exposure to Pb (Abdullah et

al. 2019). Subsequently, Lahori et al. (2021) reported an increase in peroxidase levels, particularly in liver tissue,in *Cyprinus carpio* exposed to copper nanoparticles. . During this investigation, peroxidase activity of *S. hasta*, decreased in response to an increase in Se concentrations. This suggests that waterborne Se can potentially impact the immunomodulation of *H. hasta*. Based on the results of our research, it can be inferred that antioxidant enzymes can be utilized as valuable biomarkers of oxidative stress in aquatic organisms. Anti-proteases are a crucial component of the innate defense system in vertebrates' non-specific immunity (Ellis 2001).Fish plasma contains various protease inhibitors that likely play a significant role in preventing bacterial invasion and growth (Magnadóttir et al. 1999). According to Thilagam et al. (2009), it was observed that the level of protease inhibitors in Japanese sea bass decreased following exposure to 17b-estradiol. Kim & Kang (2015) reported the notable decreased level of protease inhibitors in *P. major* exposed to different waterborne Se. The significant reduction observed at elevated concentrations indicates that exposure to waterborne selenium can potentially impact the in vivo regulatory capacity of the immune defense system to hydrolyze proteins.

To summarize, the findings of this study indicate that exposure of juvenile S. *hasta* to varying concentrations of Se led to a significant increase in antioxidant enzymes (SOD, GST and GSH levels) in gill and kidney tissue. At the same time exposure to increased concentration of Se resulted in the inhibition of AChE activity in both the brain and muscle tissues of the juvenile fish. Furthermore, the study indicated that exposure to Se significantly affects the immune responses of *H. hasta*, including lysozyme, peroxidase, and anti-protease activity.

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