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REGULAR ARTICLES

Dietary supplementation with melatonin: influence on growth performance, oxidative stress status, and amelioration of silver nanoparticles-induced toxicity in Nile tilapia (*Oreochromis niloticus*)

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Abstract

Excessive use of silver nanoparticles (AgNPs) due to antibacterial properties can raise concerns about their release into environment and potential toxicity in aquatic organisms. Melatonin has several physiological functions especially antioxidant potential against oxidative stress. The current study was conducted to investigate the potential effects of two doses of dietary melatonin on growth performance, plasma biochemistry, and liver enzyme activity in Nile tilapia (*Oreochromis niloticus*) juveniles. We also investigated the potential ameliorative effect of melatonin in AgNPs-induced biochemical alterations in tilapia fish. The results showed that melatonin-supplemented diets had no significant effect on growth performance of fish ($P>0.05$). The liver GPx activity increased in fish fed melatonin-supplemented diets ($P<0.05$), but the SOD activity showed no significant difference in comparison with the control ($P>0.05$). The administration of melatonin-supplemented diets reduced the activity of liver MDA compared to the control ($P<0.05$). Feeding fish with high melatonin-supplemented diet (200 mg kg^{-1} of diet) decreased the plasma glucose, total protein, and AST levels ($P<0.05$). The liver GPx and SOD activities were higher in high melatonin-treated fish exposed to AgNPs than the control group ($P<0.05$). Dietary melatonin decreased the liver MDA activity in AgNPs-exposed fish. The plasma glucose, AST, and ALT levels in melatonin-treated fish exposed to AgNPs decreased compared to the untreated exposed fish ($P<0.05$). Melatonin-treated fish exposed to 0.05 and 0.5 mg L^{-1} of AgNPs had lower plasma LDH level than the control group ($P<0.05$). The results showed that consumption of melatonin-supplemented diets could modulate some of the biochemical indices of plasma and liver in Nile tilapia. The findings also indicated the ameliorative effect of dietary melatonin on AgNPs-induced toxicity in Nile tilapia.

Keywords Fish · Growth · Melatonin · Metal nanoparticles · Oxidative stress · Toxicity alleviator

Highlights
- Dietary melatonin increased the activity of antioxidant enzyme of GPx in the liver of Nile tilapia (*Oreochromis niloticus*).
- The liver MDA level as a lipid peroxidation biomarker decreased by melatonin supplemented diets in a dose-dependent manner.
- High melatonin dose (200 mg kg^{-1} of diet) reduced the plasma glucose, total protein, and AST levels.
- Dietary melatonin effectively ameliorated the AgNPs-induced toxicity in Nile tilapia.

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Introduction

"Nanoparticle" is a term that refers to particles in size range from 1 to 100 nm (Bhuvaneshwari et al. 2016). Nanoparticles (NPs) have particular and interesting features like higher surface area to volume ratio, which increase their chemical reactivity and physical absorption capacity. These properties may affect the toxicity of NPs when interact with biological systems (Ates et al. 2016). Silver nanoparticles (AgNPs) because of unique bactericidal and fungicidal activity are extensively used in detergents, wound dressings, cosmetic products, textiles, food packaging, and water disinfection filters (Asz et al. 2006; Cho et al. 2009; Johari et al. 2015; Sarkheil et al. 2016). AgNPs most probably release from consumer products into environment during their production, consumption period, and after final disposal (Benn and Westerhoff 2008; Kim et al. 2010; Gottschalk and Nowack 2011). Blaser et al.

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(2008) investigated the release of silver from textiles and plastics into freshwater ecosystem in the European Union (EU). They reported that silver release was in the range of 110–230 t/year. Water quality criteria values set by the US Environmental Protection Agency (USEPA) for silver in freshwater and saltwater are 3.4 and 1.9 μg L^{-1}, respectively (Reidy et al. 2013). Excessive entering of AgNPs into the aquatic environment can strongly affect and damage the aquatic organisms (Batley et al. 2012; Angel et al. 2013).

In the recent years, many studies have been evaluated AgNPs toxicity in aquatic organisms including fish. Most of these studies have addressed toxicity induced by AgNPs in fish which includes embryonic deformation and lethality (Wu et al. 2010; Cho et al. 2013), fish mortality (Hedayati et al. 2012; Rajkumar et al. 2015), alteration in the blood indices (Johari and Kalbassi 2016; Shaluei et al. 2013; Rajkumar et al. 2015), alteration in the metabolic enzymes in different tissues (Lee et al. 2012; Taju et al. 2014; Rajkumar et al. 2015), oxidative stress (Schrand et al. 2008; Bacchetta et al. 2017; Kanwal et al. 2019), lipid peroxidation (Massarsky et al. 2014; Xiang et al. 2020), silver bioaccumulation (Bruneau et al. 2015; Clark et al. 2019), DNA damage, and alteration in gene expression (Pham et al. 2012; Johari et al. 2016; Massarsky et al. 2014; Thummabancha et al. 2016). Valerio-Garcia et al. (2017) showed that exposure of adult goodeid fish (*Chapalichthys pardalis*) to silver nanoparticles for 21 days resulted in oxidative stress, reduction in antioxidant enzymes and glucose levels, and elevation of thiobarbituric acid reactive species (TBARS) and oxidized proteins. Fish defend themselves against oxidative stress through enzymatic and non-enzymatic mechanisms (Toma's-Zapico and Coto-Montes 2005a, 2005b; Jung et al. 2016). It is found that the capacity of the antioxidant defense system of cultured fish is insufficient (Nakano et al. 1999). It is well known that feeding behavior and nutritional factors influence on antioxidant defenses (Mourente et al. 2002; Martinez-Alvarez et al. 2005). To ameliorate oxidative stress and maintain the healthy life, use of non-enzymatic antioxidants such as glutathione, β-Carotene, selenium, vitamin C, vitamin E, and recently, melatonin has gained immense interest (Toma's-Zapico and Coto-Montes 2005a, 2005b; Abdelazim et al. 2018; Asghar et al. 2018).

Melatonin (N-acetyl-5-methoxytryptamine) is mostly synthesized in pineal organ and has several functions especially the regulation of circadian rhythms (Arendt 2003; Falcón et al. 2010). Moreover, melatonin is directly involved in antioxidant defense mechanism through elimination of reactive oxygen species (ROS) and regulation of oxidative damage in fish (Hardeland et al. 2003; Allegra et al. 2003). Melatonin is also known as an indirect antioxidant, as it is involved in promoting gene expression and eventually activation of the major antioxidant enzymes including glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) (Hardeland et al. 2003; Tomas-Zapico et al. 2002). Gesto et al. (2016) found that melatonin adding to the tank water attenuated the stress response of *Solea senegalensis* to stressful conditions such as stoking fish at high density. Administration of melatonin as intramuscular implants in female Prussian carp (*Carassius gibelio*) exposed to two concentrations of waterborne cadmium (0.4 or 4.0 mg L^{-1}) for 7 or 13 weeks increased the activity of GPx enzyme and reduced the accumulation of cadmium, copper, zinc, and iron metals in the hepatopancreas tissue (Drąg-Kozak et al. 2019). However, there is no enough information on ameliorative effect of melatonin on toxicity-induced by nanomaterials in aquatic animals.

Nile tilapia (*Oreochromis niloticus*) is one of the most important economic source of food, and its cultivation is rapidly expanding in the world because of tolerance of a wide range of environment conditions and intensification of cultivation systems (Ponzoni et al. 2011; Santos et al. 2013). Entrance of pollutants such as silver nanoparticles as a stressor into the water bodies may threaten the health condition, welfare, and growth performance of this species. Therefore, the current study was aimed to (1) investigate the dietary administration of melatonin on growth performance, plasma biochemical parameters, and liver enzymes activities and (2) investigate the potential alleviative effect of melatonin on aquatic toxicity-induced by different concentrations of AgNPs by measuring the plasma and liver biochemical indices in Nile tilapia.

Materials and methods

Silver nanoparticles and characterizations

AgNPs were purchased from Nano Nasb Pars Co. (Tehran, Iran) in a form of a water-based colloidal suspension of citrate-capped AgNPs with nominal concentration of 4000 mg L^{-1} of metallic silver. Transmission electron microscopy (TEM; Carl Zeiss AG - Zeiss EM900 model) was used to determine the shape, size, and surface morphology of particles. To determine the average diameter of particles on TEM images, the diameter of 119 individual particles or their aggregates/agglomerates was randomly measured using AxioVision digital image processing software (Release 4.8.2.0, Carl Zeiss Micro Imaging GmbH, Germany). The sock suspension of AgNPs used in this study was exactly from the same batch, which its characterizations were recently reported by Behzadi Tayemeh et al. (2020).

Preparation of experimental diets

A commercial diet (Nutra-MP) as a basal diet was purchased from Skretting Co., Stavanger, Norway. The proximate composition of the basal diet is shown in Table 1. The experimental diets were prepared by supplementation of the basal diet with two low and high doses (50 and 200 mg kg^{-1} of dry diet)

Table 1 Chemical analysis and proximate composition of the basal diet used in the feeding experiment

Proximate composition	(%)
Crude protein	54
Crude fat and oils	18
Crude fiber	0.6
Crude ash	8.8
Digestible energy	19.2 (MJ/kg)
Minerals and vitamins	
Calcium	2.5 (%)
Phosphorus	1.4 (%)
Vitamin A	6000 UI/kg
Vitamin D3	1125 UI/kg
Manganous sulfate monohydrate	46.2 mg/kg
Ferrous sulfate monohydrate	121.6 mg/kg
Zinc sulfate monohydrate	246.6 mg/kg
Cupric sulfate pentahydrate	19.7 mg/kg
Potassium iodine	2.6 mg/kg

of melatonin (Sigma-Aldrich) and were considered as LDM and HDM, respectively. In this order, melatonin powder dissolved into 100 mL of ethanol was sprayed over the basal diet. Then, the prepared pellets were air-dried at room temperature. The surfaces of pellets were coated with bovine gelatin solution (10%) and dried in a freeze-dryer (DENA Vacuum FD-5005-BT, Iran) to prevent the release of melatonin into the water during feeding period. The basal diet without adding melatonin and coated with gelatin was considered as control.

Feeding experiment

Healthy broodstock of Nile tilapia (*Oreochromis niloticus*) fish were purchased from Nam Sai Farms Co. Ltd., Prachinburi province, Thailand, and transferred to a local fish farm in Tehran city, Iran. After spawning and cultivation of larvae to juvenile stage, two hundred healthy juveniles of Nile tilapia (*O. niloticus*) with average weight of 20.36±1.47 g and average length of 10.53±0.84 cm were transferred to the Aquatic Nanobiotechnology laboratory of University of Kurdistan, Sanandaj, Kurdistan province, Iran. Fish were stocked in two 1000-L fiberglass tank under a good aeration condition and fed the basal diet for 2 weeks to acclimatize to laboratory condition. Afterwards, the weight of fish individually measured and randomly stocked in 9-glass aquarium (100 L) at a density of 20 fish per each aquarium (three replicates for each experimental diet). The temperature and dissolved oxygen of water in each aquarium were adjusted using an aquarium heater, a central aerator pump, and an air stone, respectively. Tilapia fish were fed on experimental diets three times daily to apparent satiation for 56 days. During the feeding period, 20% of water of each aquarium was replaced with freshwater and uneaten feeds, and feces were siphoned daily. Fish were kept under water temperature of 25±1.5° C, dissolved oxygen of 7.2±0.42 mg L^{-1}, and pH of 7.6±0.64. Fish were kept and manipulated according to Animal Welfare Act and Interagency Research Animal Committee guidelines (Nickum et al. 2004).

Growth performance assay

On the 57th day of the experiment, the fish of each aquarium were individually anesthetized using clove powder (500 mg L^{-1}) to measure their final weight and length. The growth performance and feed utilization parameters of fish were calculated using the following equations:

$$\text{Weight gain (g)} = W_f - W_i$$
$$\text{Specific growth rate (\%)} = \left(\text{SGR; \%Body weight day}^{-1}\right) = [(\text{Ln}W_f - \text{Ln}W_I)/\text{Time}] \times 100$$
$$\text{Condition factor (CF)} = \left(W_f/L_f{}^3\right) \times 100$$
$$\text{Daily growth index (DGI)} = [(W_f - W_i)/\text{Time}]$$
$$\text{Hepatosomatic index (HSI)} = (\text{Liver weight}/\text{Body weight}) \times 100$$

where W_i, W_f, W_{gain}, L_f, and time refer to initial weight, final weight, weight gain, final length, and feeding period of fish, respectively.

Exposure of Nile tilapia (*O. niloticus*) to AgNPs

At the end of feeding fish with melatonin-supplemented diets for 56 days, they were exposed to three concentrations of AgNPs for 24 h. The exposure concentrations (0, 0.05, 0.1, and 0.5 mg L^{-1}) were selected based on a series of pre-tests (data not shown). For each exposure concentration, three glass aquarium (50 L) were filled with 40 L of dechlorinated freshwater, and desired concentrations of AgNPs were poured into each glass aquarium. Then, 5-starved fish for 24 h were randomly transferred into each exposure vessel under 16:8 light/dark and aeration conditions. After 24 h of exposure, fish were

collected for further biochemical analysis. The experimental groups were as follows: untreated-melatonin fish exposed to AgNPs (control), treated-LDM fish exposed to AgNPs (LDM), and treated-HDM fish exposed to AgNPs (HDM).

Plasma biochemical assay

Biochemical indices of plasma were measured in fish at the end of 56th day of feeding trail as well as 24-h waterborne exposure to AgNPs. In each experiment, five fish were randomly sampled from each replicate and anesthetized using clove powder (500 mg L^{-1}), and the blood samples were taken from the caudal vein one by one without pooling using heparinized syringes. For plasma isolation, the blood samples were centrifuged at 5000 rpm for 10 min (Eppendorf 5804R, Germany). The samples were kept at −80 °C up to measure the biochemical indices. Plasma glucose was determined based on the colorimetric glucose oxidase method at a wavelength of 546 nm using a commercial kit (Ziest Chem Diagnostic Co., Tehran, Iran) following the manufacturer's protocol. Total protein content of the plasma was measured according to the Biuret method at a wavelength of 550 nm (Annino and Giese 1976). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined based on the colorimetric Frankel-Reitman method (Reitman and Frankel 1957) using commercial kits (Ziest Chem Diagnostic Co., Tehran, Iran) at an optical density (OD) of 505 nm. Lactate dehydrogenase (LDH) level in plasma samples was determined according to the quantitative sandwich enzyme-linked immunosorbent assay (ELISA) method using a commercial kit (Fish LDH ELISA Kit MyBioSource, Inc., USA). The OD was read at 450 nm using an ELISA reader.

Liver enzymatic analysis

Enzymes activity was assayed in liver of fish sampled at the end of feeding trail and AgNPs exposure test. After the blood sampling from anesthetized fish, their livers were removed for the preparation of homogenate. The tissue was rinsed with phosphate buffered saline (PBS) and homogenized in ice-cold PBS (pH 7.4) with a glass homogenizer. The liver homogenate was centrifuged at 10,000 rpm for 10 min at 4 °C, and the obtained supernatant was stored in liquid nitrogen for further analysis (Jindal et al. 2018). Malondialdehyde (MDA) activity was determined using the thiobarbituric acid reactive substance based on the method explained by Buege and Aust (1978). Briefly, a reagent containing 15% w/v trichloroacetic acid (TCA), 0.37% w/v thiobarbituric acid, and 0.25 mol L^{-1} of hydrochloric acid was prepared. Then, 1 mL of supernatant was added to 2 mL of reagent in a tube and mixed thoroughly. In following, 1 mL of distilled water (dH_2O) was added to 2 mL of reagent and mixed very well. After adding dH_2O, tube was heated for 15 min in boiling water bath. The tube was cooled and then centrifuged at 1000 rpm for 10 min. The precipitate was removed, and the absorbance of the supernatant was read at 535 nm using a spectrophotometer (HACH DR/4000, USA). The MDA concentration of samples was calculated using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}.

The superoxide dismutase (SOD) level in the liver was evaluated based on the method described by Marklund and Marklund (1974). Briefly, 30 μL of homogenate supernatant was added to 2 mL of Tris-HCl (50 mM, pH= 8.2) into a test tube. After few seconds, 20 μL of pyrogallol solution (10 mM, pH=7.4) was added into tube. Pyrogallol autoxidation in test tube compared to the control was quantified at wavelength of 420 nm. The SOD activity was calculated based on the one unit of SOD that causes 50% inhibition of pyrogallol oxidation.

The glutathione peroxidase (GPx) activity was determined using protocol of Paglia and Valentine (1967). Briefly, 50 μL of the homogenate supernatant was transferred into tube test. Then, the reaction medium composed of 100 μL of 150 mM reduced glutathione, 100 μL of 8 mM NADPH, 20 μL of 0.12 M sodium azide, 20 μL of glutathione reductase (30 U mL^{-1}), and 2.65 mL of 50 mM potassium phosphate buffer (pH 7.5 mM EDTA) was added to test tube and incubate at 37 °C for 30 min. Then, 100 μL of 2 mM H_2O_2 solution was added and mixed rapidly. The absorbance of samples was read every 15 s for 5 min at 340 nm using a spectrophotometer (HACH DR/4000, USA). The decrease in absorbance was expressed as enzyme activity.

Statistical analysis

Data were presented as mean ± standard deviation (SD). All statistical analysis was performed using SPSS software (Version, 19, IBM SPSS, Armonk, NY, USA). Kolmogorov–Smirnov test was employed to examine normality assumption of data. One-way analysis of variance (ANOVA) and two-way (dose of melatonin × concentration of AgNPs) ANOVA followed by Duncan's new multiple range test was applied to determine the significant differences between the means at 95% confidence level.

Results

Characterization of AgNPs

TEM micrograph of experimental AgNPs is shown in Fig. 1. Based on TEM, AgNPs were spherical in shape with an average diameter of 11.17±7.83 nm and a size distribution ranged from 2.00 to 47.52 nm.

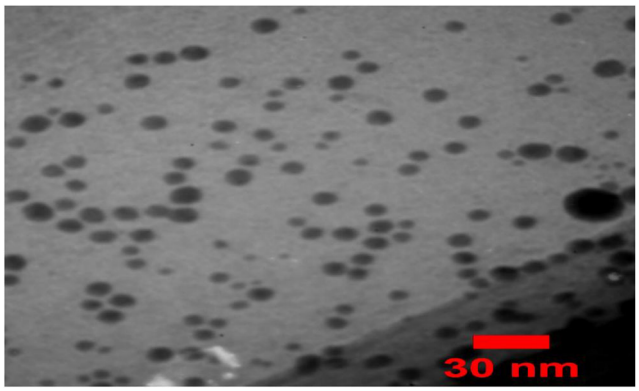

Fig. 1 TEM micrograph of AgNPs from 4000 mg L^{-1} stock suspension

Growth performance

Growth performance and feed utilization indices of tilapia (*O. niloticus*) juveniles fed on diets supplemented with different levels of melatonin are shown in Table 2. The growth performance parameters except condition factor (CF%) showed no significant differences between dietary groups (P>0.05). The CF% value increased in HDM dietary group compared to the other dietary groups (P<0.05).

Biochemical analyses

After feeding fish with melatonin

The enzymes activity in liver of tilapia (*O. niloticus*) fed on melatonin-supplemented diets are shown in Table 3. The glutathione peroxidase (GPx) activity in LDM and HDM dietary groups increased in comparison with the control (P<0.05). The malondialdehyde (MDA) activity in LDM and HDM dietary groups decreased compared with the control (P<0.05). No significant difference was observed in superoxide dismutase (SOD) activity between different dietary groups (P>0.05).

The variations of plasma biochemical indices in fish fed on melatonin-supplemented diets are presented in Table 3. The glucose level in the HDM and LDM dietary groups was lower than in the control (P<0.05). Fish fed on diet supplemented with high level of melatonin (HDM) showed lower levels of the total protein and aspartate aminotransferase (AST) than the other dietary groups (P<0.05). The alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) levels showed no significant differences between the dietary groups (P>0.05).

After exposure of fish to AgNPs

The activity of enzymes in the liver of fish exposed to four concentrations of AgNPs for 24 h after feeding with melatonin-supplemented diets for 56 days are shown in Fig. 2a–c. For GPx enzyme, there was a significant main effect of melatonin dose (F=84.87; P=0.00) and AgNPs concentration (F=127.48; P=0.00), while melatonin dose × AgNPs concentration interaction effect (F=1.73; P=0.15) was not significant. In all dietary groups, the GPx activity decreased significantly with increasing exposure concentration of AgNPs from 0 to 0.5 mg L^{-1} (P<0.05). The GPx activity in fish exposed to 0 and 0.05 mg L^{-1} of AgNPs was higher in HDM and LDM dietary groups than the control group (P<0.05). In 0.1 and 0.5 mg L^{-1} of AgNPs, the GPx activity increased in HDM group in comparison with the control and LDM groups (P<0.05) (Fig. 2a). For MDA enzyme, the main effects of melatonin dose (F=34.10; P=0.00) and AgNPs concentration (F=25.09; P=0.00) were statistically significant, while their interaction (F=1.62; P=0.18) was not significant. The MDA activity in the all-dietary groups increased with elevation of exposure concentration of AgNPs (P<0.05). In the all exposure concentrations of AgNPs except 0.1 mg L^{-1}, the MDA activity in the LDM and HDM dietary groups decreased compared to the control group (P<0.05). The MDA activity showed no significant difference between the LDM and HDM dietary groups (P>0.05) (Fig. 2b). For SOD enzyme, the results of main effects of melatonin dose (F=26.75; P=0.00), AgNPs concentration (F=269.43; P=0.00), and their interaction (F=3.94; P=0.007) were significant. The SOD activity decreased coincidence with increasing exposure concentration of AgNPs in all dietary groups (P<0.05). In the exposure concentrations of 0.05, 0.1, and 0.5 mg L^{-1} of AgNPs, the SOD level in the HDM and LDM dietary groups increased significantly compared to the control (P<0.05). The SOD level in 0 mg L^{-1} of AgNPs showed no significant difference between dietary groups (P>0.05). In the 0.05 mg L^{-1} of

Table 2 Growth performance and feed utilization parameters of Nile tilapia (*O. niloticus*) juveniles fed on diets supplemented with different doses of melatonin for 8 weeks (mean ±SD, n=3)

Treatment	Initial weight (g)	Final weigh (g)	Weight gain (g)	SGR% (%BW day^{-1})	CF%	DGI	HSI%
Control	20.44±1.78^a	53.22±1.78^a	32.77±1.58^a	3.91±0.032^a	1.66±0.054^a	0.65±0.031^a	1.11±0.13^a
LDM (50 mg kg^{-1})	21.80±1.56^a	53.89±2.93^a	32.08±1.87^a	3.92±0053^a	1.73±0.060^a	0.64±0.037^a	1.32±0.34^a
HDM (200 mg kg^{-1})	22.30±2.80^a	55.23±0.12^a	32.92±2.93^a	3.94±0.004^a	1.94±0.001^b	0.65±0.058^a	1.24±0.17^a

The values with different letters in the same column are significantly different (ANOVA, P<0.05). *SGR* specific growth rate, *CF* condition factor, *DGI* daily growth index, *HSI* hepatosomatic index, *LDM* low dose of melatonin, *HDM* high dose of melatonin

Table 3 Biochemical indices in the liver tissue and the plasma of Nile tilapia (*O. niloticus*) fed on diets supplemented with different doses of melatonin for 56 days (mean ± SD; *n*=3)

Treatment	Parameters							
	Liver tissue			Plasma				
	GPx	MDA	SOD	Glucose	Total protein	AST	ALT	LDH
Control	5.445±0.27^{a}	1.8±0.10^{c}	7.15±0.31^{a}	71.11±1.04^{b}	4.26±0.17^{b}	51.63±0.36^{b}	19.82±0.25^{a}	163.99±0.2^{a}
LDM (50 mg kg^{-1})	5.86±0.05^{b}	1.49±0.04^{b}	7.40±0.42^{a}	69.46±0.52^{a}	4.21±0.02^{b}	51.2±0.61^{b}	19.82±0.28^{a}	163.68±0.46^{a}
HDM (200 mg kg^{-1})	6.14±0.17^{b}	1.32±0.05^{a}	7.37±0.03^{a}	69.10±0.02^{a}	3.96±0.04^{a}	50.24±0.09^{a}	19.42±0.12^{a}	163.545±0.28^{a}

AgNPs, the SOD level in the HDM group was significantly higher than the LDM group (P<0.05) (Fig. 2c).

The variations of the plasma biochemical indices in melatonin-treated fish exposed to three concentrations of AgNPs for 24 h are presented in Fig. 3a–e. For glucose, total protein (TP), AST, ALT, and LDH parameters, the main effects and their interaction effects were statistically significant (P<0.05). In all dietary groups, the highest glucose level was observed in the exposure concentration of 0.5 mg AgNPs L^{-1} (P<0.05). In exposure concentrations of 0.05, 0.1, and 0.5 mg L^{-1} of AgNPs, the glucose level in HDM dietary group was significantly lower than the LDM and

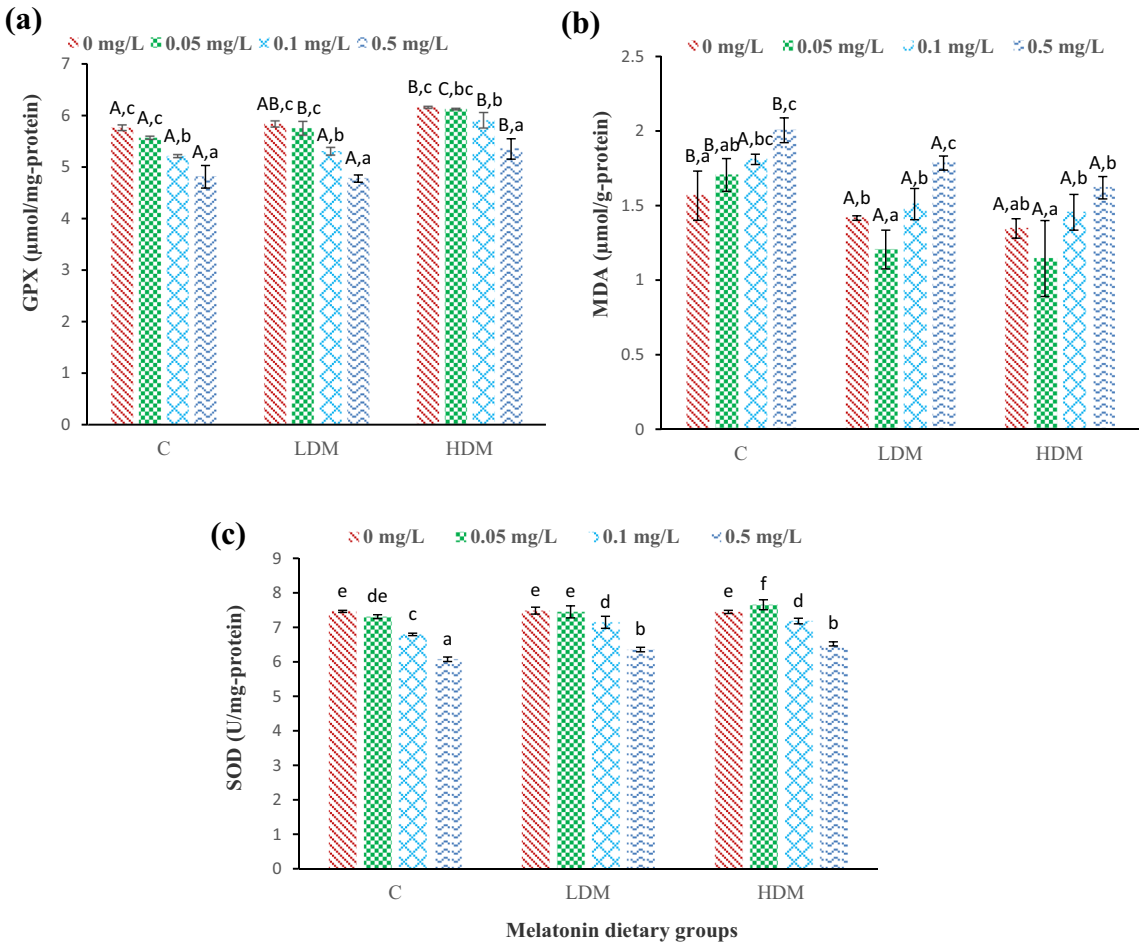

Fig. 2 Glutathione peroxidase (GPx) (**a**), malondialdehyde (MDA) (**b**), and superoxide dismutase (SOD) (**c**) levels in the liver of melatonin-treated Nile tilapia (*O. niloticus*) exposed to three concentrations (0, 0.05, 0.1, and 0.5 mg L^{-1}) of silver nanoparticles (AgNPs) for 24 h. C, untreated-melatonin fish exposed to AgNPs (control); LDM, treated-LDM fish exposed to AgNPs; HDM, treated-HDM fish exposed to AgNPs. Bars with different lowercase letters in each melatonin dietary groups are significantly different (mean ± SD, one-way ANOVA, and two-way ANOVA, P<0.05). Bars with different capital letters in each AgNPs concentration are significantly different (mean ± SD, one-way ANOVA, P<0.05)

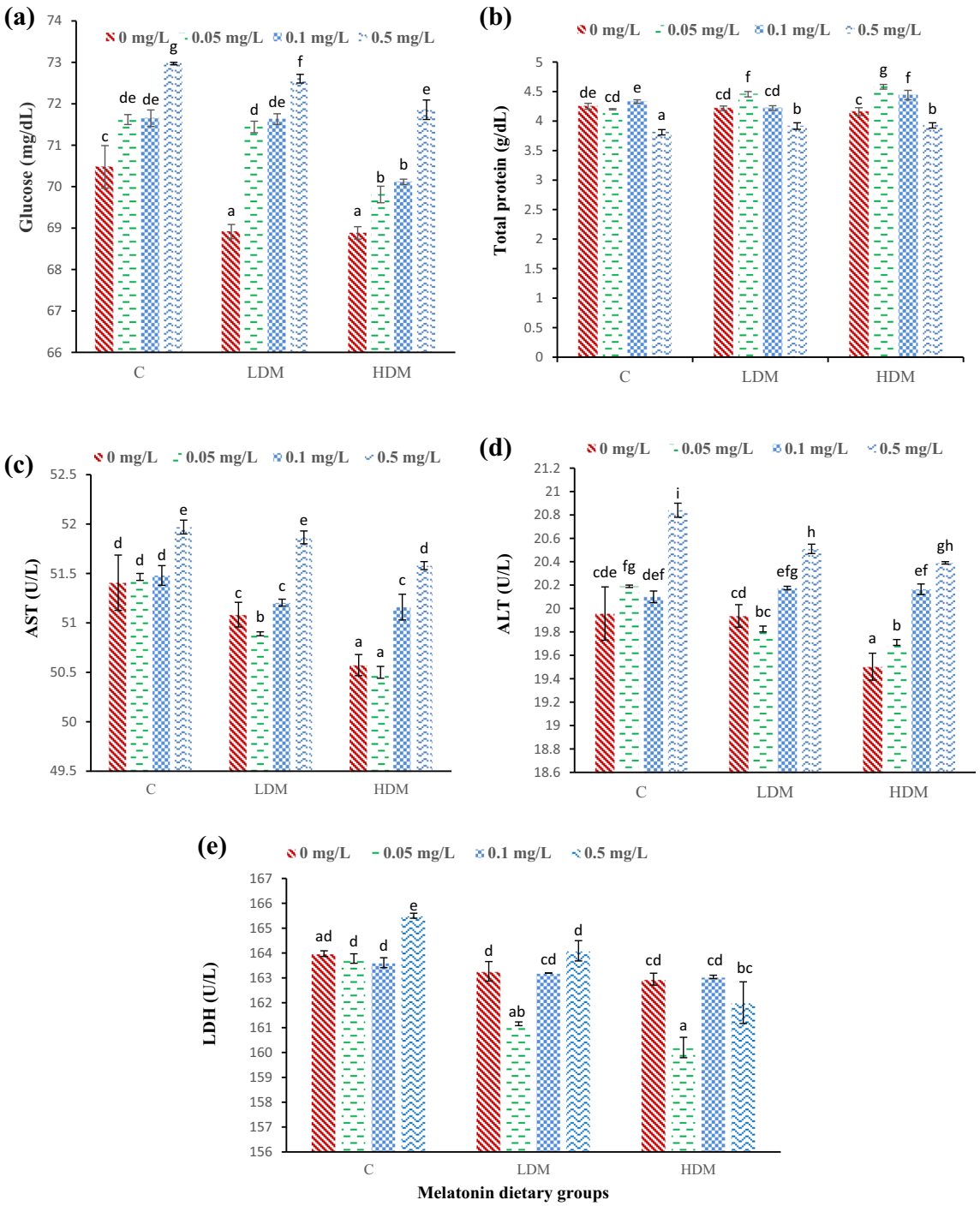

Fig. 3 Glucose (**a**), total protein (**b**), aspartate aminotransferase (AST) (**c**), alanine aminotransferase (ALT) (**d**), and lactate dehydrogenase (LDH) (**e**) levels in the plasma of melatonin-treated Nile tilapia (*O. niloticus*) exposed to four concentrations (0, 0.05, 0.1, and 0.5 mg L^{-1}) of silver nanoparticles (AgNPs) for 24 h. C, untreated-melatonin fish exposed to AgNPs (control); LDM, treated-LDM fish exposed to AgNPs; HDM, treated-HDM fish exposed to AgNPs. Bars with different letters are significantly different (mean ± SD, two-way ANOVA, $P< 0.05$)

control groups ($P<0.05$). In 0 mg L^{-1} of AgNPs, the glucose level in LDM and HDM groups was significantly lower than the control ($P>0.05$) (Fig. 3a). The plasma total protein (TP) level decreased coincident with increasing exposure concentration of AgNPs in the all-dietary groups ($P<0.05$). In 0 mg L^{-1} of AgNPs, the TP level in HDM group decreased significantly compared to the control and LDM groups ($P<0.05$). The TP level in HDM dietary group was significantly higher than the control group in the 0.05, 0.1, and 0.5 mg L^{-1} of AgNPs ($P<0.05$). The highest TP level was observed in the HDM dietary group in the 0.05 mg L^{-1} of AgNPs ($P<0.05$) (Fig. 3b).

The AST activity increased with elevation of exposure concentration from 0 to 0.5 mg L^{-1} of AgNPs in the all dietary groups ($P<0.05$). In the 0, 0.05, and 0.1 mg L^{-1} of AgNPs, the AST activity in the LDM and HDM dietary groups was significantly lower than the control ($P<0.05$). In the 0.5 mg L^{-1} AgNPs, the lowest AST activity was observed in the HDM dietary group ($P<0.05$) (Fig. 3c).

In the all-dietary groups, the ALT activity increased with elevation of exposure concentration from 0 to 0.5 mg L^{-1} of AgNPs ($P<0.05$). In fish exposed to 0 mg L^{-1} of AgNPs, the ALT activity increased in HDM group in comparison to the control and LDM groups ($P<0.05$). In the 0.05 and 0.5 mg L^{-1} of AgNPs, the ALT activity was lower in the both melatonin dietary groups than the control ($P<0.05$). In the 0.1 mg L^{-1} of AgNPs, there was no significant difference in the ALT activity between the dietary groups ($P>0.05$) (Fig. 3d). The LDH activity in the control group increased significantly with elevation of AgNPs concentrations from 0 to 0.5 mg L^{-1} ($P<0.05$). In the LDM and HDM dietary groups, the LDH activity decreased with increasing AgNPs concentration from 0 to 0.05 mg L^{-1}, while this value increased significantly with higher elevation of AgNPs concentration from 0.05 to 0.5 mg L^{-1} ($P<0.05$). The lowest LDH activity was observed in melatonin treated fish exposed to 0.05 mg L^{-1} of AgNPs ($P<0.05$). In fish exposed to 0.05 and 0.5 mg L^{-1} of AgNPs, the LDH level in LDM and HDM groups was significantly lower than the control ($P<0.05$) (Fig. 3e).

Discussion

In the first section of present study, the Nile tilapia (*O. niloticus*) juveniles were fed on diets supplemented with low (50 mg kg^{-1} of diet) and high (200 mg kg^{-1} of diet) doses of melatonin for 56 days. The results indicated that feeding the fish with melatonin-supplemented diets had no significant influences on growth performance and feed utilization parameters. Fish fed on diet supplemented with high level of melatonin showed only the higher condition factor (CF%) than the control. Administration of melatonin as intraperitoneal injection in tench (*Tinca tinca*) and goldfish (*Carassius auratus*) species showed significant reduction in food intake in both species (López-Olmeda et al. 2006). Studies in rainbow trout, *Oncorhynchus mykiss* (Conde-Sieira et al. 2012), and zebrafish, *Danio rerio* (Piccinetti et al. 2010), found that treatment with melatonin alters gene expression involved in feeding regulation. Effect of melatonin on the growth of fish is contradictory in literatures. Treatment of goldfish (*C. auratus*) and Atlantic salmon (*Salmo salar)* parr with melatonin increased weight gain and growth of these fish (De Vlaming 1980; Singh and Lal 1994). However, contradictory result was reported in Nile tilapia (*O. niloticus*) by Singh et al. (2012). They found that the growth rate (SGR % per day) reduced by 36.6% in fish treated with melatonin (25 μg L^{-1} for 21 days) as compared to the untreated fish. De Pedro et al. (2008) also showed that chronic (10 days) intraperitoneal treatment of goldfish (*Carassius auratus*) with melatonin (10 μg g^{-1} body weight) reduced the specific growth rate and body weight gain by 76% and 74%, respectively.

It is found that melatonin modulates antioxidant enzyme activities including SOD, CAT, and GPx probably via melatonin receptors (Mayo et al. 2002; Tomas-Zapico et al., 2005). Melatonin interaction with calmodulin can activate antioxidant enzymes through inhibition of downstream processes and subsequently activation of nuclear RORα melatonin receptor (Toma's-Zapico and Coto-Montes 2005a, 2005b). Based on the results, the dietary treatment of fish with low and high doses of melatonin increased the GPx activity in the liver tissue. In contrast, melatonin treatment had no significant effect on the SOD activity. Evaluating the effect of dietary melatonin supplementation (10 mg kg^{-1} body wt. for 4 weeks) on erythrocytic antioxidant enzymes in common carp (*Cyprinus carpio*) showed that the SOD activity increased significantly, whereas alterations in catalase (CAT) activity were not significant (Ghodrati Azadi et al. 2013). Gulcin et al. (2009) also reported that melatonin injection (10 mg kg^{-1} fish) activated SOD, CAT, and peroxidase (POD) enzymes in erythrocytes of rainbow trout (*O. mykiss*).

Malondialdehyde (MDA) is a low molecular weight end product produced during the peroxidation of lipid by the action of reactive oxygen species and is often known as an oxidative stress biomarker (Ateş et al. 2017; Kapusta et al. 2018). In the current study, feeding Nile tilapia (*O. niloticus*) juveniles with melatonin-supplemented diets led to decreasing the liver MDA enzyme activity. The MDA activity decreased coincident with increasing melatonin dose from 50 to 200 mg kg^{-1} of diet. These findings suggest the protective effect of the dietary melatonin against lipid peroxidation probably through direct elimination of ROS or activation of antioxidant enzymes. In agreement with these findings, Ghodrati Azadi et al. (2013) found decreased levels of erythrocytic MDA activity in common carp (*C. carpio*) after feeding with melatonin-supplemented diet. It has also been reported that intraperitoneal injection of melatonin (10 mg kg^{-1} fish) decreased plasma MDA activity in the rainbow trout (*O. mykiss*) compared to the control group (Gulcin et al. 2009). Maitra and Hasan (2016) showed that there was an inverse relationship between the levels of melatonin and MDA in ovary of carp *Catla catla* under natural photo-thermal conditions indicating the antioxidant function of melatonin during the spawning phase.

The effect of experimental diets on some plasma biochemical indices showed that the glucose level decreased significantly in fish fed on melatonin-supplemented diets. Similarly, dietary melatonin supplementation (10 mg kg^{-1} body weight) decreased blood glucose level in common carp (*C. carpio*) (Ghodrati Azadi et al. 2013). Melatonin administration also

decreased blood glucose in diabetic rats (Anwar and Meki 2003). Reduction in blood glucose concentration may be attributed to melatonin role on catecholaminergic responses (Maitra et al. 2000). In contrast, treatment of Nile tilapia (*O. niloticus*) adults with diet supplemented with low-dose melatonin (0.3 mg kg^{-1} BW) increased blood glucose level (Kim et al. 2017). Intraperitoneal injection of melatonin (0.1 and 0.5 μg kg^{-1} body weight) also increased glucose in plasma of rainbow trout (*O. mykiss*) after 1 or 3 h of injection (Sangiao-Alvarellos et al. 2007). These conflicting results may be related to the experimental animals used in the studies, dosage of used melatonin, and treatment period (Nishida 2005; Peschke 2008). In the present study, the plasma total protein content decreased in fish fed on high level of melatonin (200 mg kg^{-1} of diet) in comparison with other dietary groups. It has been reported that melatonin decrease protein level in blood of fish (Falcon et al. 2007). However, Singh et al. (2012) found that the blood protein level increased in Nile tilapia (*O. niloticus*) after water treatment with melatonin in dose of 25 μg L^{-1} for 3 weeks.

Alanine aminotransferase (ALT) and aspartate amino transferase (AST) enzymes are normally localized in the cells of the liver, gills, heart, muscles, kidneys, and other organs. The presence and elevation of these enzymes in blood circulation reflect damage to tissue cells and organ dysfunction (Shahsavani et al. 2010; Rather 2015). Therefore, these enzymes can be used as general biomarkers under stressful conditions or environmental pollutions (El-Shehawi et al. 2007; Ozmen et al. 2008). The findings of present study revealed that the plasma AST level was lower in fish fed diet supplemented with high dose of melatonin than in the control. Fish treatment with melatonin also did not significantly alter the plasma ALT and LDH levels. These finding revealed that melatonin-supplemented diets had no toxic effect on liver tissue of Nile tilapia. Ghodrati Azadi et al. (2013) attributed the higher plasma AST and ALT activities to cytotoxic effects of dietary melatonin on liver cells of common carp (*C. carpio*).

Exposure of melatonin-treated Nile tilapia (*O. niloticus*) juveniles to four concentrations of AgNPs for 24 h showed that the liver antioxidant enzymes activities including GPx and SOD decreased coincident with increasing the exposure concentration from 0 to 0.5 mg L^{-1}. The activity of liver SOD and GPx decreased in goodeid fish (*C. pardalis*) exposed to two concentrations (1.93 and 4.08 mg L^{-1}) of AgNPs (Valerio-Garcia et al. 2017). The GPx and SOD activities were higher in AgNPs-exposed Nile tilapia fed on high melatonin-supplemented diet than the control groups indicating the ameliorative effect of melatonin on toxicity of AgNPs. Drąg-Kozak et al. (2019) showed that co-exposure of Prussian carp females (*C. gibelio*) to sub-lethal concentration of waterborne cadmium and melatonin increased the GPx and SOD levels in the hepatopancreas tissue. It was also found that injection of melatonin (5 or 10 μg g^{-1} body mass) to goldfish (*C. auratus*) effectively decreased the oxidative stress induced by thermal stress (Jung et al. 2016).

In the present study, the liver MDA activity as a production of lipid peroxidation increased in melatonin-treated Nile tilapia exposed to AgNPs in a dose-dependent manner. Exposure of *Tilapia zillii* and *O. niloticus* to 4 mg L^{-1} of AgNPs led to a highly significant increase of MDA in brain tissue in comparison to the control (Afifi et al. 2016). In the current study, low and high dose of melatonin decreased the MDA activity in fish exposed to the all concentrations of AgNPs except 0.1 mg L^{-1}. Moniruzzaman et al. (2018) showed that melatonin treatment reduced the MDA activity in highest dose of H_2O_2-exposed carp (*Labeo rohita*) hepatocytes.

Exposure of Nile tilapia to AgNPs resulted in increasing the plasma glucose level in a dose dependent manner, while treatment with melatonin especially at dose of 200 mg kg^{-1} of diet reduced the glucose level suggesting the melatonin attenuating effect. Gesto et al. (2016) reported that exposure of sole *Solea senegalensis* to stressful conditions increased the plasma glucose level coincident with increasing time exposure. In contrast to our findings, the glucose level in melatonin-treated sole fish was higher than in untreated fish. In the current study, the plasma total protein (TP) content was lower in Nile tilapia exposed to higher AgNPs concentration. The TP level clearly increased in AgNPs-exposed fish pre-treated with high dose of melatonin. The Nile tilapia exposed to higher concentration of AgNPs showed the higher plasma AST and ALT levels. The serum levels of ALT and AST increased significantly by increasing exposure concentration of AgNPs from 3 to 1000 mg L^{-1} in common carp (*C. carpio*) juveniles (Monfared et al. 2015). The AST and ALT activities in melatonin-treated Nile tilapia exposed to AgNPs were significantly lower than in untreated fish. Similarly, melatonin treatment significantly reduced the elevated AST and ALT levels in serum of rats induced by CCl_4 injection (Ogeturk et al. 2004). Hashish and Elgaml (2016) also showed that melatonin decreased the serum hepatic enzymes (AST and ALT) in albino rats treated with potassium dichromate ($K_2Cr_2O_7$). In the current study, the plasma LDH activity increased with increasing exposure concentration of AgNPs in the control group. It has been reported that enhanced LDH level in fish species exposed to AgNPs may lead to an increase in conversion rate of lactate to pyruvate and then to glucose (Abdel-Hameid 2007; Monfared et al. 2015). Our results also showed that the plasma glucose level increased in fish exposed to four concentrations of AgNPs compared to the control. The reduced plasma LDH level in melatonin-treated Nile tilapia exposed to 0.05 and 0.5 mg L^{-1} of AgNPs in comparison to the control groups reflecting ameliorative effect of melatonin. Administration of melatonin ameliorated the elevation of LDH level in the serum of diabetic rats induced by streptozotocin (Amin et al. 2014).

Conclusions

The present study investigated the effects of two doses of dietary melatonin on growth performance, plasma biochemical indices, and liver enzymes activities as well as potential toxicity alleviation caused by AgNPs in Nile tilapia juveniles. The results revealed that melatonin-supplemented diets had no significant influence on the growth performance but modulated the liver GPx and MDA activities and the plasma glucose, total protein and AST levels. Our finding also showed that toxicity of waterborne AgNPs on the biochemical parameters of plasma and liver increased coincident with elevation of exposure concentration. This study revealed that dietary melatonin was capable to ameliorate the toxicity effects induced by AgNPs in Nile tilapia.

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Declarations

Ethics approval Ferdowsi University of Mashhad (FUM) and University of Kurdistan animal ethic rights were applied to all experiments on fish.

Consent to participate Not applicable

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Conflict of interest The authors declare no competing interests.

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