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Effects of Silver Nanocolloid and Copper Oxide (I) on HSP70 Expression and GST Activity in the Caspian Kutum

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Abstract

The present study investigated the lethal and semi-lethal effects of silver nanocolloid and copper (I) oxide nanoparticles on the expression of HSP70 and glutathione S-transferase (GST) in the gill, liver, and serum of the Caspian kutum fry (*Rutilus kutum*). Fish were exposed to different concentrations of silver nanocolloid (0.05, 0.35, and 0.7 of LC_{50}) and copper (I) oxide nanoparticles (0.25 and 0.5 of LC_{50}). The results showed a high expression of the HSP70 gene on the 7th and 21st days of exposure to silver nanocolloid and copper (I) oxide nanoparticles. Significant changes in GST levels were observed under the influence of silver nanocolloid in the gills and serum. In conclusion, variations of the HSP70 gene expression and GST activity show that the presence of nanoparticles could be affecting the antioxidant system in the fish body; indicating that their excessive influx brings disruptive danger to aquatic health.

Introduction

The possible danger of nanomaterials to the aquatic environment has drawn attention because of their common usage in industry, medicine, and aquaculture (Fei et al., 2009). Silver nanoparticles (AgNPs) are increasingly used in medicine, healthcare products, food production, household hygiene, and other industrial purposes due to their unique physical and biological properties. These include optical, electrical, and thermal conductivity as well as bactericidal effects (Lee et al., 2012; Luthuli, 2012). Recent studies have shown that silver nanoparticles can interact with macromolecules like proteins, altering the mitochondrial functionality as well as modulating levels and activity of reactive oxygen species (ROS) (Liu, 2006).

Copper (I) oxide (Cu_2O) is a red-colored inorganic compound with a cubic structure that possesses both semiconducting as well as antimicrobial properties (Abdel-Khalek et al., 2015). Cuprous oxide is commonly used as a pigment, a fungicide, and an antifouling compound in the production of anti-corrosive paints for marine purposes (Kiaune and Singhasemanon, 2011). The most important copper toxicity pathways include inhibiting ATP pumps, ion channels and enzymes; and inducing ROS synthesis in organisms (Kiaune and Singhasemanon, 2011).

Changes in biochemical status are usually the first detectable response to environmental disorders and stress (Safari et al., 2014). Heat shock proteins (HSPs) are an evolutionary family of conservative and commonly occurring chaperone proteins in eukaryotic

organisms. Their expression is quickly and continuously modulated in response to metabolic changes, a fundamental mechanism protecting proteins from numerous shocks (Yamashita et al., 2010). Based on their molecular weight, HSPs are divided into three main groups that comprise different families, i.e., (1) macromolecular proteins (75-110 kDa), which include families HSP90/100/110, (2) medium-molecular proteins (47-78 kDa), which include families HSP47/60/70 and (3) small heat shock proteins (sHSP), that comprise molecular mass proteins of 8.5 - 43 kDa (Luthuli, 2012). One of the largest families and most studied stress-related proteins distributed throughout cells, are HSP families HSP70 and HSP90 (Jing et al., 2013; Mohanty et al., 2018; Sarkar and Roy, 2017). The biological activities of HSPs mainly include facilitating the synthesis and/or folding of proteins in the cell. In addition, HSPs play an important role in the production, secretion, transport, and degradation of proteins, particularly with regard to activity modulation of transcription factors and protein kinases (Abdolhay et al., 2011; Heredia-Middleton et al., 2008; Whitley et al., 1999). Depending on the degree of stress, the expression level of HSPs is subjected to significant and continuous changes, playing an essential role in maintaining the cell's homeostasis. Consequently, the increase and accumulation of HSPs protects cells from stress and ensures cell survival (Safari et al., 2014; Zhang and Zuiderweg, 2004).

Changes in the activity of antioxidant enzymes in aquatic organisms can be considered a potential indicator of stressful conditions. Glutathione S-transferase (GSTs) enzymes are a large family of dimer proteins that are found in all aerobic organisms and are currently known as a major detoxification factor. The GST family is divided into three main subfamilies, namely: cytosolic, mitochondrial, and microsomal, which can constitute up to 10% of the cytosolic protein in mammalian organs (Afifi et al., 2016; Rudneva et al., 2010). The main function of GST is to catalyze the conjugation of reduced glutathione (GSH) tripeptide with internal and external toxic xenobiotics and, subsequently, converting them and/or their metabolites into hydrophilic compounds that can be easily eliminated from the cell (Carvalho-Neta and Abreu-Silva, 2013; Habig et al., 1974; Zhang et al., 2012).

The Caspian kutum, *Rutilus kutum*, is a species of the cyprinid family inhabiting brackish waters of the Caspian Sea and its freshwater tributaries (Gharedaashi et al., 2013). The Caspian kutum is migratory and endemic to the Caspian Sea. The Caspian kutum represents a very common and one of the most economically important teleost fishes of Iranian fisheries (Heidari et al., 2009).

To this date, several studies have been carried out on changes in the expression levels of the HSP70 gene under the influence of various nanoparticles in selected aquatic organisms, such as Persian sturgeon (*Acipenser persicus*) (Safari et al., 2014), rainbow trout (*Oncorhynchus mykiss*) (Ceyhun et al., 2010; Feng et al., 2003), common carp (*Cyprinus carpio*) (Jiang et al., 2016); yellow perch (*Perca flavescens*) (Pierron et al., 2009) and Japanese medaka (*Oryzias latipes*) (Chae et al., 2009). Moreover, some studies have been conducted on the measurement of GST in aquatic animals, namely: marine catfish (*Arius arius*) (Mani et al., 2014), Pemecou sea catfish (*Sciades herzhergii*) (Carvalho-Neta and Abreu-Silva, 2013); brown trout (*Salmo trutta*) (Almli et al., 2002), Nile tilapia (*Oreochromis niloticus*) (Oruç and Üner, 2000), *Brycon caphalus* (Monteiro et al., 2009) and the other Black Sea teleosts (Rudneva et al., 2010).

Today, the use of nanoparticles and their entry into the aquatic ecosystem is a growing trend. Concentrations of copper (I) oxide and silver nanocolloid nanoparticles have not been measured in the Caspian Sea and its basin; although concentrations of different heavy metals including copper and silver were measured in the Caspian sea by (Pakzad et al., 2016; Raeisi et al., 2014). This study aimed to investigate the effects of copper (I) oxide and silver nanocolloid nanoparticles on expression levels of HSP70 genes in the liver and GST activity in the gill and serum of the Caspian kutum.

Materials and Methods

Fish

The Caspian kutum fry (N 240) with an average weight of 17.1±3.6 g obtained from the Fish Rearing, Propagation and Restocking Center of Dr. Yousefpouri (Rasht, Guilan Province, Iran). The fish were kept in a fiberglass tank for 7 days in a volume of 1000 liters of dechlorinated water (pH 7.6±0.2, temperature 19±1°C, oxygenation 7.4±0.1 mg/L) without a re-circulation system. The fish were fed approximately 1% of their body weight two times a day during the experiment with the feeding powder purchased from Isfahan Mokammel Co. (Isfahan, Iran).

Silver Nanocolloid

Preparation of the Nanocolloid

Silver nanocolloid was purchased from Nano Nasb Pars (Tehran, Iran) with a concentration of 4000 mg/L. The silver colloid nanoparticles were spherical, 16.6 nm in diameter, and dark brown in color.

Determination of LC_{50} Concentration

To determine the LC_{50}, 10 fish (with 3 replicates) were transferred to glass aquaria (volume of 80L) and kept under constant conditions (photoperiod 12L12D; water hardness 263±4 ppm; temperature 19±1°C and pH 7.6±0.2) for 96 hours. After the acclimatization period, different concentrations of silver nanocolloid were

tested to determine its LC_{50} concentration level. As a result, the concentration of 0.25 mg/L was determined as LC_{50}96h for silver colloid nanoparticles.

Copper (I) Oxide Nanoparticle (Cu_2O)

Preparation of Cu_2O

Based on the protocol described by Bai et al. (2012), Cu_2O was synthesized in the Marine Biology Laboratory at the University of Guilan (Bai et al., 2012). 0.05 g of copper (II) acetate and 0.125 g of PVP (Poly Vinyl Pyrolidon) were dissolved in 100 ml of deionized water to make the aqueous solution. Then, 20 ml of 0.25 M sodium hydroxide (NaOH) water solution was added drop by drop to the starting solution under intense shaking. Next, 15 ml of 0.05 M ascorbic acid water solution was slowly added to the resulted solution under the same conditions. The color of the mixture changed from blue through green to orange after 30 minutes of shaking. In the end, the obtained solution was centrifuged for 15 minutes at 4000 rpm and the separated orange deposits were dried in an oven.

X-ray Diffraction Analysis

X-ray diffraction (XRD) was used to determine the type of synthesized material (Cu_2O), as well as the phase and its crystalline properties. For this purpose, a sample of synthesized nanoparticles (0.1g) was sent to Beam Gostar Taban Research Laboratory located in Tehran, Iran for the specified analysis.

Determination of LC_{50} Concentration

Based on (Mazarei et al., 2015), a concentration of 1.7 mg/L of Cu_2O was considered as LC_{50}. The specific procedure was the same as for silver colloid nanoparticles.

Treatments

The following concentrations of silver nanocolloid were selected for treatments used in the present study: 0.0125, 0.0875, and 0.175 mg/L, which consist of 5, 35, and 70% of determined LC_{50} concentration, respectively. For Cu_2O, the applied concentrations were as follows: 0.425 and 0.850 mg/L, which consist of 25 and 50% of determined LC_{50} concentrations, respectively. A control group comprised of fish that were not exposed to any treatment.

In total, the experiments were carried out in six groups, i.e., 3 groups for silver nanocolloid, two for Cu_2O, and one control group. Each experimental group was carried out in triplicates that give a total number of 18 tanks used. In general, the number of fish used under each experimental variant was 30, including each replicate (10 fish per tank).

The fish were kept in glass aquariums of 80 liters volume with constant conditions (photoperiod 12L12D; pH 6.6–7.2, Temperature 21±2^{o}C; oxygen 7.7±0.5 mg/L). After the adaptation period, feeding was stopped and the experiments commenced in 24 hours. Then, the determined concentrations of silver nanocolloid and Cu_2O were added to the respective aquaria. Before adding the nanoparticles, the nanoparticles were homogenized by sonication for 5 minutes.

The exposure of fish to the silver colloid and Cu_2O nanoparticles was continuously carried out for 21 days. To maintain good sanitary conditions of each aquarium during the experiment, the fish were transferred to a fresh aquarium with the same conditions and the same concentration of nanoparticles on every 3rd day of treatment.

Sampling

The sampling of fish was done randomly on days 7 and 21 of exposure to the nanoparticles used. Blood samples were collected from randomly selected fish within each treatment. To this end, the fish were subjected to anesthesia by transferring to a smaller container of water mixed with clove oil in a dosage of 30 mg/L (Neiffer and Stamper, 2009). Then, the fish were placed in a wet cotton cloth and the heparinized syringe with a 23 g needle was used to collect the blood samples via vertical way through the spine after the anterior fin. 0.2 ml of drawn blood was centrifuged at 4°C and 4000 rpm. The separated serum was stored at -70°C in the freezer. Next, new specimens were dissected and the gill and liver tissues were collected. For euthanasia, the fish were first anesthetized with benzocaine and then frozen (Underwood and Anthony, 2013). The samples of gill were mixed with sodium phosphate buffer (pH 6.5) at a 1:5 ratio and then centrifuged (Centurion Scientific, UK) at 6000 rpm and 4°C for 10 minutes. The homogenized extract was finally transferred to a freezer and stored at -70°C.

RNA Extraction

The Trizol method was used to extract RNA. Liver tissue (50-100 mg) was homogenized in liquid nitrogen and added to a 1 ml solution of Trizol and incubated for 5 minutes at room temperature. Then, 400µl of chloroform was added, shortly vortexed, and left at room temperature for 2 to 3 minutes. Next, the solution was centrifuged at 10,000 rpm for 15 minutes at 4°C and the supernatant containing RNA was slowly separated and transferred to a new microtube. Isopropanol (500 µl) was then added to the microtube containing RNA and stored at room temperature for 10 minutes. The precipitate was separated from the isopropanol by centrifugation (10,000 rpm for 10 minutes at 4°C). After that, 1000 µl of 75% ethanol was added to the precipitate and centrifuged for 5 minutes (7,800 rpm at 4°C). The isolated RNA was then stored at -70°C until further analyses.

Complementary DNA Synthesis

Before complementary DNA synthesis, the isolated RNA was subjected to the RNase-free DNase I (Thermo Fisher Scientific, USA) to remove the remnant DNA present in the RNA samples. For this end, 2 µl DNase and 2 µl DNase buffer was added to 16 µl extracted RNA and incubated for 30 minutes at 37°C. Then, 1 µl EDTA was added and again incubated at 65°C for 10 minutes. Finally, 4 µl of the sample was collected and stored at -70°C. The NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) was used to determine the quality and quantity of resulted RNA at absorption 260 and 280 nm.

The BIOFACT kit (South Korea) was used to synthesize cDNA, carried out in two steps as recommended in the manufacturer's protocol. First, 50 µM of Oligo dT Primer (1µl), 10 mM of each dNTP and 2 µl of template RNA (1 µg) were mixed, filled to 10 µl with diethylpyro- carbonate (DEPC) water and was incubated for 5 min at 65 °C; then cooled immediately on ice. Afterward, 4 µl of 5X Prime Script Buffer, 0.5 µl RNase Inhibitor (40U/µl), 1 µl Prime Script RTase (200U/µl) and 5.5 µl DEPC treated water was added to the above mixture, and the mix was incubated for 30 minutes at 50°C. In the end, it was immediately incubated at 95°C for 10 minutes and kept at -70°C for further analysis.

Polymerase Chain Reaction (PCR)

For PCR primer design, the sequence of the Caspian kutum HSP70 nucleotide was searched in the NCBI database (Accession number: KT380686) and the FASTA aligned sequences were transferred to Gene Runner software (Version 6.5.52 x64 Beta). The maximum specificity of designed primers was validated with an online primer blast (Table 1). PCR reaction was carried out within a 20 µl reaction mix that consisted of 100 ng of liver tissue synthesized cDNA, 2 µl of PCR buffer, 1.75µl MgCl2, 1.2µl dNTP, 0.2µl Taq polymerase, and 0.2 µM of each primer. The desired volume of the reaction mix was obtained by adding nuclease-free water. The PCR program comprised the following steps: initial denaturation at 95°C for 5 minutes followed by 35 cycles of 15 seconds at 95°C (denaturation), 30 seconds at 60°C (annealing), 40 seconds at 72°C (extension). In the end, the final extension step at 72°C for 5 minutes was applied. The target PCR products were analyzed by electrophoresis in a 2% agarose gel stained with safe-stain.

Glutathione S-Transferase (GST) Assay

The assay method was based on the use of substrate containing 1-chloro, 2-nitrobenzene (CDNB) and reduced glutathione (GSH) (Habig et al., 1974). Ten microliters of each substrate at a concentration of 100µM were mixed with 25µl of samples (gill and serum) and diluted to a volume of 1000µl by adding sodium phosphate buffer (pH 6.5). The absorbance of the samples was read at 340 nm for 5 minutes using a spectrophotometer (lab Unlimited, UK).

Statistical Analysis

The data were analyzed using SPSS version 19 in Windows 10. Kolmogorov-Smirnov test was used to measure the normality of the data. In the case of normal data, one-way ANOVA with Duncan's post hoc test was used to evaluate the differences between treatments at the confidence level of 95%. Independent T-test was performed to determine the difference between day seven and day twenty of the experiment. In addition, the combined effects of the exposure time and concentration of the nanoparticles were analyzed by two-way ANOVA. All data were expressed in mean ± SD.

Results

XRD of Copper (I) Oxide Nano Synthesis

Analysis of the X-ray diffraction and "the Joint Committee on Powder Diffraction Standards (Jcpbs) file No-77-0199" showed that the synthesized material was the copper (I) oxide (Cu_2O) nanoparticle (Figure 1).

Silver Nanocolloid

Expression of the HSP70 Gene

The recorded patterns in expression levels of HSP70 were similar both on the 7^{th} and 21^{st} day of experimental exposure on silver nanocolloid ($r=0.90$, $p<0.05$). The expression level of HSP70 increased significantly in all treatments compared to the control on the 7^{th} day of exposure ($p<0.05$) (Figure 2). The highest expression level was reported for the silver nanocolloid concentration at the level of 0.0875 mg/L and differed significantly from other exposure variants ($p<0.05$). The applied two-way ANOVA test evidenced the significant influence of exposure time (days) and tested concentrations on observed levels of HSP70 gene expression ($p<0.05$). According to the independent T-test, the observed differences in HSP70 gene expression on the 7^{th} and 21^{st} day of exposure under all concentrations tested were statistically significant (0.0125, 0.0875, and 0.125 mg/L) ($p<0.05$).

Changes in GST Activity of the Gills

According to the obtained results, a specific trend toward increased GST activity in gill tissue of the fish treated by the silver nanocolloid in the concentration of 0.175 mg/L compared to the control group at both the 7^{th} and 21^{st} day of exposure ($p<0.05$) (Figure 3) was observed. The maximum GST activity was recorded at 0.0125 mg/L concentration on the 7^{th} day of experimental exposure. Based on two-way ANOVA, the interaction of days of exposure and different

concentration had no significant effect on GST activity ($p>0.05$) but there were significant differences in GST activity between different concentrations ($p<0.05$) and between days of exposure ($p>0.073$). According to the independent T-test, a significant difference was observed in GST activity under the concentration of 0.0125 mg/L between the 7th and 21st day of experimental exposure ($p<0.05$).

Changes in GST Enzyme of the Serum

The performed analysis on GST in the serum showed an increase in activity under the 0.175 mg/L concentration of silver nanocolloid, both on the 7th and 21st day of exposure ($p<0.05$) (Figure 4). Based on two-way ANOVA there were no significant differences in GST activity between each day of exposure ($p>0.073$) and their interaction (days of exposure and different concentration) ($p>0.05$). In turn, the significant differences in GST activity were recorded between different concentration variants ($p<0.05$). In addition, there was a significant correlation in variations of GST activity between the 7th and 21st day of exposure at 0.0125 mg/L concentration ($r=0.89$; $p<0.05$). The maximum GST activity was recorded at 0.175 mg/L of silver nanocolloid. According to the independent T-test, a significant difference was also observed in GST activity at a concentration of 0.0125 mg/L between the 7th and 21st day of exposure ($p<0.05$).

Copper (I) oxide (Cu2O)

HSP70 gene expression on 7th and 21st day

An increase in HSP70 gene expression was observed at different concentrations of copper (I) oxide

Table 1. Primer sequences of HSP70 genes of the Caspian kutum.

Gene	Forward	Reverse
HSP70	AGTTGTCACAGTTCCTGCC	GTCTCTCAGGGCTTTCTCC

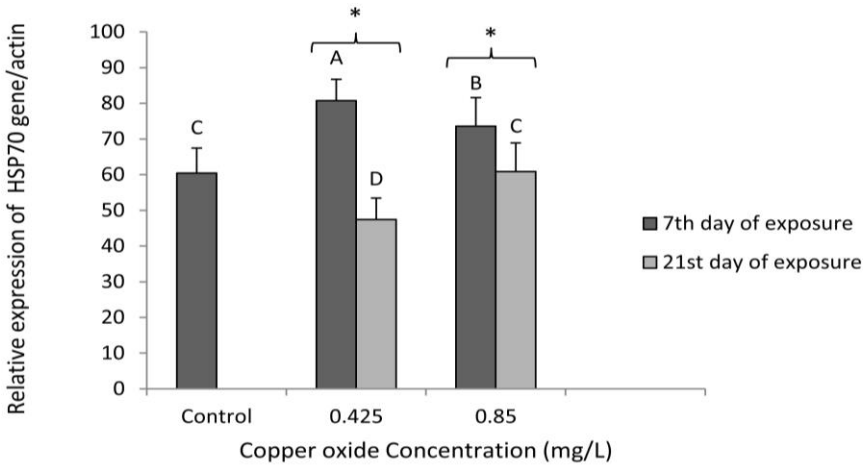

Figure 1. XRD analysis of synthesized copper (I) oxide nanoparticle.

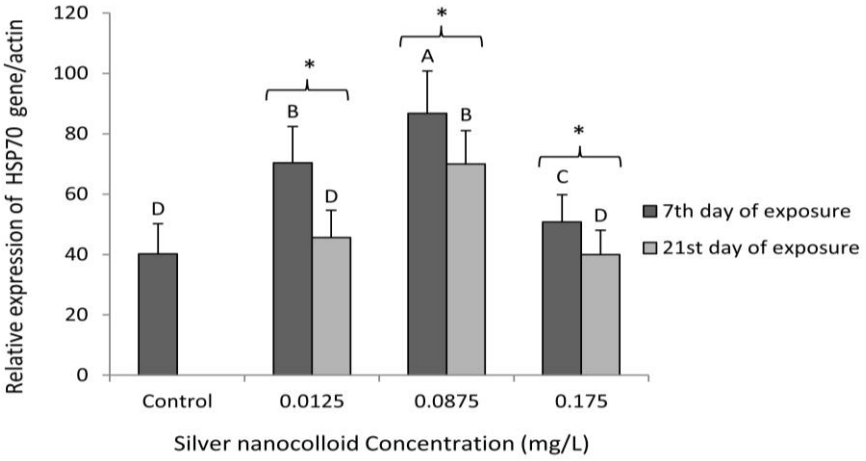

Figure 2. HSP70 gene expression in the liver of Caspian kutum exposed to different concentrations of silver nanocolloid. The letters above columns are showing significantly different based on one-way ANOVA and the symbols, *, are showing a significant difference between the 7th and 21st day of exposure in each concentration based on independent T-test ($p<0.05$).

nanoparticles (0.425 and 0.85 mg/L) compared to the control on the 7th day of exposure ($p<0.05$) (Figure 5). On the 21st day of exposure, the highest level of HSP70 was recorded at 0.85 mg/L ($p>0.05$). Gene expression of HSP70 was significantly higher in all treatments on the 7th day of exposure compared to the 21st day of exposure ($p<0.05$). Based on two-way ANOVA, the interaction between days of exposure and different concentration had a significant effect on HSP70 gene expression ($p<0.05$). According to the independent T-test, a significant difference was observed in HSP70 gene expression in both concentrations of copper (I) oxide nanoparticles (0.425 and 0.85 mg/L) on the 7 and 21 days of exposure ($p<0.05$).

Discussion

In the present study, changes in HSP70 gene expression in the liver of Caspian kutum fry were significantly increased in all treatment variants compared to the control group on the 7th and 21st days of exposure, which suggests the essential role of the HSP70 gene in reducing shock outcomes caused by exposure on nanomaterials in fish. Contrary to the present study, (Safari et al., 2014) observed a significant decrease in HSP70 expression in *Acipenser persicus* liver on the 7th day and a significant increment on the 14th day exposure to different concentrations of cadmium chloride. In turn, (Girilal et al., 2015) showed a significant increase in HSP70 gene expression in the liver of *Oreochromis niloticus* exposed to chemically synthesized silver compound derivatives. In this study, the observed increase in HSP70 expression at 0.0875 mg/L following silver nanocolloid exposure at both sampling days compared to the concentration of 0.175 mg/L may be due to a reduction of metabolic capacity of the fish due to toxicity (Safari et al., 2014). Moreover, nanoparticles and silver nanocolloid in the aquatic environment can accumulate at high concentrations, reducing the efficiency of their displacement and consequently, leading to adverse interaction and alterations in the metabolism of living organisms (Behra

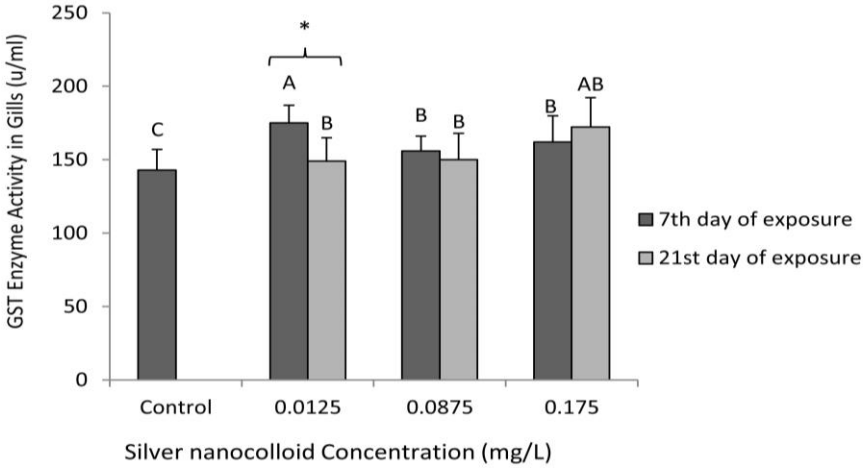

Figure 3. GST activity of gills affected by different concentrations of silver nanocolloid on the 7th and 21st days of exposure. The letters above columns are showing significantly different based on one-way ANOVA and the symbols, *, are showing a significant difference between the 7th and 21st day of exposure in each concentration based on independent T-test ($p<0.05$).

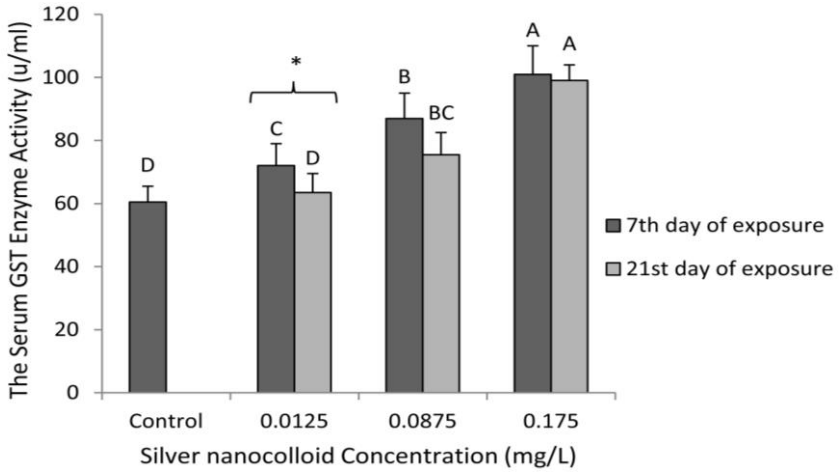

Figure 4. The serum GST activity was affected by different concentrations of silver nanocolloid on the 7th and 21st days of exposure. The letters above columns are showing significantly different based on one-way ANOVA and the symbols, *, are showing a significant difference between the 7th and 21st day of exposure in each concentration based on independent T-test ($p<0.05$).

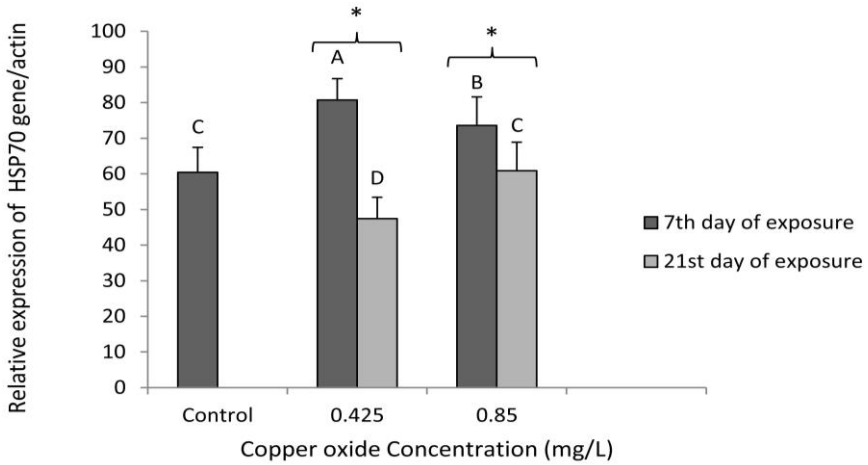

Figure 5. HSP70 gene expression in the liver of Caspian kutum exposed to different concentrations of Cu2O nanoparticle. The letters above columns are showing significantly different based on one-way ANOVA and the symbols, *, are showing a significant difference between the 7th and 21st day of exposure in each concentration based on independent T-test ($p<0.05$).

et al., 2013). Silver nanoparticles in the aqueous medium are capable of inducing apoptosis and ROS that probably caused the increased gene expression of HSP70 in the Caspian kutum's liver, observed in this study.

In the case of copper (I) oxide, the results obtained showed that changes in HSP70 gene expression generally exhibited a significant increase on the 7th day, which subsequently decreased on the 21st day compared to the control group. (Jiang et al., 2016) observed a similar decrease in gene expression in the common carp under simultaneous exposure to cadmium and copper metals, compared to the control. Moreover, the authors observed a significant increase in gene expression at high doses of copper and cadmium after 96 h. (Safari et al., 2014) reported a considerable decrease at different concentrations of endosulfan toxin on the 7th day of exposure and found a significant increase in HSP70 gene expression in *Acipenser persicus*'s liver on the 14th day of treatment. It was evidenced that high levels of copper can cause severe changes in the internal protein system. The process can take place directly by denaturing the proteins or indirectly by producing elevated levels of ROS (Kiaune and Singhasemanon, 2011). The increase of HSP70 expression in the liver may be due to its chaperone function, which plays a vital role in the cellular response process, and it is critical for protecting cells from copper-induced toxicity (Feng et al., 2003). Therefore, it seems probable that the increase in HSP70 levels in the present study may be due to a requirement for the re-folding of denatured proteins and restoring homeostasis in the Caspian kutum. The observed higher expression levels of the HSP70 may also be a secondary after-effect of shock caused by nanoparticles, as heat shock proteins display an evolutionarily-determined high sensitivity to occurring stresses.

A relatively higher increase in liver HSP70 gene expression in all treatments was observed on the 7th day of exposure compared to the 21st day treatment, which was different from the results of time-dependent changes in HSP70 in Iranian sturgeon fry affected by cadmium chloride (Safari et al., 2014). The toxicity of copper nanoparticles inhibits ATP bypass pumps and enzymes, and chronic exposure can affect ATP-dependent functions in HSP70 (Kiaune and Singhasemanon, 2011). Therefore, the accumulation of the nanoparticles may be responsible for a lowering expression level of HSP70 during prolonged exposure on the 21st day compared to the 7th day of treatment. It also seems probable that the lower expression on the 21st day of the experiment in the Caspian kutum fry may be a result of acclimatization to stress induced by the nanoparticles.

In the present study, a significant increase in GST was observed in different silver nanocolloid treatments compared to the control. (Lee et al., 2012) found no significant change in GST activity at low concentrations of silver nanoparticles coupled with citrate after 48 hours of exposure in *Cyprinus carpio*. Moreover, a considerable decrease in GST activity at high concentrations of nanoparticles after 96 hours of exposure was also recorded. (Srikanth et al., 2014) reported a significant time-dependent increase in GST up to 72 hours of exposure to the oxide nanoparticles (IO-NP). Silver nanoparticles can enter the cell during division or endocytosis and as a result, cause disturbances in mitochondrial functionality, leading to oxidative stress through elevated levels of ROS (Afifi et al., 2016). Increased levels of GST activity in gill may be linked with detoxification and cell defense activity, due to its antioxidant effect against oxidative stress caused by metal nanoparticles (Lee et al., 2012). Differences in the activity of antioxidant enzymes are dependent on the species, tissue type and composition of the stressor, its dosage and, exposure time. It is probable that exposure to oxidative conditions due to the presence of silver nanocolloid, increases the GST activity in the gill.

In the present study, a significant increase in GST activity levels was observed in the serum at both the 7th and 21st day of exposure to silver nanocolloid, compared to the control. Aliko et al. (2018) observed a significant

increase in the GST activity of goldfish's (*Carassius auratus*) plasma after 96 hours of exposure to manganese compounds (Aliko et al., 2018). GST is found in erythrocytes and plays a role in cell defense against stressful conditions. Increased serum GST in the Caspian kutum fry could directly affect metabolic disorders and erythrocyte injury.

In the present study, a significantly higher increase in GST activity was observed both in gill and serum on the 7th day of exposure, compared to the 21st day. The level of induced GST was differential and mainly depended on tissue type and the nature of the inducer. An increase in GST activity on the 7th day of treatment evidences the role of GST in the antioxidant system and defense against free radicals, most probably taking part in the adaptation process of fish to stressful conditions (Oliveira et al., 2009). It seems that increases in gill and serum GST activity on the 7th day exposure (acute toxicity) in Caspian kutum fry are associated with its antioxidant function, playing a significant role in defense mechanisms against free radicals caused by exposure to silver nanocolloids.

It is probable that the decrease in GST activity in Caspian kutum fry on the 21st day is due to prolonged exposure to silver nanocolloids, which may indicate adaptation to stressful conditions in semi-chronic toxicity.

Conclusion

In conclusion, it seems that the presence of metal nanoparticles such as silver and copper (I) oxide adversely affects the health of aquatic organisms. Therefore, further studies are needed on the effects of nanoparticles on the expression of health-promoting genes and antioxidant enzymes, which maintain the homeostasis and survival of organisms.

Ethical Statement

Animal handling and tissue sampling procedures were carried out under the standard principles of laboratory animal care to reduce animal suffering; and the study was approved by the local ethics committee of the Faculty of Science, University of Guilan (reference number 2949436). For euthanasia, the fish were first anesthetized with benzocaine and then frozen (Underwood and Anthony, 2013).

Funding Information

Not applicable.

Author Contribution

Razieh Amani prepared equipment and performed the experiments. Behrooz Heidari was the corresponding author, supervisor and designed the experiment and he was in charge of all stages of the project. Hosein Ghafoori was the advisor. AbdolMajid Valipour wrote the manuscript, prepared figures, and tables.

Conflict of Interest

Ther is no conflict of interest.

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