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Study of effects of silver nanoparticles on oxidative stress level and antioxidant in *Cyprinus carpio* fish under some ecological factors

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ABSTRACT

The present study was aimed to investigate the median lethal concentration of silver nanoparticles (AgNPs) toward common carp (*Cyprinus carpio*) prepared by chemical methods. Then, treating fish with silver nanoparticles under different environmental conditions (heat and ventilation). Then to study the functional, physiological changes after acute and chronic exposure to these concentrations. Where the lethal concentration was found for half of the experimental fish (1763 ppm) and that concentration was used to calculate safety concentrations. The presence of significant at $P < 0.05$ in each of the values in vitamins (A, E and C), and well in (GSH, SOD, CAT). Where there was a marked increase in the values of each of (A, E, SOD), and decrease in the values of each of (GSH, CAT and vit C). While there were no significant differences in (MDA) comparing with the control group.

Key words: *Oxidative stress, AgNPs, Antioxidan0t, Common carp.*

Introduction

Nanotechnology is a known field of research since last century. Nanoparticles (NPs) are wide class of materials that include particulate substances, which have one dimension less than 100 nm at least (Stebounova *et al.,* 2011). Nano-silver particles (AgNPs) are generally smaller than 100 nm, contain 20–15,000 silver atoms, and have unusual physical, chemical and biological properties. When using AgNPs for therapeutic and diagnostic purposes, there in vivo fate and toxicity are crucial aspects that need to be evaluated (Li and Chen, 2011). Silver nanoparticles have gained great interests due to their unique properties such as chemical stability, catalytic and excellent conductivity. It can be incorporated into composite fibers, cryogenic superconducting materials, cosmetic products, food industry and electronic components (Magudapathy *et al.,* 2001). Common carp (*Cyprinus carpio* L.) considered as the most common type of fish cultured in Iraq, this fish species have many characteristics which make it suitable for breeding in aquatic environment of Iraq included rapid increase in weight during short time as well as resistance to many pathogens in aquatic environment of Iraq.

UV–vis absorbance spectroscopy analysis:

According to (Raut Rajesh *et al.,* 2009), silver nitrate (AgNO3) bioreduction to AgNPs was regularly tracked by UV-vis spectroscopy (Shimazu 2401PC) following test dilution with deionized water. It is used for purification and quality management of air, biosensing, imaging, drug delivery system. AgNPs have unique optical properties, which make them strongly interact with specific wavelengths of light. In addition, UV-vis spectroscopy is fast, easy, simple, sensitive; selective for different types of NPs, needs only a short period time for measurement, and finally a calibration is not required for particle characterization of colloidal suspensions (Huang *et al*., 2007).

TEM analysis of AgNPs

The suspension containing AgNPs of *C. murale* was sampled by TEM analysis using JEOL model 1200 EX electron microscope. TEM samples were prepared by placing a drop of the suspension of AgNP solutions on carbon-coated copper grids and allowing water to evaporate. The samples on the grids were allowed to dry for 4 min. The shape and size of silver nanoparticles from *C. murale* were determined from TEM micrographs (Elavazhagan and Arunachalam, 2011). X-ray diffraction (XRD) is a popular analytical technique, which has been used for the analysis of both molecular and crystal structures, qualitative identification of various compounds, quantitative resolution of chemical species, measuring the degree of crystallinity, isomorphous substitutions, particle sizes (Waseda *et al*., 2011). Recently, the field of nanoscience and nanotechnology has provided a driving force in the development of various high-resolution microscopy techniques in order to learn more about nanomaterials using a beam of highly energetic electrons to probe objects on a very fine scale (Yao and Kimura, 2007). Using SEM, we can probe the morphology of particles and derive a histogram from the images by either by measuring and counting the particles manually, or by using specific software. The combination of SEM with energy-dispersive X-ray spectroscopy (EDX) can be used to examine silver powder morphology and conduct chemical composition analysis (Yao and Kimura, 2007). Generally, AFM is used to investigate the dispersion and aggregation of nanomaterials, in addition to their size, shape, sorption, and structure; three different scanning modes are available, including contact mode, non-contact mode, and intermittent sample contact mode. AFM can also be used to characterize the interaction of nanomaterials with supported lipid bilayers in real time, which is not achievable with current electron microscopy (EM) techniques (Koh *et al*., 2008; Picas *et al*., 2012).

Materials and Methods

Acclimatization of the *Cyprinus carpio* L.

Common carp (*Cyprinus carpio*) collected from the culture of Heet city fish (west of Iraq in Al-Anbar Governorate) were used in this study. The average length of these fish between 10 -14 cm and weight between 24 -30 g. They were acclimatized in a 45 L aquarium (60×40×30 cm) with constant aeration, light/dark 12 h/12 h. Air pumps were applied to preserve the dissolved oxygen concentration at the optimal level .Collected fish were fed for 45 days during the experiment with a manufactured standard diet.

The chemical methods for preparation of Silver nanoparticles (AgNps)

(used $AgNO_3$ that source of Ag ion) that used in the experiments were prepared by Described method from Stebounova *et al*., (2011). After three experiments to find the LC_{50} of AgNps to *Cyprinus Carpio* fish, we find the concentration was 1763 ppm (LC50), then was treated the fish in acute toxicity.

Determination of Acute toxicity of AgNps

The exposed fish samples were sorted into two main groups, every group from this was acting as a treatment by four concentration of AgNPs with economic factors (temperature and ventilation). The fish mortality was recorded after 24, 48, 72 and 96 hours. The concentration of AgNPs shown in Table (1).

The concentration that appeared in its half-fish experiment death was 1763 ppm that used in the chronic toxicity experiment.

Subchronic toxicity test for AgNPs

The fish were exposed to three concentrations of AgNps which were calculated according by $1/5^{th}$, $1/20^{th}$ and $1/100^{th}$ of the 1763 LC 50 value which was calculated in this study which were = 352 ppm, 88 ppm and 17.65 ppm respectively. Two groups of fishes were used in each aquarium subjected (every group contains 10 fish) to 352, 88 and 17.65 ppm to the selected concentrations and left for 4 and 6 weeks. Water was refreshed every 48 hr. to remove any wastes. This group was treated with two economic factors (heating and ventilation), the temperature degree divided into three sections with each treatment (25±2, 30±2, 35±2) respectively.The ventilation is divided into three sections with each treatment (15-minute ventilation and 15-minute interruption, 15-minute ventilation and 30-minute interruption, continuous ventilation without interruption) respectively. Sampling was done after 3 weeks and 6 weeks of exposure to the silver nanoparticles. 6 fish were removed from each treated community of AgNPs after the specified exposure periods. Fishes were fed once per day, which referred to the importance of water changed due to decrease the concentration because of the adsorption on the glass aquaria, evaporation, of absorption by the animal tissues and accumulation of fecal residues in the aquaria.

Determination of vitamins

Vitamin A, vitamin C and Vitamin E concentration were estimated using a vitamin-dependent method to absorb light highly at wavelength in the ultraviolet region (López-López *et al*., 2011; Omaye *et al*., 1979)

Assay of Serum Glutathione (GSH)

Reduced glutathione is determined according to the method based on the reaction of thiol compounds in glutathione with 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB) at pH=8 (Aebi, 1983).

Assay the activity of serum SOD

This method is based on the ability of SOD to inhibit oxidation of epinephrine to adrenochrome or (carbazochrome) (Fridovich, 1989).

Determination of catalase

Enzyme activity according to procedure describing that used for measurement of catalase activity (Omaye *et al.,* 1979).

Determination of Malondialdehyde (MDA)

The amount of MDA, which is the lipid peroxidation index, is calculated on the basis of the thiobarbituric acid (TBA) reaction forming the compound TBA2-MDA, which according to the standard Guidet and Shah Process, is a pink coloured product.

Statistical analysis

Analyses of data were completed by using statistical software SPSS program, version 22, for windows, USA, Levesque, 2007 and Prism program version 7. The mean and standard deviation of each variable were estimated. Differences between the mean of different groups were done by using ANOVA one way with Duncan multiple comparison tests. In addition to the use of Gen-Stat, 2012 for the analysis of Multiple Linear Regression between different groups.

Results and Discussion

Silver nanoparticles (AgNPs) preparation and characterization

In this study, AgNPs were prepared by the chemicals methods and depending on the color and the other indicators. The accident of silver ion reduction at temperatures (47, 57, 65 and 70) °C was examined. The Appropriate temperature of 70 °C for 30-45min considered the optimum temperature in biological synthesis. The result suggested that high temperature accelerated the reduction process (Gou, Zhou *et al.,* 2015) in chemicals synthesis; we observed the optimum temperature was 50°C for 10 min (Zia *et al*., 2016). The pH showed that pH 9.0 is the best conditions for the synthesis of silver nanoparticles, observe that the maximum absorbance had increased when pH increased from 6-10. The results indicate that an alkaline pH favored the formation of AgNPs.

X-ray diffraction (XRD) Analysis

XRD was tested for the phase purity and crystalline nature confirmation of AgNPs as seen in Fig. 1. Which illustrates the evolution of X-ray diffraction patterns of dried silver nanoparticles showed Bragg reflections representative of the fcc structure of AgNPs. XRD peaks at 2θ of 38.45° ,44.39°, 64.57°, and 77.54°. They could be attributed to the 111, 200, 220 and 311 crystallographic planes. The intensity of the peak of (111) at 38.18° diffraction was much stronger than those peaks of (200) at 44.39°, (220) at 64.57° and (311) at 77.54°. And it is compliant with the mainstream 19-3501 JCPDS Data Card No. The average particle size of the Debye-Scherrer formula was determined (Khan *et al*., 2013).

Atomic Force Microscopy (AFM) Analysis

The results of AFM measurement were revealed for-

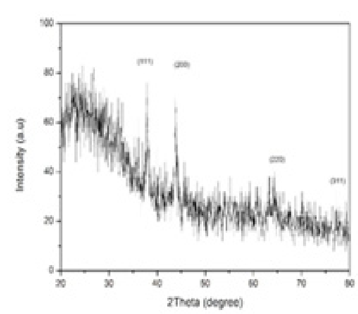

Fig. 1. X-ray diffraction pattern of synthesis AgNps

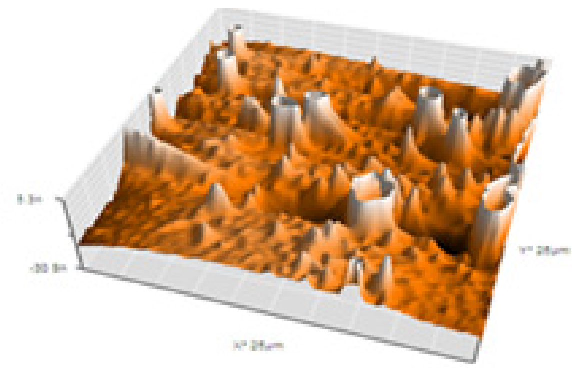

Fig. 2. AFM analysis for AgNPs 3D image. Mean grain size: 30.5 nm. Total grain volume (zero): 17.6

mation of nanoparticles with different sizes as shown in Figure 2. Granularity accumulative distribution chart obtained from AFM software indicated that the average diameter of AgNPs .

Transmission Electron Microscopy (TEM)

Results of morphological and nanostructure were studied by TEM. The morphology of green synthesized Ag NPs was viewed by TEM. The TEM image, as represented in (Fig. 3) showed that the Ag NPs formed were well dispersed with a spherical shape and particle sizes ranging from 10 to 17 nm. It is known that the optical and electronic properties of metal nanoparticles are largely affected by the shape of nanoparticles (Fig. 3)

UV–Vis Absorbance Spectroscopy Analysis:

Silver nanoparticle solutions (AgNPs) were characterized by using UV-Vis spectroscopy (UV-Vis sp 6405, jenway). A 1.5 ml of each sample solution input in quartz cuvette, shows UV-Vis absorption spectrum of Ag nanoparticles to know the formation of AgNPs. The UV-Vis spectrometric readings were recorded at a scanning speed of 200 to 800 nm. Absorption spectra peaks for AgNP solutions exhibit characteristic peaks around 420nm, as shown in (Figures 4). The result of UV absorption peaks of synthesized AgNPs occurred at range (420 – 425) nm for each sample during two weeks.

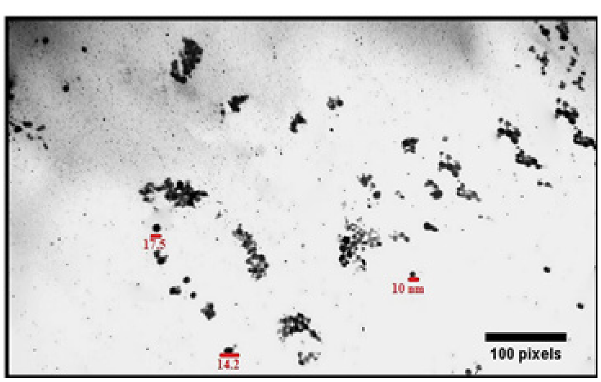

Fig. 3. TEM micrograph of chemically synthesized AgNps.

The AgNPs used in this experiment was characterized by using a number of methods of the study, including UV/Vis spectroscopy, AFM and TEM. The solutions were observed to change color from its colorless and transparent form to a slightly yellowish one after a few minutes during the ablation of the silver plate. The dark yellow would be achieved for higher concentration (Tajdidzadeh *et al.*, 2014). TEM microscopy is a technique to characterize the nano composite structure. TEM microscopy is used to obtain detailed information about the microstructure, morphology and the size distribution of nanoparticles embedded within a polymer matrix or deionized distilled water (Abd El-kader *et al.*, 2015). TEM images as (Figs. 3) showed that the AgNPs for each type were spherical in shape and nanoscale range 1-100 nm, this was confirmed by the results of UV-vis and AFM.

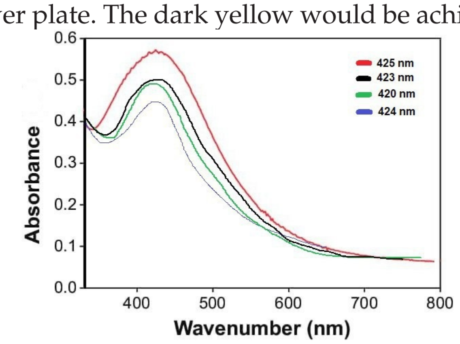

Fig. 4. UV–Vis spectra of chemically synthesized AgNps

The results show specific activities in the averages (increase) of vit A, vit E and SOD) in the fish blood exposed to AgNPs at different conditions (Figure 3-1), (Fig. 3-2), (Figure 3-3).

While the results show specific activities in the averages(decrease) of (vit C, GSH and CAT) in the fish blood exposed to AgNPs at different conditions (Figure 3-4), (Figure 3-5), (Figure 3-6).

The results of the present study show that there is a difference in the average of MAD concentration in the serum of fish blood exposed to different concentrations of AgNPs comparing with the control group and figure (3-7) show that there are not significant differences in the averages of MAD.

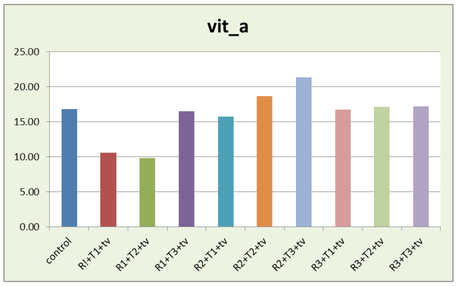

Fig. 3-1. Vitamin A concentration in *Cyprinus carpio* blood with different concentration of AgNPs

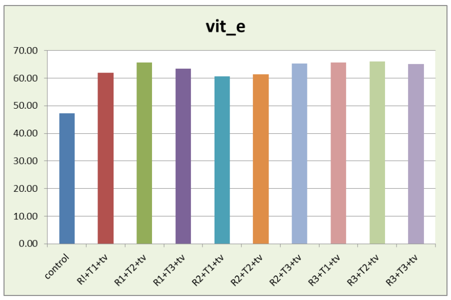

Fig. 3-2. Vitami E concentration in *Cyprinus carpio* blood with different concentration of AgNPs

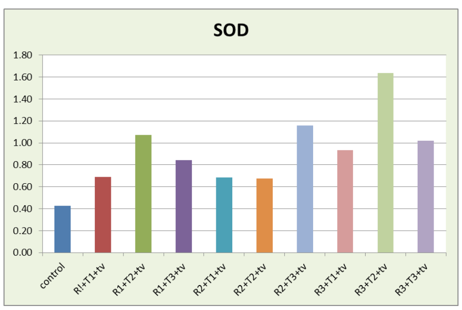

Fig. 3-3. SOD activity in blood *Cyprinus carpio* with different concentration of AgNPs

Discussion

Oxidative stress is a condition of an excess of reac-

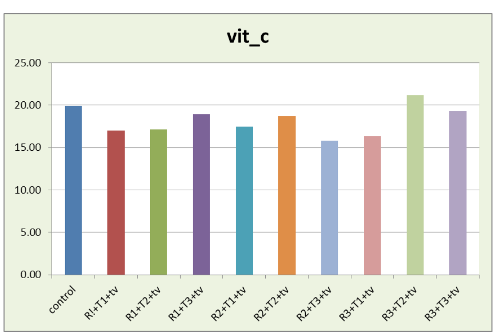

Fig. 3-4. Vitami C concentration in *Cyprinus carpio* blood with different concentration of AgNPs

tive oxygen species (ROS), which through disrupting or destroying homeostasis, interferes with biological processes. This leads to an imbalance between ROS production and the ability of the biological system to detoxify the reactive intermediates readily or restore the resulting damage (Lin *et al.*, 2007; Mittler, 2002) Via intracellular ROS development involving mitochondrial respiration and activation of NADPH-like enzyme systems, NPs react with cells and activate their pro-oxidant effects (Driscoll *et al.*, 2001). The gills are the first line of protection against any pollutant by mucous secretion in fish (Handy *et al.*, 2009). Subsequently, by binding to Na/K-ATPase and inhibiting toxin entry,

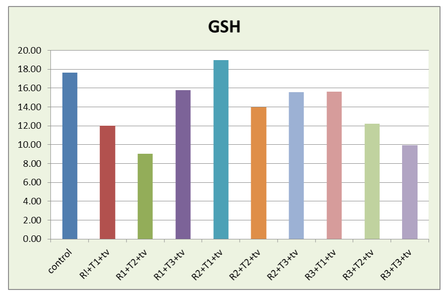

Fig. 3-5. GSH activity in blood *Cyprinus carpio* with different concentration of AgNPs.

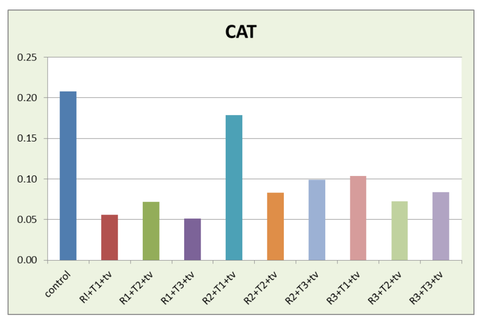

Fig. 3-6. CAT activity in blood *Cyprinus carpio* with different concentration of AgNPs

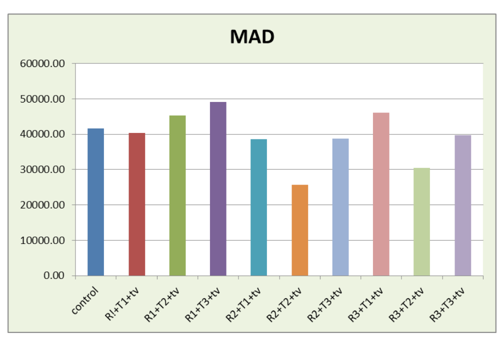

Fig. 3-7. MAD activity in blood *Cyprinus carpio* with different concentration of AgNPs

gills respond by producing oedema with the raising of gill lamellar epithelium. It is recognized that the introduction of toxins into the marine environment causes adverse effects on the atmosphere and on living organisms, bringing considerable importance to the study of toxicant-induced oxidative stress responses in aquatic species. By producing free radicals and/or modifying antioxidant enzyme systems of reacting oxygen species (ROS) many contaminants can result in some degree of oxidative damage (Huang *et al*., 2007). In a number of marine and freshwater species, antioxidant resistance enzymes have been suggested as biomarkers of contaminant or seasonally induced oxidative stress, and their activation represents a particular reaction to contaminants (Borkoviæ *et al*., 2005). SOD, which catalyzes the conversion of the radical superoxide anion to molecular oxygen and hydrogen peroxide (H_2O_2), has been called the first protection line of the cell against ROS and could defend against biochemical superoxide-induced harm (Fridovich, 1989). In biological environments, vitamin E has a beneficial effect on the stabilization of the metabolic process (Heydarnejad *et al*., 2013)**.** The inactivation of toxic oxy radicals induced by stress and environmental contamination plays a crucial role in fish welfare. Decreased vitamin E may be due to violent activation in the liver of chelated heavy metals. Several studies have indicated that vitamin E has the best defense against toxicants metal in people and animals. The increased vitamin E in the present study may be due to its use as the first line of defense in the protection of subcellular organelles and the stability of liver cell membranes against toxic reactive metabolites caused by heavy metals. These results are consistent with previous studies demonstrating the sensitivity of guinea pigs to disturbances in antioxidant status. Particularly, Se deficiency combined with vitamin E or C deficiency has been reported to cause skeletal muscle damage (Pretto *et al*., 2014). Further, vitamin E combined with vitamin C deficiency has been shown to promote limb paralysis and death due to severe damage in the brainstem and spinal cord. A discernable decreasing trend for vitamin C concentrations in tissues with decreasing dietary Se was observed for week 5, 48 hrs and week 12, 24 hrs guinea pigs. In contrast, no trend was observed for week 5, 24 hrs and week 12, 48 hrs guinea pigs. Glutathione It is suspected that detoxifies copper and Ag through two mechanisms. Firstly, by GPx operation, it scavenges reactive oxygen species formed by copper in the Fenton reaction. Secondly, the cysteinyl group was active in copper sequestration, then reducing its intracellular concentration (Stohs and Bagchi, 1995). The GSH/GSSG proportion is widely used as a thiol status biomarker for marine species (Van der Oost *et al*., 2003)**.** While our research did not impact the GSH/GSSG ratio, variations in overall GSH were observed in the liver and gills. As needed, glutathione produced in the liver could be transported to the gills, which could explain the elevated total GSH concentrations in the gills observed in our sample. Subsequently, GSH synthesis may be interrupted directly in the liver, with a resulting decline in overall GSH in this tissue (Kretzschmar, 1996). CAT as an inducible enzyme that protects is against reactive oxygen species in the biological system (Oruc *et al*., 2004). Most of the superoxide anions (O2-) released in the cell are converted into hydrogen peroxide (H2O2) molecules created inside the peroxisomes when oxidative stress occurs, and are killed exclusively by CAT (Babo and Vasseur, 1992; Monteiro *et al*., 2009). Studied the impact of methyl parathion on the function of Brycon cephalus liver, white muscle and gills on antioxidant enzymes. They stated that when compared to placebo, methyl parathion dramatically improved CAT activity in all tissues. Another research, (Yonar *et al*., 2014), demonstrated antioxidant enzymatic effects in the liver, kidney and gill of Cyprinus carpio of the pesticide malathion, which is an organophosphate pesticide. Malathion was found to induce a substantial improvement in *Cyprinus carpio's* CAT behaviors over all tissues. Our Discovery Demonstration C. The operations of Umbla liver, kidney and gill CAT Increased in multiple concentrations following AgNPs exposure. This change in the activity of these enzymes is same as a reaction to the increased generation of ROS caused by pesticide

Table 1. The concentration of silver nanoparticles that used in acute toxicity test of common carp and median lethal concentration (LC50) of experiment fish and control

Group	AgNPs conc.ppm	Total No. of fish	% Mortality
1	221	8	50
2	442	8	25
3	882	8	zero
4	1763	8	100
Control	zero	8	zero

toxicity (John *et al.,* 2001). SOD as an antioxidants enzyme is essential for oxyradical formation inhibition and is used to show oxidative stress as a biomarker. The transition of superoxide radicals to H_2O_2 and O_2 is catalyzed by SOD and is the first enzyme to treat oxyradicals (Zhang *et al.*, 2004). The impact of cypermethrin on antioxidants enzymes in the kidney and liver of Oreochromis niloticus and Cyprinus carpio were investigated by (Üner *et al*., 2001). They documented that, relative to controls, cypermethrin greatly improved the SOD activity of Oreochromis niloticus and *Cyprinus carpio* in the liver and kidney (Yonar *et al*., 2014). Reported that in the liver, kidney and gills of Cyprinus carpio of the malathion-exposed populations, the SOD activity was substantially increased. The present findings showed that in the groups exposed to AgNPs at various concentrations, SOD activity was increased. SOD is the first enzyme to respond against oxygen radicals and is the one that offers the greatest response to oxidative stress (Winston and Di Giulio, 1991) CAT activity, found mainly in peroxisomes, is associated with elevated concentrations of H_2O_2. Depending on the dosage, length, and route of exposure, antioxidant enzymes have been shown to be either triggered or inhibited by AgNPs in popular carps. The thesis addressed the influence of exposure to Ag-NPs in the physiology of the common carp through biochemical histological analysis. Analysis of enzymes associated with oxidative stress such as CAT, SOD and GSH in blood, regulate the histological alteration of the kidney, liver and gill and exposed classes of AgNPs showed deleterious effects of this substance in a dose-related manner. The CAT level increased in the treated fish dealing with AgNPs in the second and fourth weeks. This result indicates an increase in the level of peroxide hydrogen (H_2O_2), which may be the result of SOD enzyme activity to neutralize superoxide radicals. According to (Xing *et al.*, 2012) induction of subacute dose of Atrazine and chlropyrifos either separately or combined caused an increase in CAT activity level in the liver tissues and gills of *Cyprinus carpio* after 40 days. In goldfish, liver SOD activity has been increased, meaning that nickel activates SOD activity. In exchange, SOD could scavenge O-2 to defend cells from injuries and strike the balance between oxidant and antioxidant for a significant number of contributions in defense against the toxic effects of ROS. In the toxicity of atmospheric substances, free radicals play a central role. LPO induces MDA and is known as an example of oxidative stress arising from free radical disruption to cell membrane materials (Amin and Hashem, 2012; Yonar and Sakin, 2011), recorded a spike in rainbow trout gill and liver MDA levels caused by copper sulphate. In addition (Yan *et al*., 2015), studied the impact of various doses of AgNPs and exposure periods on the level of zebrafish liver MDA. AgNPs have been reported to induce a rise in zebrafish liver MDA levels in a time- and dose-dependent method. Many researchers have also documented a link between stress caused in fish by MDA and AgNPs (Kavasoðlu *et al*., 2015; Oruc *et al*., 2004; Üner *et al*., 2001) Exposure of C.carpio In my study, A pronounced increase in MDA in the liver, gill, and kidney in a dose-dependent pattern is seen in umbla for all classes of AgNPS concentration. The level of cellular disruption, called lipid peroxidation, through diazinon induction, which was calculated by the MDA index, was investigated in the current research. Based on measurements of MDA levels in serum samples between two and four weeks, this index improved in the DZN relative to the control group, which demonstrated a rise for peroxidation. This increase may be the result of the development of hydroxyl radicals (OH) as a strong oxidizing agent and a key factor at the beginning of the process of lipid oxidation produced by the metabolism of diazinon.

References

Abd El-kader, F., Hakeem, N., Elashmawi, I. and Menazea, A. 2015. Synthesis and characterization of PVK/ AgNPs nanocomposites prepared by laser ablation. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy.* 138 : 331-339.

Aebi, H. 1983. Methods of enzymatic analysis. *Catalase.* 673-686.

Amin, K.A. and Hashem, K.S. 2012. Deltamethrin-induced oxidative stress and biochemical changes in tissues and blood of catfish *(Clarias gariepinus*): antioxidant defense and role of alpha-tocopherol. *BMC Veterinary Research.* 8 (1): 45.

Babo, S. and Vasseur, P. 1992. *In vitr*o effects of Thiram on liver antioxidant enzyme activities of rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology.* 22(1) : 61-68.

Borkoviæ, S. S., Šaponjiæ, J. S., Pavloviæ, S. Z., Blagojeviæ, D.P., Miloševiæ, S.M., Kovaèeviæ, T. B. and Saièiæ, Z. S. 2005. The activity of antioxidant defence enzymes in the mussel *Mytilus galloprovincialis* from the Adriatic Sea. *Comparative Biochemistry and Physi-*

ology Part C: Toxicology & Pharmacology. 141(4) : 366-374.

Driscoll, K.E., Howard, B. W., Carter, J. M., Janssen, Y. M., Mossman, B. T. and Isfort, R. J. 2001. Mitochondrial-derived oxidants and quartz activation of chemokine gene expression *Biological Reactive Intermediates* VI (pp. 489-496): Springer.

Elavazhagan, T. and Arunachalam, K. D. 2011. Memecylon edule leaf extract mediated green synthesis of silver and gold nanoparticles. *International Journal of Nanomedicine.* 6 : 1265.

Fridovich, I. 1989. Superoxide dismutases. An adaptation to a paramagnetic gas. *Journal of Biological Chemistry.* 264 (14) : 7761-7764.

Gou, Y., Zhou, R., Ye, X., Gao, S. and Li, X. 2015. Highly efficient *in vitro* biosynthesis of silver nanoparticles using *Lysinibacillus sphaericus* MR-1 and their characterization. *Science and Technology of Advanced* materials.

H Saber, T. 2011. Histological adaptation to thermal changes in gills of common carp fishes *Cyprinus carpio* L. *Rafidain Journal of Science.* 22 (1) : 46-55.

Handy, R.D., Bury, N. and Flik, G. 2009. Osmoregulation and Ion Transport: Integrating Physiological Molecular and Environmental Aspects.

Heydarnejad, M.S., Khosravian-Hemamai, M. and Nematollahi, A. 2013. Effects of cadmium at sub-lethal concentration on growth and biochemical parameters in rainbow trout (*Oncorhynchus mykiss*). *Irish Veterinary Journal.* 66 (1) : 11.

Huang, X., Jain, P. K., El-Sayed, I. H. and El-Sayed, M. A. 2007. Gold nanoparticles: interesting optical properties and recent applications in cancer diagnostics and therapy.

John, S., Kale, M., Rathore, N. and Bhatnagar, D. 2001. Protective effect of vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes. The *Journal of Nutritional Biochemistry*. 12 (9) : 500-504.

Kavasoðlu, M., Sarioðlu, Y., Uysal, K., Dönmez, M., Altikat, S., Yetek, Ý. and Kuru, H.Ý. 2015. Effect of sodium cyanide on antioxidant enzyme activities and lipid peroxidation in some tissues of mirror carp (*Cyprinus carpio*). *Pakistan Journal of Zoology.* 47(6) : 1777-1782.

Khan, A., Asiri, A. M., Rub, M. A., Azum, N., Khan, A. A. P., Khan, S. B. and Khan, I. 2013. Synthesis, characterization of silver nanoparticle embedded polyaniline tungstophosphate-nanocomposite cation exchanger and its application for heavy metal selective membrane. *Composites Part B: Engineering.* 45 (1) : 1486-1492.

Koh, A. L., Hu, W., Wilson, R. J., Wang, S. X. and Sinclair, R. 2008. TEM analyses of synthetic anti-ferromagnetic (SAF) nanoparticles fabricated using different release layers. *Ultramicroscopy*. 108 (11) : 1490-1494.

Kretzschmar, M. 1996. Regulation of hepatic glutathione metabolism and its role in hepatotoxicity. *Experimental and Toxicologic Pathology.* 48(5) : 439-446.

Li, Y. F. and Chen, C. 2011. Fate and toxicity of metallic and metalcontaining nanoparticles for biomedical applications. *Small*. 7 (21) : 2965-2980.

Lin, A.J., Zhang, X.H., Chen, M.M. and Qing, C. 2007. Oxidative stress and DNA damages induced by cadmium accumulation. *Journal of Environmental Sciences.* 19 (5) : 596-602.

López-López, E., Sedeño-Díaz, J. E., Soto, C. and Favari, L. 2011. Responses of antioxidant enzymes, lipid peroxidation, and Na+/K+-ATPase in liver of the fish Goodea atripinnis exposed to Lake Yuriria water. *Fish Physiology and Biochemistry.* 37 (3) : 511-522.

Magudapathy, P., Gangopadhyay, P., Panigrahi, B., Nair, K. and Dhara, S. 2001. Electrical transport studies of Ag nanoclusters embedded in glass matrix. *Physica B: Condensed Matter.* 299 (1-2) : 142-146.

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science.* 7 (9) : 405-410.

Monteiro, D. A., Rantin, F. T. and Kalinin, A. L. 2009. The effects of selenium on oxidative stress biomarkers in the freshwater characid fish matrinxã, *Brycon cephalus* exposed to organophosphate insecticide Folisuper 600 BR®(methyl parathion). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology.* 149 (1) : 40-49.

Omaye, S.T., Turnbull, J.D. and Sauberlich, H.E. 1979. Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids Methods in Enzymology. 62 : 3-11): Elsevier.

Oruc, E. O., Sevgiler, Y. and Uner, N. 2004. Tissue-specific oxidative stress responses in fish exposed to 2, 4-D and azinphosmethyl. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 137 (1): 43-51.

Picas, L., Milhiet, P.E. and Hernández-Borrell, J. 2012. Atomic force microscopy: A versatile tool to probe the physical and chemical properties of supported membranes at the nanoscale. *Chemistry and Physics of Lipids*. 165 (8) : 845-860.

Pretto, A., Loro, V.L., Silva, V.M.M., Salbego, J., de Menezes, C.C., de Freitas Souza, C. and Baldisserotto, B. 2014. Exposure to sublethal concentrations of copper changes biochemistry parameters in silver catfish, Rhamdia quelen, Quoy & Gaimard. *Bulletin of Environmental Contamination and Toxicology*. 92 (4) : 399-403.

Raut Rajesh, W., Lakkakula Jaya, R., Kolekar Niranjan, S., Mendhulkar Vijay, D. and Kashid Sahebrao, B. 2009. Phytosynthesis of silver nanoparticle using *Gliricidia sepium* (Jacq.). *Curr. Nanosci.* 5 (1) : 117-122.

Stebounova, L. V., Adamcakova-Dodd, A., Kim, J. S., Park, H., T O'Shaughnessy, P., Grassian, V.H. and Thorne, P.S. 2011. Nanosilver induces minimal lung toxicity or inflammation in a subacute murine inhalation model. *Particle and Fibre Toxicology.* 8 (1) : 1-12.

Stohs, S. J. and Bagchi, D. 1995. Oxidative mechanisms in the toxicity of metal ions. *Free Radical Biology and Medicine.* 18 (2) : 321-336.

Tajdidzadeh, M., Azmi, B., Yunus, W. M. M., Talib, Z. A., Sadrolhosseini, A., Karimzadeh, K. and Dorraj, M. 2014. Synthesis of silver nanoparticles dispersed in various aqueous media using laser ablation. *The Scientific World Journal.* 2014.

Üner, N., Oruç, E. Ö., Canli, M. and Sevgler, Y. 2001. Effects of cypermethrin on antioxidant enzyme activities and lipid peroxidation in liver and kidney of the freshwater fish, *Oreochromis niloticus* and *Cyprinus carpio* (L.). *Bulletin of Environmental Contamination and Toxicology.* 67(5) : 657-664.

Van der Oost, R., Beyer, J. and Vermeulen, N. P. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology*. 13(2) : 57-149.

Waseda, Y., Matsubara, E. and Shinoda, K. 2011. X-ray diffraction crystallography: introduction, examples and solved problems: Springer Science & Business Media.

Winston, G. W. and Di Giulio, R.T. 1991. Prooxidant and antioxidant mechanisms in aquatic organisms. *Aquatic Toxicology*. 19 (2) : 137-161.

Xing, H., Li, S., Wang, Z., Gao, X., Xu, S. and Wang, X. 2012. Oxidative stress response and histopathological changes due to atrazine and chlorpyrifos exposure in common carp. *Pesticide Biochemistry and Physiology.* 103 (1) : 74-80.

Yan, S., Wang, J., Zhu, L., Chen, A. and Wang, J. 2015. Toxic effects of nitenpyram on antioxidant enzyme system and DNA in zebrafish (*Danio rerio*) livers. *Ecotoxicology and Environmental Safety.* 122 : 54-60.

Yao, H. and Kimura, K. 2007. Field emission scanning electron microscopy for structural characterization of 3D gold nanoparticle superlattices. *Modern Research and Educational Topics in Microscopy*. 2 : 568-576.

Yonar, M. E. and Sakin, F. 2011. Ameliorative effect of lycopene on antioxidant status in *Cyprinus carpio* during pyrethroid deltamethrin exposure. *Pesticide Biochemistry and Physiology*. 99 (3) : 226-231.

Yonar, S.M., Ural, M., Silici, S. and Yonar, M.E. 2014. Malathion-induced changes in the haematological profile, the immune response, and the oxidative/ antioxidant status of *Cyprinus carpio*: Protective role of propolis. *Ecotoxicology and Environmental Safety.* 102 : 202-209.

Zhang, J., Shen, H., Wang, X., Wu, J. and Xue, Y. 2004. Effects of chronic exposure of 2, 4-dichlorophenol on the antioxidant system in liver of freshwater fish *Carassius auratus. Chemosphere.* 55 (2) : 167-174.

Zia, F., Ghafoor, N., Iqbal, M. and Mehboob, S. 2016. Green synthesis and characterization of silver nanoparticles using Cydonia oblong seed extract. *Applied Nanoscience.* 6 (7) : 1023-1029.