# The subacute toxicity of silver nanoparticles on some immunology and physiology responses in Shabut (*Tor grypus*)

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**ABSTRACT**

*The present study aimed to determine the acute and subacute toxicity and evaluate the effects of subacute concentrations (10%, 20%, 40%, and 80% of 96-h lethal concentration 50 of nanoparticles silver; 0.0 as control, 0.012, 0.025, 0.05, and 0.101 mg/L) of silver nanoparticles on some immunology and physiology responses of Shabut (Tor grypus) after 0, 7, 14, 21, and 28 days of exposure. The results showed that the total white blood cell counts and lysozyme activity were significantly higher in silver nanoparticle-exposed groups (P<0.05). The hemoglobin values in 10% and serum bactericidal in 20% and 40% of 96-h lethal concentration 50 of silver nanoparticles exposed groups were significantly higher compared to the control group (P<0.05). A significant increase was observed in the serum levels of alanine aminotransferase in the 40% and 80% silver nanoparticles exposed groups (P<0.05). Aspartate aminotransferase levels in the 10% silver nanoparticles exposed group were significantly higher compared to the control group (P<0.05). Results suggest that silver nanoparticles induce alterations in the serum biochemical and immunology parameters and stress responses in Shabut (Tor grypus).*

***Keywords: immunology, physiology responses, silver nanoparticles, Tor grypus, toxicity***

## 1. *INTRODUCTION*

*Silver nanoparticles (as a part of the commercial revolution due to their antimicrobial properties) are increasingly used in various products [1] to [4]. Incorrect administration of antibiotics against controlling disease outbreaks could result in the development of resistant bacteria strains. Therefore, there is much interest in finding a new way to use types of safe and cost-effective biocidal material [2].*

*In recent years, silver nanoparticles (AgNPs) have been increasingly used in medical applications [5]. Silver nanoparticles are widely used worldwide in many fields. Their dimensions are 100 nm or less in size [6]. They have some properties that cause them to make potential value in inks, microelectronics, and medical imaging [6] and [7]. However, because of the bactericidal activity of silver [2] and the relatively low cost of manufacturing of AgNPs [8] to [13], they have made extremely popular in a diverse range of field. Commercialization of nanoparticles is developing at a much faster rate than the understanding of the harmful impact on the environment [7]. Presently, there are over 600 commercialized products on the market from nanomaterial, and AgNPs are included in one-quarter of these products [14] (Laban et al. 2010). Data indicate that manufactured nanomaterials used in consumer goods [1] and aquaculture [15] will accumulate in waters [16].*

*However, there are limited data regarding their potentially harmful effects on aquatic organisms. There are several works of literature regarding the toxicity of nanoparticles. Nevertheless,*

*there is an emerging gap in the physiological and immunological effect of nanoparticles on aquatic organisms and the ecotoxicology community is only at the beginning of understanding the potential risks to wildlife associated with NPs Ag [17] and [4]. Several strategies have been used to determine the effect of undesirable materials on aquatic organisms [18].*

*Shabut, Tor grypus (Cyprinidae), is a commercial fish found in rivers and estuaries. According to the FAO (Food and Agriculture Organization), it is known as one of the most significant fish species in freshwaters [19]. The present study aims to evaluate some immunology and physiology responses in Shabut (Tor grypus) exposed to silver nanoparticles.*

## 2. MATERIAL AND METHODS

### 2.1 Experimental animal and condition

*This study was conducted at the Department of Aquatic Animal Health of Chamran University with Shabut (Tor grypus) purchased from the bony fish propagation and rearing center of Azadegan (Ahvaz, Iran). Fish (n=240; total weight of 50±6.32 g; total length of 25±2.7 cm) were transferred to the aquatic animal laboratory of veterinary medicine at Chamran University. They were adapted for two weeks in glass aquaria filled with chlorine-free water. Electronic air-pumping compressors were used for supplying continuous aeration. Fish fed with commercial pelleted diet twice a day. The water quality parameters (pH: 8.7±0.50, dissolved oxygen: 8±0.25 mg/L, temperature: 27±1ºC, total hardness: 870±2.50 mg/L as CaCo3, NO2<0.01, and NO3<0.1 mg/L) were measured twice a week during the study period by portable water quality monitoring (YSI Incorporated, Ohio, USA).*

### 2.2 Preparation and characterization of AgNPs

*In this study, the colloidal silver nanoparticles (AgNPs) type L2000 with the commercial name Nanocid® were purchased from NanoNasb Pars Co., Ltd (Tehran, Iran). It was concentrated at 4000 mg/L spherical silver nanoparticles with water-based nanoparticles. Also, transmission electron microscopy (TEM) results of silver nanoparticles from NanoNasb Pars Co. Ltd. revealed that the size was about 7.1±1.63 nm (Figure 1). The product was synthesized by a United States Patent Application (No: 20090013825). For this purpose, 4.5 g of Linear Alkyl Benzene Sulfonate (LABS) was dissolved in 95 ml of distilled water. Thereafter, 0.32 g of silver nitrate and 0.2 g of hydrazine (0.03 M) were added to the solution. Eventually, the yellowish silver colloidal solution was formed. Physicochemical properties of this colloidal product are: average zeta potential of 53.33±7.86 mV, pH of 2.4, and geometric average diameter of 12.65±1.46 nm and according to inductively coupled plasma atomic emission spectrometry (ICP-AES) results, the actual concentration of Silver in the colloid of silver nanoparticles has been measured as 3988 mg/L [20].*

### 2.3 Acute toxicity assay

*During the two-week acclimation period, the healthy condition of the fish was controlled by their activity and external appearance. The acute toxicity test was conducted in Shabut (Tor grypus) for the first time (50g and 25cm) following the Organization for Economic Cooperation and Development Guideline (OECD) under static renewal test conditions [21] and [22]. Groups of 30 fish (10 fish in three replicates) were exposed for 96h in each prepared concentration (0, 0.15, 0.3, 0.6, 1.25, 2.5, and 5 mg/L) in triplicate control groups. During the study, the water of the aquaria was renewed after 24h and followed by re-dosing. The LC10, LC50, and LC90 values (with 95% confidence limits) were calculated using EPA Probit Analysis by Version 1.5 and the data obtained in acute toxicity bioassay by Finny's method of Probit analysis [23]. The mortality rates were recorded at 24, 48, 72, and 96h after exposure to calculate the 96h-LC50 for silver nanoparticles with 95% confidence limits [24]. During exposure, dead fish were immediately removed. Four concentrations (10%, 20%, 40%, and 80% of 96-h LC50) were selected to estimate the hematological and biochemical effects of AgNPs on Shabut (Tor grypus) in four groups.*

### 2.4 Subacute toxicity assay

*For the subacute toxicity assay, 150 Shabut (body weight: 250±5.43 g) were randomly disturbed in 15 glasses aquariums (five treatment groups in three replicates). For the control group, 10 fish were randomly selected after acclimation. Every tank containing 10 fish was exposed to the test solution with concentrations of Nanocid 0.0 as control, 0.012, 0.025, 0.05, and 0.101 mg/L. The test water in this study was renewed daily. The concentration of AgNPs was kept constant by re-dosing. After three days of exposure to a subacute dose*

*of AgNPs, five fish were taken from the control and treatment groups for hematological and biochemical study. Fish were anesthetized with 2-phenoxyethanol at 0.5 ml/L. Then, blood samples were taken from the caudal vein using nonheparinized syringes. The same protocol was applied on days 7, 14, 21, and 28.*

**2.5 Hematological and immunological analysis**

*For hematology tests, the blood samples were placed into heparinized tubes as anticoagulant material. The leukocyte counts were determined by a Neubaeurhemocytometer with Natt-Herring fluid and hemoglobin (Hb) assayed by cianometahemoglobin method with Drobkin solution [25]. Serum bactericidal assay was done according to Misra et al. [26]. Briefly, sera samples were diluted three times with 0.1% gelatin-veronal buffer (GVB+2) that consisted of pH 7.5, 0.5 mM/ml mg2+, and 0.15 mM/ml Ca2+. The bacteria A. hydrophila were suspended in the GVB2+ to make a concentration of 1×105 CFU/ml. The bacteria and diluted sera were mixed at 1:1 and incubated for 90 min at 25ºC. The viable bacterial counts were calculated by counting the colonies from the resultant incubated mixture on TSA plates after 24h incubation. The bactericidal activity of test serum was expressed as the percentage of colony-forming units in the test groups to that in the control group. The lysozyme activity of plasma was evaluated spectrophotometrically according to the method of Ellis [27].*

**2.6 Biochemical analysis**

*For biochemical analysis, the blood was immediately placed in non-heparinized tubes and left to clot at 4ºC for 15 min. Afterward, tubes were centrifuged at 3000 rpm for 10 min to obtain serum. Lactate dehydrogenase (LDH), Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were measured by kinetic enzyme assays with the standard kit (ZiestChemie, Iran).*

**2.7 Statistical analysis**

*The statistical analysis was carried out using the software SPSS version 17.0. Normality was assessed via the Shapiro-Wilk test, and homoscedasticity was evaluated using Levene's test. One-way analysis variance (ANOVA) was used to compare different AgNP concentrations in various groups. Duncan's test was subsequently employed to determine which group differed significantly.*

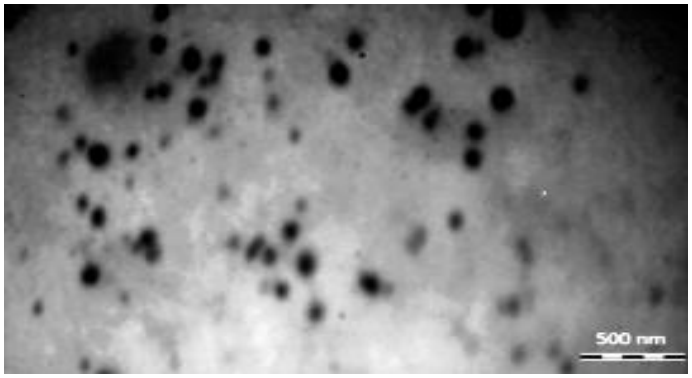


**Fig. 1.** *TEM micrographs of silver nanoparticles from NanoNasb Pars Co. Ltd.*

### *3. RESUTS AND DISCUSSION*

*Acute toxicity test results for the Nanocid on Tor grypus are presented in Tables 1 and 2. Fish mortality increased significantly when exposure concentration and time were increased. Median lethal concentration tests of 10%, 50%, 90%, and 99% are shown in Table 2. The 96-h LC50 value obtained for Tor grypus was 0.127 mg/L (confidence interval 0.45–0.26 mg/L).*

**Table 1.** Time courses of lethal concentrations (LC10-LC99) of AgNPs (mg/L) in Tor grypus.

| Point | 24 h | 48 h | 72 h | 96 h |
|---|---|---|---|---|
| $LC_{10}$ | 0/129 | 0/058 | 0/039 | 0/034 |
| $LC_{50}$ | 0/188 | 0/131 | 0/205 | 0/127 |
| $LC_{90}$ | 2/73 | 2/319 | 2/294 | 1/258 |
| $LC_{99}$ | 2/99 | 2/471 | 2/473 | 1/458 |

**Table 2.** Median lethal concentration (LC50) of AgNPs (mg/L) at 96 h in Tor grypus.

| Time of exposure (h) | $LC_{50}$ (mg/L) | Lower limit | Upper limit |
|---|---|---|---|
| 96 | 0.127 | 0.45 | 0.26 |

*During 28 days of the study, no mortality was recorded during subacute exposure for all groups. Results of hematological values and immunological activities analysis of the controls and experimental fish under subacute exposure are presented in Table 3. At the end of the experiment, WBC values were significantly higher in 10%, 20%, 40%, and 80% concentrations compared to the control group (P<0.05). The results of this study are consistent with the findings of Shaluei et al. [28] and Mekkawy et al. [29]. The white blood cells of fish respond to various stressful factors including chemical stimuli and substances that alter their normal physiological processes [30]. Therefore, a normal response to toxicant exposure is increasing or decreasing numbers of white blood cells [31]. In the present study, the increase in WBC may have resulted from the stimulation of the defense mechanism of the fish to eliminate the effect of the toxicant [32].*

*The content of hemoglobin (Hb) showed significantly higher values at concentrations of nanocid 10 in comparison to the other groups (P<0.05). Shaluei et al. [28] studied the subacute exposure to AgNPs in silver carp (Hypophthalmichthys molitrix) and reported hemoglobin (Hb) count were significantly reduced at both concentrations tested (0.04 and 0.02 mg/L). Also, Mekkawy et al. [29] and Imani et al. [32] found a significant increase in Hb in Clarias gariepinus and Oncorhynchus mykiss after exposure to AgNPs. Compensation for decreased oxygen-carrying capacity of Hb and dilution of blood due to damages of gill epithelium may be responsible for increasing Hb content [33].*

*At the end of the experiment, there was a significant increase in serum bactericidal between 20 and 40 mg/L AgNPs exposed groups compared to the control group (P<0.05). These results are consistent with the findings of other researchers that demonstrate the antibacterial properties of silver nanoparticles [34] and [35]. Kumar et al. [36] also reported that silver nanoparticles synthesized from Sargassum tenerrimum seaweed have antibacterial activity against gram-negative and positive bacteria. Silver nanoparticles have better bactericidal activity against a variety of gram-negative and gram-positive bacteria due to their unique biological properties, shape, size, and contact surfaces [37] and [38]. Silver nanoparticles are likely to cause disorder in the function of the bacteria (thereby causing death) by binding to the surface of the cell membrane or penetrating the cell and binding to the DNA.*

*Lysozyme, a vital bacteriostatic protein, is found in fish skin mucus, blood, gill, digestive tract, and eggs. This enzyme has a key role in the safety of fish which is one of the most important factors in the innate immune system [39]. Therefore, lysozyme activity is an indicator of fish health. At the end of the exposure experiments, a significant increase was found in lysozyme activity in the groups exposed to 10, 20, and 40 AgNP concentrations compared to the control group (P<0.05). Otherwise, the elevated serum lysozyme activity in the present study could be due to nanosilver cytotoxicity. Lysozyme's activity was elevated in Takifugu fasciatus exposed to copper nanoparticles for 30 days [40].*

**Table 3.** Hematological and immunological values in five groups of Tor grypus after exposed to AgNPs for 28 days experimental period.

| Parameters | | 0 Days | 7 Days | 14 Days | 21 Days | 28 Days |
|---|---|---|---|---|---|---|
| **WBC ($10^3$cells/mL)** | | | | | | |
| | **Control** | 13.95±2.35 $^{b}$ | 14.00±1.00 $^{b}$ | 7.35±0.05$^{a}$ | 7.05±0.15$^{a}$ | 6.85±0.05$^{a}$ |
| | **Nanocid 10** | 6.70±0.50 $^{a}$ | 9.05±0.15 $^{b}$ | 7.30±0.20$^{a}$ | 8.05±0.85$^{ab}$ | 9.95±0.35$^{b}$ |
| | **Nanocid 20** | 8.70±0.80 $^{b}$ | 7.15±0.05 $^{a}$ | 8.00±0.20$^{a}$ | 8.95±0.65$^{ab}$ | 8.93±0.04$^{b}$ |
| | **Nanocid 40** | 8.20±0.10 $^{b}$ | 13.00±0.50 $^{b}$ | 7.20±0.10$^{a}$ | 7.20±0.10$^{a}$ | 8.25±0.05$^{b}$ |
| | **Nanocid 80** | 9.25±0.35 $^{b}$ | 11.75±0.75 $^{b}$ | 8.10±0.20$^{a}$ | 9.25±0.65$^{b}$ | 8.10±0.50$^{b}$ |
| **Hb (g/dL)** | | | | | | |
| | **Control** | 0.15±0.01 $^{a}$ | 0.14±0.00 $^{a}$ | 0.17±0.01 $^{a}$ | 0.22±0.01 $^{a}$ | 0.23±0.00$^{a}$ |
| | **Nanocid 10** | 0.16±0.03 $^{a}$ | 0.18±0.01 $^{a}$ | 0.21±0.01 $^{a}$ | 0.38±0.11 $^{b}$ | 0.37±0.01$^{b}$ |
| | **Nanocid 20** | 0.21±0.03 $^{a}$ | 0.18±0.06 $^{a}$ | 0.18±0.02 $^{a}$ | 0.21±0.01 $^{a}$ | 0.27±0.08$^{a}$ |
| | **Nanocid 40** | 0.23±0.08 $^{a}$ | 0.16±0.01 $^{a}$ | 0.20±0.00 $^{a}$ | 0.15±0.01 $^{a}$ | 0.19±0.01$^{a}$ |
| | **Nanocid 80** | 0.19±0.07 $^{a}$ | 0.24±0.03 $^{a}$ | 0.23±0.02 $^{a}$ | 0.22±0.02 $^{a}$ | 0.21±0.01$^{a}$ |
| **Serum bactericidal activity (%cfu/control)** | | | | | | |
| | **Control** | 66.66±4.58$^{a}$ | 38.66±9.81$^{a}$ | 69.33±5.16$^{c}$ | 48.33±9.39$^{a}$ | 44.66±4.03$^{a}$ |
| | **Nanocid 10** | 33.66±2.57$^{a}$ | 25.00±4.47$^{a}$ | 29.33±6.71$^{a}$ | 49.00±9.4$^{a}$ | 52.66±4.58$^{a}$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| | **Nanocid 20** | 36.33±4.58$^{a}$ | 80.33±4.03$^{b}$ | 66.66±6.34$^{bc}$ | 76.33±8.59$^{b}$ | 83.33±2.58$^{b}$ |
| | **Nanocid 40** | 36.33±8.31$^{a}$ | 83.33±2.58$^{b}$ | 50.66±1.50$^{b}$ | 43.00±6.44$^{a}$ | 88.33±2.58$^{b}$ |
| | **Nanocid 80** | 55.00±7.74$^{a}$ | 42.00±8.53$^{a}$ | 70.33±6.94$^{c}$ | 48.66±2.87$^{a}$ | 55.33±3.61$^{a}$ |
| **Lysozyme activity (U/mg protein)** | | | | | | |
| | **Control** | 90±4.47$^{a}$ | 93.33±2.58$^{a}$ | 120±10.49$^{a}$ | 88.33±2.5$^{a}$ | 78.33±5.16$^{a}$ |
| | **Nanocid 10** | 98.33±11.25$^{ab}$ | 119.33±5.24$^{a}$ | 83.33±2.58$^{a}$ | 135±7.92$^{a}$ | 128.33±9.3$^{d}$ |
| | **Nanocid 20** | 110±4.47$^{ab}$ | 101.66±6.83$^{a}$ | 85±4.47$^{a}$ | 96.66±11.25$^{a}$ | 96.66±2.58$^{bc}$ |
| | **Nanocid 40** | 110±7.74$^{ab}$ | 94±7.95$^{a}$ | 110±7.92$^{a}$ | 128.33±6.95$^{a}$ | 111.66±5.16$^{c}$ |
| | **Nanocid 80** | 119.33±5.24$^{b}$ | 135±7.92$^{a}$ | 96.66±4.37$^{a}$ | 98.33±2.58$^{a}$ | 86.66±2.58$^{ab}$ |

Different letters in the same columns show significant differences among groups ($P$<0.05).

*The effects of subacute Nanocid exposure on biochemical values between five groups of fish at 28 days of study are presented in Table 4. Biochemical results of the present experiments showed a significant increase in the serum levels of ALT in the 40 and 80 mg/L AgNPs exposed fish and AST in the 10 mg/L AgNPs exposed fish (P<0.05). This result is in accordance with the finding of Monfared et al. [41] in common carp.*

*AST and ALT are plasma non-functional enzymes that generally remain in many organs including the liver. They are also considered an important indicator in assessing liver and kidney and organ dysfunction or tissue injury [42]. Studies show that free radicals caused by nanoparticles can attack liver cells and can cause the release of enzymes stored in the serum. These results suggest that ALT and AST levels are applicable indicators for organ damage detection caused by exposure to AgNPs.*

*LDH is an enzyme found in almost all body tissues, such as the heart, kidneys, liver, skeletal muscle, brain, erythrocyte, and gills. LDH measurement is used to detect tissue disorders and as an aid in the diagnosis of tissue damage [43]. In our study, LDH was observed in the group exposed to 40 AgNP concentration at the end of experiments decreased (P<0.05). The reduction in LDH activity may be due to the higher glycolysis rate under AgNP stress. AgNPs may inhibit the fish's aerobic and anaerobic metabolism, thus reducing LDH activity. However, Changes in LDH activity are as a good diagnostic tool in toxicology studies of fish [44] (Min and Kang 2008).*

**Table 4.** AST, ALT, ALP, and LDH (IU/L) enzymes changes in five groups of Tor grypus after exposed to AgNPs for 28 days experimental period.

| **Parameters** | | **0 Days** | **7 Days** | **14 Days** | **21 Days** | **28 Days** |
|---|---|---|---|---|---|---|
| **AST (IU/L)** | | | | | | |
| | **Control** | 106.33±12.08 $^{a}$ | 91.91±21.6 $^{ab}$ | 63±41$^{a}$ | 39.5±13.5$^{a}$ | 31.5±8.5$^{a}$ |
| | **Nanocid 10** | 158.5±41.5 $^{a}$ | 119.5±15.5 $^{ab}$ | 68±10$^{a}$ | 86±3$^{a}$ | 121.5±5.5$^{b}$ |
| | **Nanocid 20** | 131.5±12.5 $^{a}$ | 139 ±44 $^{b}$ | 212.5 ±31.5$^{b}$ | 83±5.5$^{a}$ | 88±33$^{ab}$ |
| | **Nanocid 40** | 164.5±26.5 $^{a}$ | 60.00±20 $^{a}$ | 158 ±14$^{b}$ | 157.5±0.10$^{b}$ | 89.5±23.5$^{ab}$ |
| | **Nanocid 80** | 167 ±44 $^{a}$ | 76.5±12.5 $^{ab}$ | 78±12.5$^{a}$ | 60±0.65$^{a}$ | 76.5±20.50$^{ab}$ |
| **ALT (IU/L)** | | | | | | |
| | **Control** | 6.33±1.52 $^{a}$ | 5.49±0.73 $^{a}$ | 5.5±0.5 $^{ab}$ | 6±1.73 $^{a}$ | 4.5±1$^{a}$ |
| | **Nanocid 10** | 5±2 $^{a}$ | 7±1 $^{a}$ | 3.5±0.5 $^{a}$ | 7.5±0.5 $^{a}$ | 4.5±2.5$^{a}$ |
| | **Nanocid 20** | 7±2 $^{a}$ | 7.5±3.5 $^{a}$ | 8.5±2.5 $^{ab}$ | 5.5±0.5 $^{a}$ | 9±3$^{ab}$ |
| | **Nanocid 40** | 10±3 $^{a}$ | 4.5±0.05 $^{a}$ | 10.5±1.5 $^{b}$ | 8.5±1.5 $^{a}$ | 15±2.5$^{b}$ |
| | **Nanocid 80** | 9±3 $^{a}$ | 3.5±1.5 $^{a}$ | 8.5±1.5 $^{ab}$ | 16.5±1.5 $^{b}$ | 12±0.5$^{b}$ |
| **ALP (IU/L)** | | | | | | |
| | **Control** | 129.33±29$^{ab}$ | 140.89±10.15$^{a}$ | 121.5±15.5$^{c}$ | 149±13$^{a}$ | 133.5±15$^{a}$ |
| | **Nanocid 10** | 100±20$^{a}$ | 102±17$^{a}$ | 149.5±20.5$^{a}$ | 104±21$^{a}$ | 153±9.5$^{a}$ |
| | **Nanocid 20** | 234.5±28.5$^{a}$ | 118.5±5$^{b}$ | 132.5±20.5$^{bc}$ | 84±19$^{b}$ | 129.5±20$^{b}$ |
| | **Nanocid 40** | 195±20$^{ab}$ | 132±7$^{b}$ | 112±25$^{b}$ | 120±22$^{a}$ | 123.5±5$^{b}$ |
| | **Nanocid 80** | 157.5±8.5$^{ab}$ | 93.5±28.5$^{a}$ | 152±18$^{c}$ | 119.5±23.5$^{a}$ | 159±9.5$^{a}$ |
| **LDH (IU/L)** | | | | | | |
| | **Control** | 1296.66±160.3$^{a}$ | 1173.43±109.96$^{a}$ | 685±105$^{a}$ | 1420±123$^{a}$ | 1289.5±17.5$^{b}$ |
| | **Nanocid 10** | 885±150$^{a}$ | 1170±30$^{a}$ | 1261.5±174.5$^{ab}$ | 1191.66±165.05$^{a}$ | 1421.5±174.5$^{b}$ |
| | **Nanocid 20** | 1028.5±77.5$^{a}$ | 1464±176$^{a}$ | 1628.5±107.5$^{b}$ | 1479±89$^{a}$ | 955.6±117.5$^{ab}$ |
| | **Nanocid 40** | 1175±155$^{a}$ | 1385.5±114. 5$^{a}$ | 1122.5±167.5$^{ab}$ | 1827.5±62. 5$^{a}$ | 550±38$^{a}$ |
| | **Nanocid 80** | 1350±110$^{a}$ | 1245±45.9$^{a}$ | 1284.5±65$^{ab}$ | 1394±120$^{a}$ | 1360.5±40.5$^{b}$ |

Different letters in the same columns show significant differences among groups ($P$<0.05).

*In conclusion, this study showed that AgNPs induce alterations in the serum biochemical parameters of Shabut in different concentrations. The data obtained here indicate that the presence of AgNPs in aquatic environments may be harmful to the health of Shabut (Tor grypus) and probably also to other aquatic organisms. Further stress-induced genetic studies are required to understand better AgNPs toxic effects parallel to such immunological and physiological studies.*

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**Conflict of interest**

The authors declare that they have no competing interests.

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