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Original Article

Hemato-immunological, serum metabolite and enzymatic stress response alterations in exposed rainbow trout (*Oncorhynchus mykiss*) to nanosilver

Ali Taheri Mirghaed*[1], Peyman Yarahmadi[1], Mitra Shabrang harehdasht[2], Paul M. Craig[3], Hamed Ghafari Farsani[4], Nahid Ghysvandi[5], Soheil Eagdari[2]

[1]Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
[2]Department of Fisheries, Faculty of Natural Resources, University of Tehran, Karaj, Iran.
[3]Department of Biology, Faculty of Science, University of Waterloo, Waterloo, Ontario, Canada.
[4]Young Reasearchers and Elite Club, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.
[5]Department of Fisheries, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

Abstract: The aim of present study was to investigate the effects of sub-lethal concentrations of silver nanoparticles (AgNP) on hematological parameters, differential tests of white blood cells, serum metabolite parameters, serum enzymes activity and serum ions in rainbow trout, *Oncorhynchus mykiss*. Healthy rainbow trout, were exposed to sub-lethal concentrations (0, 1.5 and 2.5 ppm) of nanosilver for 14 days. RBC, WBC and Hct levels were significantly (P<0.05) increased in exposed groups. Within the white blood cells, only neutrophils showed a significant increase at 7 and 14 days post exposure (P<0.05). Serum triglyceride, total serum protein, albumin and globulin levels were decreased (P<0.05) in exposed fish, however, cholesterol levels increased in the 2.5 ppm group at 7 days after exposure (P<0.05). Cortisol and glucose increased significantly at 7 and 14 days of exposure in both concentrations of AgNPs (P<0.05). Decreases in serum ions level were observed, although reduction in chloride ions occurred earlier and more severe than other measured parameters (P<0.05). Elevation in serum ALP, LDH, ALT and AST enzymes were observed during the experiment (P<0.05), although SOD and CAT activity were significantly decreased in exposed groups (P<0.05). The results revealed that AgNP can affect the hematological, serum metabolite and enzymatic parameters of *O. mykiss*, as well as AgNP exposure induce a general oxidative stress response in *O. mykiss*.

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Introduction

Nanotechnology is rapidly expanded in applications ranging from electronics to biotechnology and involves materials and process that have at least one dimension in the range of 1-100 nanometers (Moore, 2006). Although the applications of nanotechnology is broad, there is increasing concern about nanomaterial impacts on the health of the environment, especially in aquatic ecosystems that act as a sink for many pollutants, including engineering nanomaterials (ENMs) (Scown et al., 2010a). Case studies examining the toxicology effects of ENMs, have shown that the interactions between nanomaterial and all levels of biological organization, from cells to ecosystems are remarkably complex (Aschberger et al., 2011). Therefore, with worldwide increases in ENM application, it is essential to investigate their impact on humans, animal health and the environment.

Nanotechnology has a broad potential to revolutionize all scientific fields (Hood, 2004). Among all of ENMs, silver nanoparticles (AgNPs) were acclaimed for their antibacterial properties (Aschberger et al., 2011). It is well-documented that AgNPs are toxic to a variety of model and non-model aquatic organisms (Asghari et al., 2012; Asharani et al., 2008; Behra et al., 2013; Farkas et al., 2011; Griffitt et al., 2008; Lapresta-Fernández et al., 2012). The toxic effects of nanosilver has been assessed and determined in different aquatic organisms with acute

*Corresponding author: Ali Taheri Mirghaed
E-mail address: mirghaed@ut.ac.ir
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and chronic toxicity mechanisms of these compounds. These studies reported that silver nanoparticles result in decreased hatching rate, increased mortality, and hatching delay in zebrafish (*Danio rerio*) embryos and larvae (Massarsky et al., 2013; Yeo and Kang, 2008). Furthermore, it has been demonstrated in zebrafish exposed to AgNPs that there is abnormal body axes, slow blood flow, pericardial edema and cardiac arrhythmia, and increased apoptosis in the gills of juveniles and adults (Asharani et al., 2008; Griffitt et al., 2009). Furthermore, exposure of rainbow trout (*Oncorhynchus mykiss*) to different sizes of AgNPs (10 nm, 35 nm and 600-1600 nm) induced several detoxifying and reactive oxygen scavenging transcripts, such as cyp1a2, cyp3a45, hsp70a, gpx, and g6pd (Scown et al., 2010b), changes in plasma biochemical parameters (Johari et al., 2013) and resulted in oxidative stress and cellular metabolic activity in primary gill cells of rainbow trout (Farkas et al., 2011).

Although numerous studies have demonstrated the toxic effects of AgNPs, there is still a lack of understanding the impact AgNPs on aquatic ecosystems due to the difficulty in accurately quantifying AgNPs within a natural setting (Malina et al., 2010). Data, such as hematological, serum electrolytes, as well as serum enzymatic parameters are needed to assess the environmental risk posed by silver and the other nanoparticles. Hence, the main objective of this study was to determine the effects of nanosilver exposure over time on serum metabolites, including triglyceride (TG), cholesterol (Cho), total protein (TP), albumin (Albu), globulin (Glo), glucose (Glu) and cortisol, and serum ions (Ca^{++}, K^{+}, Na^{+} and Cl^{-}), serum enzymes activity (Alkaline phosphates (ALP), lactate dehydrogenize (LDH), Alnine aminitransferase (ALT), Aspartat (AST), SOD (Superoxide dismutase), and CAT (Catalase), and assess if these changes in hematogical parameters can be used as key biomarkers for AgNP exposure in rainbow trout (*Oncorhynchus mykiss*).

Materials and Methods

Characterization of silver nanoparticles: The Nanosilver colloidal product (commercial name: Nanocid®) was purchased from Nano Nasb Pars Co., (U.S Patent No. US/2009/0013825) which contained 4000 ppm nanosilver particles. Prior to use, the shape and size distribution of the AgNPs was determined using a transmission electron microscopy (TEM). Two experimental concentrations of 1.5 and 2.5 ppm were obtained from this solution.

Fish: Healthy, mixed sex rainbow trout (N=90; weight: 101.2±0.4 g; total length: 19.6±0.7 cm; mean±SD) were obtained from a local fish farm (Mahisara fish culture; Karaj, Iran). The fish were acclimatized to the laboratory conditions in 100L tanks, which were equipped with a flow-through system with dechlorinated tap water for 14 days and fed (1% of BW) commercial rainbow trout diet (Behparvar Co. Tehran, Iran) twice a day. After acclimation period, fish were distributed into nine 100L fiberglass tanks, and assigned to three doses of AgNPs; 0 mg L^{-1} (control), 1.5 ppm and 2.5 ppm, based on the 96 hr LC_{50} values obtained in the acute toxicity test for this experiment (LC_{50}=8.9 mg L^{-1}) based on Shaluei et al. (2012) and previous experiments in a number of fish species which is summarized in Table 1. Exposures were run in triplicate, at a constant water quality with a controlled photoperiod (12 hrs light; 12 hrs dark). Fish were allowed to acclimate for 48 hrs prior to the start of the exposure regime. Fish were sampled after 0, 1, 7, and 14 days exposure.

Experimental Sampling: Fish were fasted 24 hrs prior to sampling and three fish per replicate were anesthetized with clove powder at a concentration of 200 mg L^{-1}. Blood samples were collected through caudal vein puncture, and blood samples were allocated into two portions, one portion of was transferred to heparinized tubes for measurement of haematological parameters and another one to non-heparinized tubes that left to clot for 12 hrs (at 4°C), prior to centrifugation at 4ºC, 7000 rpm for 10 min. The serum was extracted from samples, placed in new tubes and stored at -80ºC until analysis.

Hematological parameters assay: Heparinized blood samples were prepared for counting the number of red

Table 1. Effective concentrations of Nanocid® obtained from toxicity experiments on fish.

Reference	Tested organism	Effect measured
Shahbazzadeh et al. (2009)	Rainbow trout (*O. mykiss*) (average weight: 1.049 *g*)	LC_{50} = 2.3 mg/l
Moaddab et al. (2011)	Osteoblast (G292) cell line	IC_{50} = 3.42 µg/ml
Shahbazzadeh et al. (2011)	Fibroblast (HF2) mesenchymal stem cells	IC_{50} = 6.68 µg/ml IC_{50} = 6.33 µg/ml
Shaluei et al. (2012)	Caspian roach (*R. caspicus*) (average weight: 3.5 *g*)	LC_{50} = 0.028 mg/l
Johari et al., 2013)	3 stages of rainbow trout (*O. mykiss*): Eleutheroembryos Larvae (average weight: 154 *mg*), Juvenile (average weight:15 *g*)	LC_{50} = 0.25 mg/l LC_{50} = 0.71 mg/l LC_{50} = 2.16 mg/l
Shaluei et al. (2013)	Silver carp (*H. molitrix*)	LC_{50} = 0.202 mg/l

blood cells (RBCs) and white blood cells (WBCs) under a light Microscope using Neubauer hemocytometer slide based on Sarder et al. (2001). The blood hematocrit (Hct) was determined by microhematocrit capillary tubes, which were centrifuged at 4ºC, 3500 g for 10 min and Hct was reported as percentage of packed cell volume (%PCV). Furthermore, to determine the white blood cells, blood was smeared on slides and air-dried after staining. White blood cells were classified under light microscope with ×1000 magnification (Härdig et al., 1988).

Serum parameters assay

Serum metabolite Parameters: Metabolite parameters, including glucose, triglycerides, cholesterol, total protein and albumin were determined with an auto analyzer Hitachi 911 with reagents provided in standard analyses kits (Pars Azmon, Tehran, Iran). The serum glucose and cholesterol concentrations were measured based on glucose oxidase and cholesterol oxidase commercial kit (Pars Azmoon). The serum total protein were measured based on commercial available procedure at 27°C and 546 nm wave length, which employed bovine serum albumin as a standard (Pars Azmoon). Serum triglycerides (TG) were determined using commercial assay based on enzymatic colorimetric method with glycerol phosphate oxidize at 546 nm and 37 °C (Pars Azmoon). The serum albumin concentrations were determined using a purchased assay (Pars Azmoon), and used acidic pH and Bromocresol Green as a reagent. The serum globulin concentration was calculated by subtracting the albumin values from the total serum protein.

Serum enzymes activity: Serum enzyme activities, including lactate dehydrogenase (LDH), alkaline phosphatase (ALP), alanine aminotransferase and aspartate aminotransferase (AST) activities were measured based on commercial kits protocol (Pars Azmoon, Tehran, Iran) and an autoanalyzer (Eppendorf, EPOS, Germany) based on Shahsavani et al. (2010). Serum CAT and SOD activity were measured based on methods previously described by Goth (1991) and Marklund and Marklund (1974), respectively.

Serum ions: For determination the serum ion concentration, commercial available reagents were used (Pars Azmon Co., Tehran, Iran). The chloride (Cl^-) were determined based on mercury thiocyanate colorimetric reaction according to available commercial kit protocol. Serum calcium (Ca^{2+}) concentration were measured by using commercial Pars Azmoon based on o-Cresolphthalein as regent. Sodium (Na^+) and potassium (K^+) were determined based on commercial Pars Azmoon kit procedure and a flame photometer (Corning 410, United Kingdom).

Statistical analysis: The results are presented as means±SD. Differences between parameters were analyzed by one-way analysis of variance and significant means were subjected to a multiple comparison test (Duncan) at the P=0.05 level.

Results

Silver nanoparticles characterization: Transmission electron microscopy images of AgNPs showed uniform and spherical structure of particles (Fig. 1A, B). The average size of particles was 23.06±1.91 nm (Fig. 1C) and based on the data, 66% of the particles

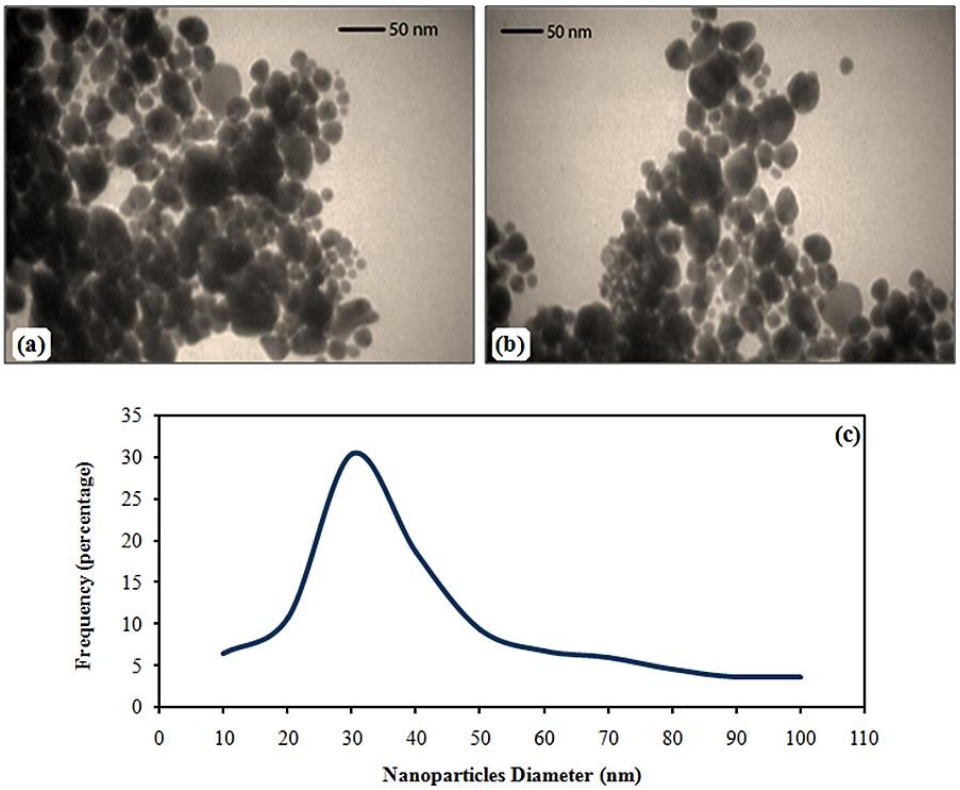

Figure 1. (A, B) Transmission Electron Microscopy images of silver nanoparticles (c) Size range of silver nanoparticles diameter.

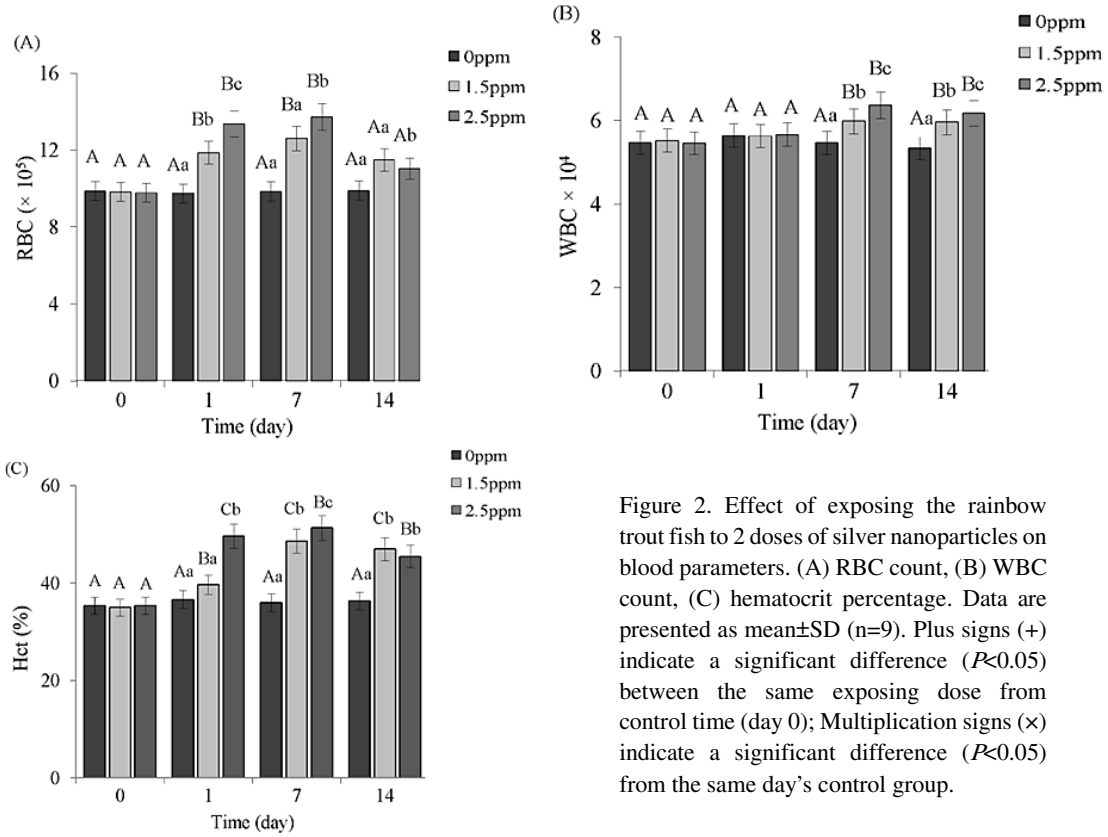

Figure 2. Effect of exposing the rainbow trout fish to 2 doses of silver nanoparticles on blood parameters. (A) RBC count, (B) WBC count, (C) hematocrit percentage. Data are presented as mean±SD (n=9). Plus signs (+) indicate a significant difference (P<0.05) between the same exposing dose from control time (day 0); Multiplication signs (×) indicate a significant difference (P<0.05) from the same day's control group.

had diameter less than 40 nm. The commercially purchased AgNPs were a water-soluble form of colloidal nanoparticles, and remained in suspension during exposure.

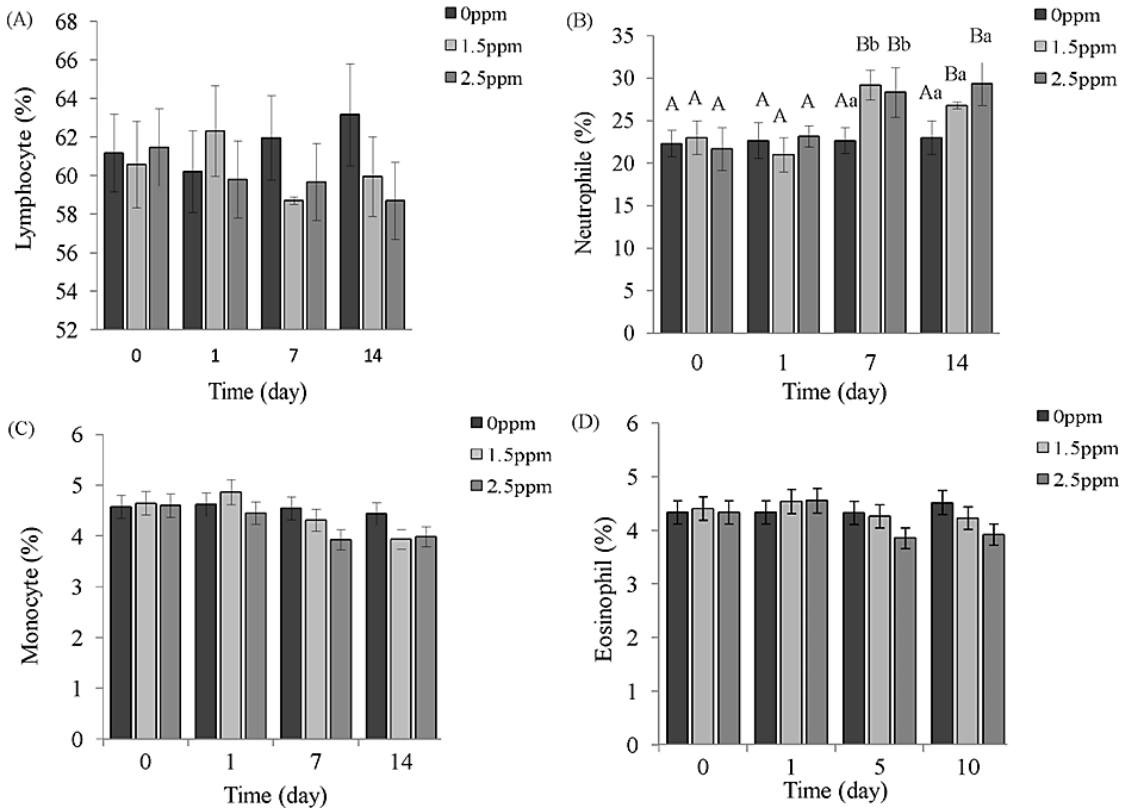

Figure 3. White blood cell differential count in rainbow trout exposed to nanosilver for 14 days. (A) Lymphocytes percentage, (B) neutrophils percentage, (C) monocytes percentage and (D) eosinophils percentage. Data are presented as mean±SD (n=9). Capital letters (A, B, C) indicate a significant difference (P<0.05) between the same exposing dose from control time (day 0); Small letters (a, b, c) indicate a significant difference (P<0.05) from the same day's control group.

Hematological parameters analysis: No mortality was observed during the sub-acute exposure for all treatments. There was a significant (P<0.05) increase in the red blood cells count in 1.5 ppm at 1, 7 and 14 days and in 2.5 ppm at 1 and 7 days exposure compared to control group (Fig. 2A). The number of white blood cells did not show changes in the first sampling time point, although in days 7 and 14 of exposure, the WBC counts were significantly increased (P<0.05; Fig. 2B). No significant changes (P>0.05) were observed in percentage of lymphocyte, monocyte and eosinophil cells (Figs. 3 B, C, D), although the number of neutrophils increased (P<0.05) at 7 and 14 days in all exposure groups.

Serum metabolite Parameters: After 1 and 7 days, triglyceride levels significantly decreased in the 2.5 ppm group. However, after 14 days of exposure, triglyceride level decreased in all doses of AgNP. Triglyceride levels in fish exposed to the 2.5 ppm dose showed a significant increase compared to previous sampling time (T_2) of this dose (Fig. 4A). Cholesterol level of blood plasma increased in the 2.5 ppm group after 7 days of exposure (Fig. 4B). A significant decrease of total protein was observed in all exposed fish during the experiment (Fig. 4C). Additionally, the plasma albumin level decreased significantly in the 2.5 ppm exposure group after 7 days and in both doses after 14 days (Fig. 4D). Furthermore, plasma glucose increased significantly in all exposure groups after 7 and 14 days (Fig. 4E).

Serum ion analysis*:* A significant decrease (P<0.05) in calcium ions level was observed solely in the high dose after 14 days of exposure (Fig. 5A). Furthermore, the amount of K^+ was significantly decreased (P<0.05) at 7 and 14 days of exposure (Fig. 5B). There were no significant changes in Na^+ concnetrations at 1 or 7 days, although after 14 days of exposure, fish in both of exposure doses were showed significant decrease (P<0.05) in Na^+ compared to control group (Fig. 5C). Moreover, AgNP exposure induced

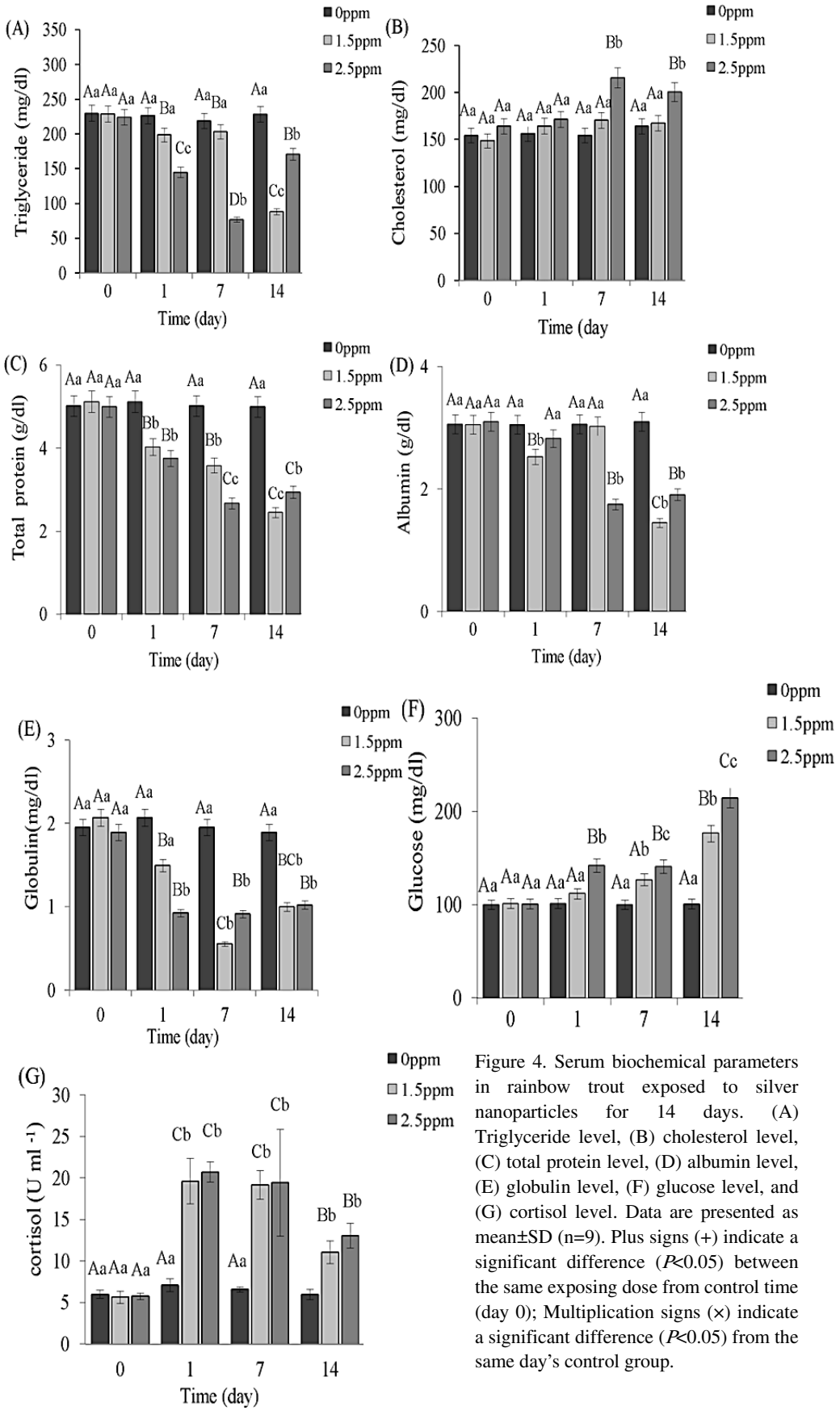

Figure 4. Serum biochemical parameters in rainbow trout exposed to silver nanoparticles for 14 days. (A) Triglyceride level, (B) cholesterol level, (C) total protein level, (D) albumin level, (E) globulin level, (F) glucose level, and (G) cortisol level. Data are presented as mean±SD (n=9). Plus signs (+) indicate a significant difference (P<0.05) between the same exposing dose from control time (day 0); Multiplication signs (×) indicate a significant difference (P<0.05) from the same day's control group.

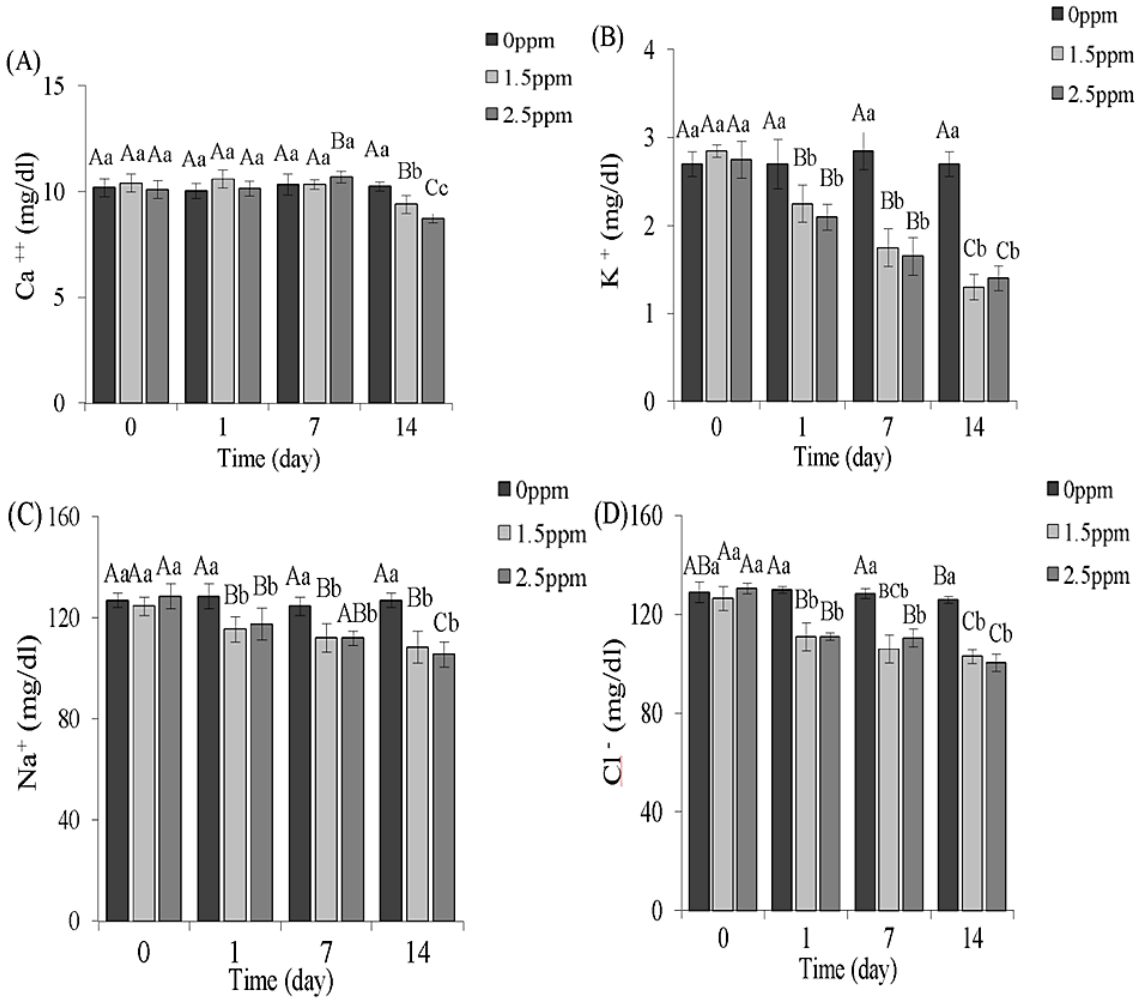

Figure 5. Serum ions concentration in rainbow trout exposed to nanosilver for 14 days. (A) Calcium, (B) potassium, (C) sodium and (D) chloride. Data are presented as mean±SD (n=9). Capital letters (A, B, C) indicate a significant difference (P<0.05) between the same exposing dose from control time (day 0); Small letters (a, b, c) indicate a significant difference (P<0.05) from the same day's control group.

significant decreases in Cl^- level at 1, 7 and 14 days (Fig. 5D). Among all investigated serum ions, Cl- ions did not change.

Serum enzymes activity: Both concentrations of ApNPs induced significantly increased (P<0.05) alkaline phosphatase level of serum in all sampling times (Fig. 6A). Likewise, LDH levels increased (P<0.05) in all exposed fish except at 1.5 ppm after one day of exposure (Fig. 6B). Both aminotransferases i.e. ALT and AST, showed significant increase (P<0.05) in all AgNP exposed fish (Fig. 6C, D). For serum antioxidant enzymes activities, CAT demonstrated a significant decrease in activity showed at 2.5 ppm of AgNP one day after exposure and there was a significant decrease in low and high dose of AgNP on days 7 and 14 (P<0.05). Likewise serum SOD activity of exposure groups exhibited a significant (P<0.05) dose-dependent decreased compared to the control group (Fig. 6E, F).

Discussion

In ecotoxicological investigations, physiological responses like hemato-immunological, serum metabolites, enzymatic activity, ion homeostasis and endocrine disturbances provide valuable insight into the affects on aquatic species (Kavitha et al., 2010; Kim and Kang, 2004; Rao, 2006b). Indeed, physiological assessments measure the responses of organisms exposed to pollutants and correlate these responses with pollutant effects (Handy and Depledge, 1999). The results presented here demonstrate that there were no mortalities in rainbow

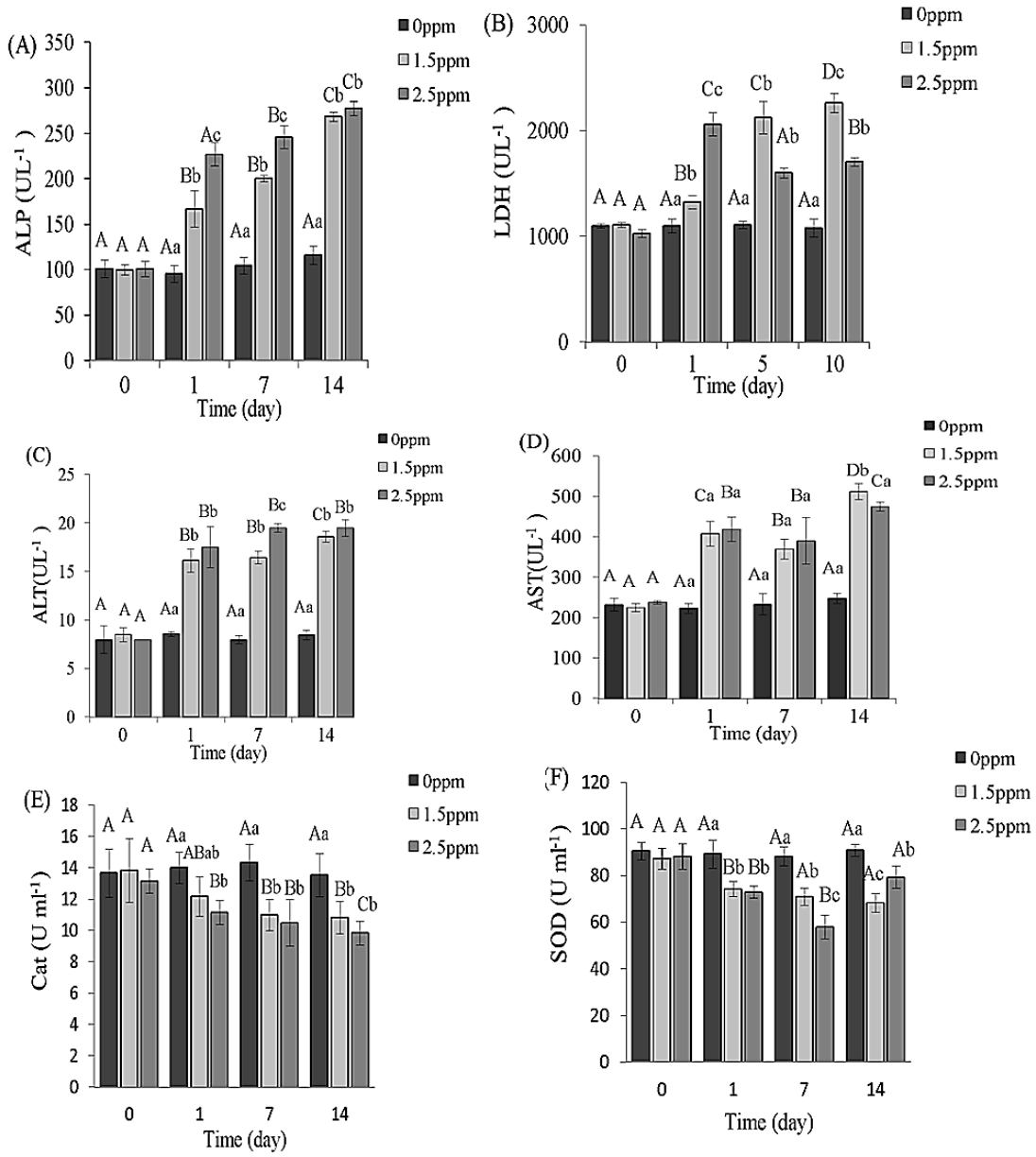

Figure 6. Serum hepatic enzymes in rainbow trout exposed to silver nanoparticles for 14 days. (A) Serum alkaline phosphatase (ALP) level, (B) serum lactate dehydrogenase (LDH) level, (C) serum alanine aminotransferase (ALT) level, (D) aspartate aminotransferase (AST) level, (E) serum catalase(Cat) activity and (F) serum superoxide dismutase (SOD) activity. Data are presented as mean±SD (n=9). Capital letters (A, B, C) indicate a significant difference (P<0.05) between the same exposing dose from control time (day 0); Small letters (a, b, c) indicate a significant difference (P<0.05) from the same day's control group.

trout after 14 days exposure with sub-lethal concentrations (1.5 and 2.5 ppm), however there were impacts on haematological parameters.

We observed a significant increase in the number of red and white blood cells and hematocrit percentage. In agreement with our results, Vinodhini and Narayanan (2009) reported that combined (Cd+Pb+Cr+Ni) metal solutions increased the level of RBCs, Htc and Hb in *Cyprinus carpio* after 32 days exposure. The increase level of RBCs could be attributed to stress in exposed fish and this is a response to supply oxygen demand in a high stress situations (Yarahmadi et al., 2015). Although in contrast, others have reported a significant decrease of RBC count, Hct percentage and hemoglobin amount in nanoparticles exposed fish (Karthikeyeni et al., 2013; Shaluei et al., 2013; Shaw et al., 2012). This could be explained by the dissimilarity in the differences in tested organism's species or differences between the sizes of nanoparticles.

There were significant increases in both WBC count and percentage of neutrophils in the 7th and 14th days of exposure. The WBCs counts and especially neutrophil cells are one of the non-specific immune indicators in fishes (Ainsworth, 1992; Neumann et al., 2001). The increase in WBC counts is known as a normal reaction to pollutants which can alter the normal physiological processes in fish (Handy and Depledge, 1999) and it has been reported in some studies after exposing the fish to pollutants (Weinreb, 1958). The increase of some hemato-immunological parameters such as leukocytes number and neutrophil level has been observed in *Hypophthalmichthys molitrix* (Shaluei et al., 2013) in *Tilapia nilotica* (Khalaf-Allah, 1999) that were subjected to nanosilver and different pesticides.

Fish exposed to AgNP showed remarkable change in the levels of serum metabolites; decreases in serum triglyceride, total protein and albumin level and conversely increases in serum cholesterol, glucose and cortisol were observed. There is limited information about effect of AgNP on serum metabolite parameters in fish, although it is well-known that serum metabolite parameters are biomarkers to evaluate the relationship between pollution and fish physiological response in ecotoxicological study (Adams et al., 1992; Adams and Ryon, 1994; Folmar et al., 1993; Öner et al., 2008). The reduction in serum triglyceride suggested that experimental animals are suffering from poor nutrition or starvation as a result of pollutant exposure-related stress (White and Fletcher, 1986). In agreement with this study, Öner et al. (2008) reported that serum triglyceride decreased in Cu-exposed *Oreochromis niloticus,* although in contrast, serum triglyceride was increased in Ag-exposed fish. However, Ag, Cd, Cr, Cu, Zn exposures increased serum cholesterol concentration (Handy and Depledge, 1999), which is in agreement with the results of this study. The serum total protein is one of the humoral innate immune parameter in fish which mainly include albumin and globulin (Zhang et al., 2013). The reduction of serum proteins in AgNP exposed fish may be related to a decrease in small proteins found in the blood, lysozymes, and suppression of innate immune response (Han et al., 2014).

Cortisol is a stress-related hormone and it is known that increased serum concentrations can be use as a first stress response bioindicator to environmental disruption (Barton, 2002). The increased serum cortisol level in the present investigation is consistent with similar observations in *H. molitrix* exposed with AgNP (nanocid) (Shaluei et al., 2013). The results demonstrated revealed an increased glucose level after 7 and 14 days of exposure. Blood glucose elevation during pollutant stress is well known as secondary stress response (Handy and Depledge, 1999). The serum glucose level is one of the secondary stress response associated with increased levels of circulating cortisol due to stress (Barton, 2002), and is demonstrated here with the strong correlation between increased circulating cortisol and glucose levels.

The results of present study indicated significant decreases in serum Ca^{2+}, K^{2+}, Na^{+} and Cl^{-}, ions levels during the experiment. Among these four ions, only serum chloride ions level changed one day after exposure. Pollutants are wel-known to disrupt the homeostatic balance of serum ions in fish (Handy and Depledge, 1999). There is limited information about effect of AgNPs on serum ion concentration. However, in agreement with our results, Johari et al. (2013) reported one dose-dependent plasma reduction of chloride and potassium ions in rainbow trout juveniles after 3 h of exposure to AgNps. Compensating for this, apical sodium channels and the basolateral Na^{+}/K^{+}-ATPase in gill epithelial cells, facilitate active uptake of Na^{+} from the dilute environment (Evans et al., 2005). These specialized cells have an important role in ion transporting including NaCl uptake. A previous study by Katuli et al. (2014) demonstrated that AgNPs inhibit the activities of Na+/K+ -ATPase in gill of zebrafish (*Danio rerio*). Conversely, gill injuries which were observed in many experiments as an effect of AgNP might lead to disruption in NaCl uptake in exposed fish (Farmen et al., 2012; Scown et al., 2010b; Wu and Zhou, 2013). Consequently, there would be a reduction in the concentration pf Na^{+} and Cl^{-} ions in

Table 2. A summary of present experiment results. Upward arrows (↑) indicate significant increases (P<0.05) and downward arrows (↓) indicate significant decreases (P<0.05) in experimental parameters. In dose-dependent group results compared with same sampling time's control group; in time-dependent column results compared with same dose's T_0 sampling data.

			dose-dependent changes								time-dependent changes					
			T_0		T_1		T_2		T_3		1.5 ppm			2.5 ppm		
			1.5	2.5	1.5	2.5	1.5	2.5	1.5	2.5	T_1	T_2	T_3	T_1	T_2	T_3
Blood cells	Red	RBC			↑	↑	↑	↑	↑		↑	↑	↑	↑	↑	
		%Hct				↑	↑	↑	↑	↑		↑	↑	↑	↑	↑
	White	WBC					↑	↑	↑	↑		↑	↑	↑	↑	↑
		Nut						↑	↑	↑			↑		↑	↑
		Mon														
		Lym														
		Eos														
Blood plasma	ions	Ca								↓						↓
		K					↓	↓	↓	↓		↓	↓		↓	↓
		Na							↓	↓			↓			↓
		Cl			↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
	Biochemical factors	Tri				↓		↓	↓	↓			↓	↓	↓	↓
		Chol						↑							↑	
		Pro_{tot}			↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
		Alb						↓	↓	↓			↓		↓	↓
		Glo			↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
		Glu					↑	↑	↑	↑		↑	↑		↑	↑
	Hepatic enzymes	ALP			↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
		LDH				↑	↑	↑	↑	↑		↑	↑	↑	↑	↑
		ALT			↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
		AST			↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
		SOD														

the blood of AgNPs exposed fish. The induced stress response found in this study may be associated with the loss of ion homeostasis, as cortisol is known to alter ionoregulatory mechanism in the gill (Barton, 2002).

Some serum enzymes such as ALP, LDH, ALT and AST were analyzed as useful biomarkers of liver injury and health status, and results revealed that the activity level of these enzymes increased in response to AgNP exposure compared to the control (Fig 6). Previous studies have shown an increase in serum ALP, LDH, ALT and AST contents in response to toxicant exposure (Das et al., 2004; Gulumian et al., 2006). ALP and LDH are cellular membrane enzyme and their activity is used as indicator of cell membrane damage (Gulumian et al., 2006). Rao (2006a) reported that the ALP activities in plasma, gill and kidney of exposed *O. mossambicus* with insecticide increased the lysosomal mobilization, cell necrosis, localized hypoxic conditions and muscular harm. Increases of LDH in serum may be a result of releasing the isozymes from the destroyed tissues and indicates cell lysis (Agrahari et al., 2007; Lemaire et al., 1991). Two serum aminotransferases (ALT and AST) which were measured in this study are well known as liver specific enzymes in hepatocellular necrosis condition (Geeraerts and Belpaire, 2010). Our results showed a significant increase in both enzymes in AgNPs exposed fish. The increase of these enzymes has been observed in the gill, liver, and kidney as a response to pollutant exposure (Oluah, 1999; Rajyasree and Neeraja, 1989; Rao, 2006a).

The antioxidant enzymes assayed in the present study revealed that AgNPs affected oxidative defense system that impacted CAT and SOD activity. SOD and CAT are two key oxidative defense enzymes that provide primary antioxidant protection against oxidative stress through catalyze dismutation of superoxide anion radicals and hydrogen peroxide, respectively (Kolayli and Keha, 1999; Wu and Zhou, 2012, 2013). In the present study, we observed a significant decrease in serum SOD and CAT activity in exposure groups from day 1 to 14. In agreement with our results, Wu and Zhou (2013) reported the dose-dependent decrease in hepatic SOD and CAT activity in Medaka (*Oryzias Latipes*) after 14 days of exposure. AgNPs induced ROS generation and damage to various cellular components in human liver (Piao et al., 2011). The reduction in the level of SOD and CAT activity lead to oxidative system damage affected by AgNP (Li et al., 2009). Further to this, ROS produced in tissue following pollutant exposure have a synergistic effect on the reduction of CAT activity (Stanic et al., 2006).

Conclusion

A summary of all results drawn from this study is presented in Table 2, in which hematological alterations of *O. mykiss*, sub-lethal exposed to AgNP, suggest that exposed fish faced a catastrophic situation that results in increased levels of RBC, WBC and neutrophils as immune response. From analysis of serum metabolites and enzymatic parameters, it was concluded that sub-lethal AgNP exposed fish had altered ion homeostasis and impacted serum enzymatic parameters. Enzymatic findings indicate an increase in serum ALP, ALT, AST and LDH concentration which may be contributed to cell and tissue damages following AgNP exposure. Furthermore, SOD and CAT activity revealed the toxicant effect of AgNPs by suppressing oxidative stress system. As result, we suggest that blood analysis, serum metabolite and enzymatic activity are used as a good biomarker of AgNP contamination. However we strongly encourage future studies in other aquatic species to confirm these findings.

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