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Ameliorative Effects of Selenium and *Chlorella vulgaris* Against Polystyrene Nanoplastics-induced Hepatotoxicity in African Catfish (*Clarias gariepinus*)

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Abstract

Polystyrene nanoplastics pollution is a global issue that has grabbed the attention of scientists and drew widespread attention owing to the possible health risks it poses. The objective of this research was to investigate the hepatotoxic effects of polystyrene nanoplastics (PS NPs) in catfish (*Clarias gariepinus*) from the histopathological, biochemical, and ultrastructural profiles. Six groups of fish were used (n= 24/group): the first group kept as control, the second group exposed to 5mg/L NPs, the third group exposed to NPs+selenium (1mg/kg diet), the fourth group exposed to NPs+*Chlorella* (25g/kg diet), the fifth group received only *Chlorella* and the sixth group received only selenium. The exposure was for 30 days. The biochemical tests showed a rise in serum ALT and AST activities after exposure to NPs compared to control group. Groups exposed to NPs and supplemented with *Chlorella* and selenium showed a significant decrease in the serum activities of these enzymes compared to NPs-intoxicated group. Results of histopathological examination demonstrated the hepatotoxic effects of NPs in NPs-intoxicated group that consisted of micro and macrovesciular steatosis, coagulative necrosis, pericentral fibrosis, periportal fibrosis, glycogen depletion and toxic changes in cellular ultrastructure. *Chlorella* and selenium supplementation alleviated the hepatotoxic effects of NPs and restored the histological and ultrastructural appearances of the liver tissue. Therefore, nanoplastics toxicity has well-defined hepatotoxic effects and these cytotoxic changes were mitigated by dietary supplementation of *Chlorella* and selenium.

KEYWORDS
Polystyrene nanoplastics, *Clarias gariepinus*, Hepatotoxicity, Selenium, *Chlorella vulgaris*

INTRODUCTION

Fish have an important role as the biggest group of vertebrates in the aquatic ecosystem (Roest Crollius and Weissenbach, 2005). *Clarias gariepinus* is a member of Claridae family and is a popular commercial species in Africa due to its good quality meat and nutritive values. *C. gariepinus* is an omnivore creature that eats anything, and has a number of distinct properties, including a high capacity to grow, higher feed conversion rate, high acclimation to low water quality and high survival rate (Iheanacho *et al*., 2019). It is one of the greatest widely recognized experimental species of fish in toxicity research (Karami *et al*., 2015).

Plastics are difficult to break down, because of their chemical stability resulting in their aggregation in the natural environment (Wang *et al*., 2016). Plastic trash is frequently degraded into microplastics (MPs, 1–5000 µm in size) and nanoplastics (NPs, less than1 µm in size). By a combination of physical, chemical, and biological mechanisms, these tiny particles become extremely hard to remove from the aquatic environment (Triebskorn *et al*., 2019; Kihara *et al*., 2021).

Exposure to NPs have been adversely affected fish, in the form of growth disorders (Manabe *et al*., 2011), increased oxidative stress (Parenti *et al*., 2019), toxicity of the reproduction system (Sarasamma *et al*., 2020), inhibition of development (Sokmen *et al*., 2020), immunotoxicity (Brandts *et al*., 2018) and neurotoxic effects (Chen *et al*., 2017). Also, microplastics caused congestion, sinusoidal dilatation, glycogen loss (Holmes *et al*., 2012), and cellular necrosis (Holmes *et al*., 2012).

The liver is the fish's primary organ for performing physiological processes such as metabolic process, storage of nutrients, and xenobiotic detoxification (Tao and Peng, 2009). As it is a major metabolic organ, affects and alters lipid metabolism in response to severe environmental conditions (Berghe, 1991). NPs have the ability to cross the intestinal barrier and enter the bloodstream, where they find their way to organs such as the liver and kidney (Lu *et al*., 2016). *In vitro* investigations revealed that NPs could penetrate the cell membranes of the hepatocytes, enter the cell via endocytosis and impair the regular physiological processes of the cell (Rossi *et al*., 2014; Jiang *et al*., 2019).

Polystyrene microplastics caused changes in metabolic profiles, energy metabolism, and induced inflammation, and lipid deposition in the liver of zebrafish (Lu *et al*., 2016), and African catfish (Karami *et al*., 2016). Furthermore, NPs caused excessive hepatic lipid accumulation in turbot *Scophthalmus maximus*

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(Wang *et al*., 2016), black rockfish *Sebastes schlegelii* (Yin *et al*., 2019) and in the large yellow croaker *Larimichthys crocea* (Lai *et al*., 2021).

Selenium is a micronutrient that is required for fish to grow steadily (Durigon *et al*., 2019). It acts as an antioxidant in stress responses and as a component of DNA and proteins (Lemly, 1993; Rider *et al*., 2009). It has an important role in the maintenance of enzymes and immune system (Patterson *et al*., 2010). Two forms of selenium had been found, including inorganic selenium compounds as sodium selenite (NaSe) and organic selenium compounds such as Se-methionine, Se-algae, Se-yeast, and Se-cysteine and Se-glycinate (Sunde, 2006). Organic Se has been found to be more rapidly absorbed and effective in terms of bioavailability and health effects in fish than inorganic ones (Wang *et al*., 2007). As shown in a study by Mechlaoui *et al*. (2019), Se-methionine was more effective than NaSe in protecting fish muscle from oxidative stress and maintaining hepatic morphology. Moreover, the development and histological features of barramundi juveniles are improved by dietary organic selenium (Ilham *et al*., 2018). Supplemental Se-methionine in the diet has been shown to reduce the stress caused by overcrowding in rainbow trout (Küçükbay *et al*., 2009).

Chlorella vulgaris is a single-celled freshwater microalgae that belongs to the Chlorophyta family (Tomaselli, 2004). In fish diets, *Chlorella* is one of the most used microalgae (Daniel *et al*., 2016). It contains many bioactive components such as carotenoids, phycobilins, fatty acids, polysaccharides, vitamins, and sterols. Higher levels of carotenoids in *C. vulgaris* have been shown to have antioxidant activities and anti-inflammatory properties (Soontornchaiboon *et al*., 2012). Its capacity to promote nutritional state, immunity, phytoremediation, stress alleviation, and resistance to disease in fish has been demonstrated in previous research. *C. vulgaris* can also decrease microbial population when administered correctly (Nicula *et al*., 2018), and had a hepatoprotective effect in rats and mice exposed to carbon tetrachloride toxicity (Hsin-yi *et al*., 2009).

To the best of the authors' knowledge, the protective effect of *C. vulgaris* on toxicity of microplastics in catfish was studied by Sayed *et al*. (2021). However, there is a shortage of studies that investigate the ameliorative effect of L-Selenomethione and *C. vulgaris* on hepatotoxicity induced by polystyrene nanoplastics (PS NPs) in catfish, which constituted the aim of this study.

MATERIALS AND METHODS

Chemicals

Polystyrene nanoplastic particles were provided from Bangs Laboratories, Inc. Transmission electron microscopy (TEM) was used to determine the size and form of the nanoplastic particles, which revealed polystyrene particles with mean diameters of (50.1 nm ±13.4 SD). The stock solution (5 g L^{-1}) of PS NPs was prepared in deionized water immediately before the experiments.

Seleno-L-methionine (≥ 98% (TLC), powder Mol. Weight 196.11 CAS Number 3211-76-5) was purchased from Sigma-Aldrich, USA.

Chlorella vulgaris microalgae in the form of dried green powder was obtained from the Institute of National Research Center, Cairo, Egypt.

Fish

A total number of one hundred and forty-four African catfish (*C. gariepinus*) with an average body weight of 160 g and a length of 25–30 cm was collected at a private fish farm in Assiut, Egypt. Fish were sent directly to Fish Biology Laboratory, Faculty of Science, Zoology Department, Assiut University, Egypt. The fish were acclimated in laboratory settings for 35 days in 100 L tanks with de-chlorinated tap water and air pumps. The fish were fed a commercial basal diet containing 30% crude protein. The study was performed between February and March 2021.

Statement of Ethics

The research was carried out in accordance with the standards and ethical rules of Faculty of Veterinary Medicine, Assiut University, Egypt.

Experimental design

After the acclimatization period, fish were randomly distributed to six experimental groups (n = 24 per group, three replicates/group): (i) the control group and fish had access to NPs-free water, (ii) NPs-intoxicated group exposed to 5 mg/L NPs in the water according to Pitt *et al*. (2018), (iii) NPs+selenium treated group (5 mg/L+ 1 mg/kg respectively), (iv) NPs+*Chlorella* treated group (5 mg/L+25 g/kg respectively), (v) *Chlorella* group (25 g/kg) according to Bai *et al*. (2001), (vi) selenium group (1mg/kg) according to Durigon *et al*. (2019). Dried *Chlorella* and selenium were both added to the diet.

Animals were kept in aquariums (30 cm length, 60 cm wide, 100 cm high) filled with 60 L of naturally dechlorinated water and aerated continuously with air compressors for 30 days. Throughout the experiment, the water quality (temperature 22°C, dissolved oxygen (6.85 mg/l), pH 7.38, and photoperiod 12:12 light: dark) was maintained. During the experiment, water was changed daily at a rate of around 50%, and PSNPs were added at the concentrations listed above. To minimize ammonia levels in the water, feces and other waste items were siphoned off on a daily basis.

PS NPs detection in GIT of C. gariepinus

PS NPs were detected in the GIT of *C. gariepinus*, the approach provided by Karami *et al*. (2016) was applied in this study.

Collection of blood samples

Six fish from each group (two fish from each replicate, n=24 per group) were caught at random with a hand net and euthanized by ice to reduce stress (Wilson *et al*., 2009). Blood was drawn from the caudal veins with a 1 mL syringe. Blood samples were collected in vacutainer tubes without anticoagulant and centrifuged at 3000 rpm for 15 minutes to obtain serum, which was kept at -20°C for further analysis.

Serum biochemical measurements

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined calorimetrically using specialized kits (Biodiagnostic Co., Giza, Egypt) according to the manufacturer's instructions (Reitman and Frankel, 1957).

Histological preparation

Liver samples were taken from each fish and preserved in a 10% neutral buffered formalin solution. The formalin-fixed samples were dehydrated in ascending concentrations of ethanol, then

cleaned in Xylol before being embedded in paraffin wax for sectioning. Paraffin sections were cut at 4 µm in thickness and were stained with the following histological stains: Ordinary Hematoxylin and Eosin stain for general histological examination (Fischer *et al*., 2008). Periodic Acid Schiff (PAS) approach for demonstration of neutral mucopolysaccharides (McManus 1946). Gomori's trichrome dye for differentiation of connective tissues and muscle fibres (Gomori, 1950). Picrosirius red stain for collagen identification (Bhutda *et al*., 2017).

The paraffin-stained sections were examined under a light microscope (Olympus, USA) by a histopathologist who was blinded to the groups' arrangement. Photos were taken by Olympus DP72 camera adapted into the microscope.

Semithin sections preparation

Two mm thick pieces of liver catfish from all groups were fixed in 5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) for 24 h. The fixed specimens were washed several times in phosphate buffer, then post-fixed in 1% osmium tetraoxide and dehydrated in ascending grades of alcohol and then embedded in epoxy resin. Semithin sections (1 µm) were stained with 1% Toluidine Blue. Semithin stained sections were examined under a light microscope (Olympus, USA) and photos were taken by Olympus DP72 camera adapted into the microscope.

Transmission electron microscopy

Small pieces of liver (2 mm thick) obtained from newly scarified fish were fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.2) for 24 h. The fixed specimens were then washed twice in 0.1 M phosphate buffer and post-fixed in 1% osmium tetroxide in the same buffer. The post-fixed specimens were dehydrated in ascending grades of alcohol and embedded in araldite resin. Semithin sections were cut and stained with 1% toluidine blue. Ultrathin sections were then obtained using a Reichert ultra-microtome, stained with uranyl acetate followed by lead nitrate (Cheville and Stasko, 2014) and examined under a Jeol Jem 1200 EX Transmission Electron Microscope at the Electron Microscope Unit of Assiut University. The obtained images were colored using Adobe Photoshop CS4 (Adobe, USA).

Negative image analysis

Negative image analysis was performed to assess the complex color micrographs that were obtained (Abd-Elkareem, 2017; Abd-Elkareem *et al*., 2020).

Statistical analysis

SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA) was used to conduct statistical analysis. For comparison between the control and exposed groups, one-way analysis of variance (ANOVA) was used, followed by a post-hoc least significant difference (LSD) multiple range test. For all experimental and control animals, all results were reported as mean± SE. The significance level was set at $p < 0.05$.

RESULTS

PS NPs detection

PS NPs was detected in GIT of *C. gariepinus* as shown in Fig. 1.

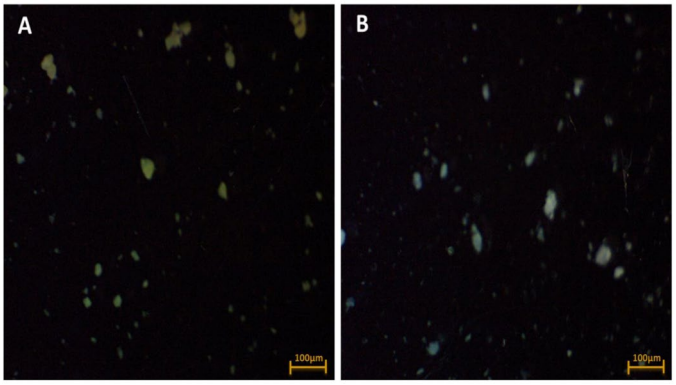

Fig. 1. Bionuclear images of A. Stock of nanoplastics (NPs) which used in experiment B. NPs particles detected in GIT of African catfish (*C. gariepinus*) after exposure.

Serum enzymes

Serum ALT activities were significantly increased in NPs-intoxicated group compared to control, Selenium treated groups ($p < 0.05$) and *Chlorella* treated group ($p < 0.01$) as shown in Table 1.

Serum ALT activities in NPs-selenium and NPs-*Chlorella* treated groups were significantly decreased compared to NPs-intoxicated group ($p < 0.05$ for NPs-selenium and $p < 0.01$ for NPs+*Chlorella*), while were non-significant when compared to control group ($p > 0.05$).

Serum AST levels were significantly increased in NPs-intoxicated group compared to control, Selenium treated and *Chlorella* treated groups ($p < 0.01$) as shown in Table 1.

Serum AST activities in NPs-selenium and NPs-*Chlorella* treated groups were significantly decreased compared to NPs-intoxicated group ($p < 0.01$). while were non-significant when compared to control group ($p > 0.05$).

Histological examination

Histological examination of the liver of control, selenium treated and *Chlorella* treated groups showed the normal histological structures, which consisted of central vein, and hepatocytes that appeared as polyhedral cells containing vacuolated eosinophilic cytoplasm and round vesicular central or eccentric nuclei (Figs. 2A, E, F). The distinct hepatic lobules were not obvious due to the presence of a scanty amount of connective tissue. The portal areas appeared without a well-defined arrangement and were

Table 1. Serum biochemical measurements of *C. gariepinus* after exposure to Polystyrene nanoplastics (5 mg/L) and treatment with selenium (1 mg/kg) and *Chlorella vulgaris* (25 g/kg) for 30 days.

Parameters	Control	NPs	NPs+Se	NPs+Ch	Ch	Se
ALT (U/L)	22.75± 2.46b	30.5± 1.94a	24.0± 1.83b	22.5± 1.85 b	19.75± 1.11b	20.75± 2.63b
AST (U/L)	18.0±0.91bc	27.25± 1.65a	21.25± 1.49b	17.75± 1.31bc	15.0± 1.15d	17.25± 1.93bc

Data are presented as the mean ± standard error. Significant differences between groups are indicated by different letters. $P < 0.05$.
NPs: Nanoplastics, NPs+Se: Nanoplastics with selenium, NPs+Ch: Nanoplastics with *Chlorella*, Ch: *Chlorella*, Se: Selenium, ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

scattered in hepatic tissue containing branches of the hepatic artery, portal veins and bile ductules and surrounded with a small amount of connective tissue (Figs. 3A, 4A, 5A, 5B).

Histopathological examination of H&E stained hepatic tissues of NPs-intoxicated group exhibited the hepatotoxic effects of NPs that appeared in form of focal fatty degeneration and change (micro and macrovesciular steatosis). Microvesicular steatosis was characterized by the presence of small-sized clear intracytoplasmic lipid droplets in the hepatocytes. Macrovesicular steatosis was characterized by the presence of large-sized intracytoplasmic lipid vacuoles that occupy most of the hepatocytes and push the nuclei to the peripheral side of the cell. Hepatic cells showed also coagulative necrosis with nuclear changes. The nuclear changes included pyknosis with nuclear shrinkage, karyorrhexis with nuclear fragmentation, karyolysis with loss of chromatin and absence of nuclei (Fig. 2B).

Disorganization of the hepatic cord was also observed. Dilatation and engorgement of central veins with blood (Fig. 3B). Proliferation of connective tissue around the central veins (pericentral fibrosis), portal veins and branches of bile ductules in the

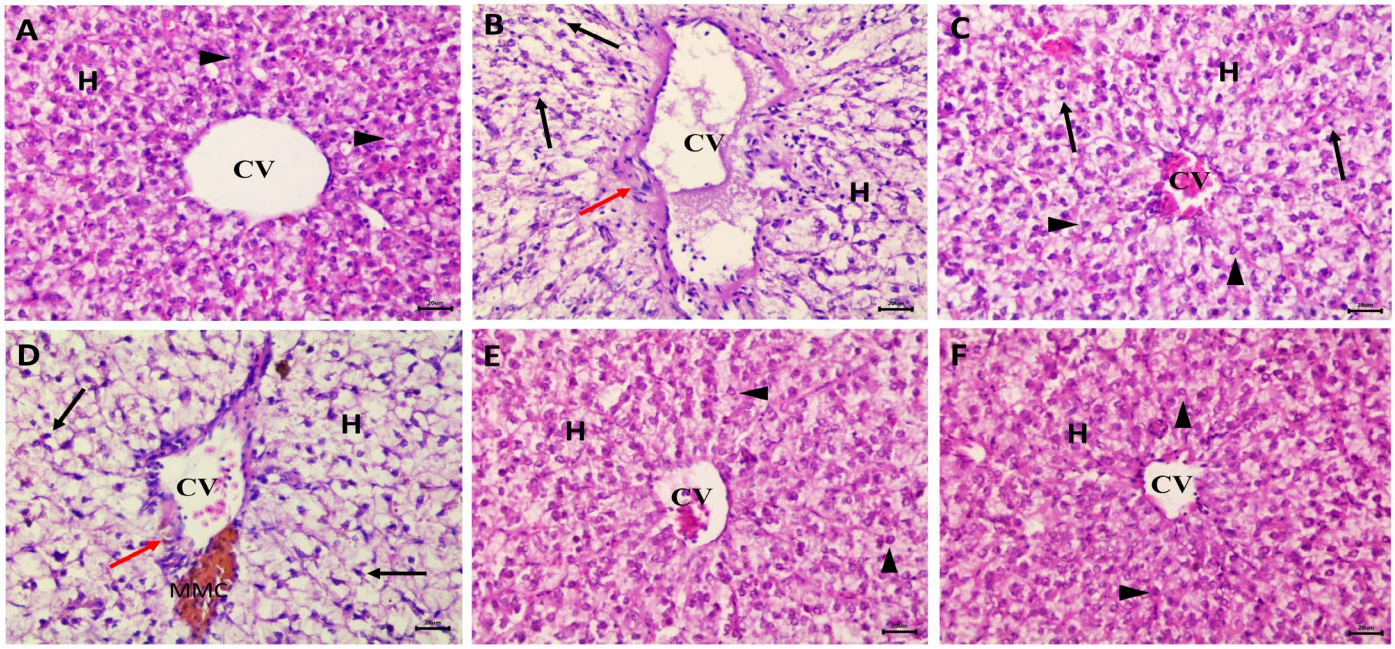

Fig. 2. Photomicrographs of paraffin sections in the liver of *C. gariepinus* exposed to NPs and NPs with *Chlorella* and selenium: Liver sections of control (A), selenium (E) and *Chlorella* (F) treated catfish showed normal histological structures that consisted of central vein (CV) and hepatocytes (H) that contain vacuolated eosinophilic cytoplasm and round central or eccentric nuclei (arrowhead). B. Liver section of NPs-intoxicated catfish showing hepatocytes with fatty change and cellular necrosis with peripherally small located pyknotic nuclei (black arrow) and proliferation of connective tissue around the central vein (pericentral fibrosis) (red arrow). C. Liver section of NPs+*Chlorella* treated catfish showed hepatocytes contained vacuolated eosinophilic cytoplasm with less fat vacuoles, normal round central and peripherally located nuclei (arrowhead), some necrotic hepatocytes with pyknotic nuclei also present (black arrow). D. Liver section of NPs+selenium treated catfish showed some hepatocytes with fatty change and cellular necrosis with small pyknotic nuclei (black arrow), pericentral fibrosis and inflammatory cells infiltration also found (red arrow). Hematoxylin and Eosin stain, scale bar = 20 µm.

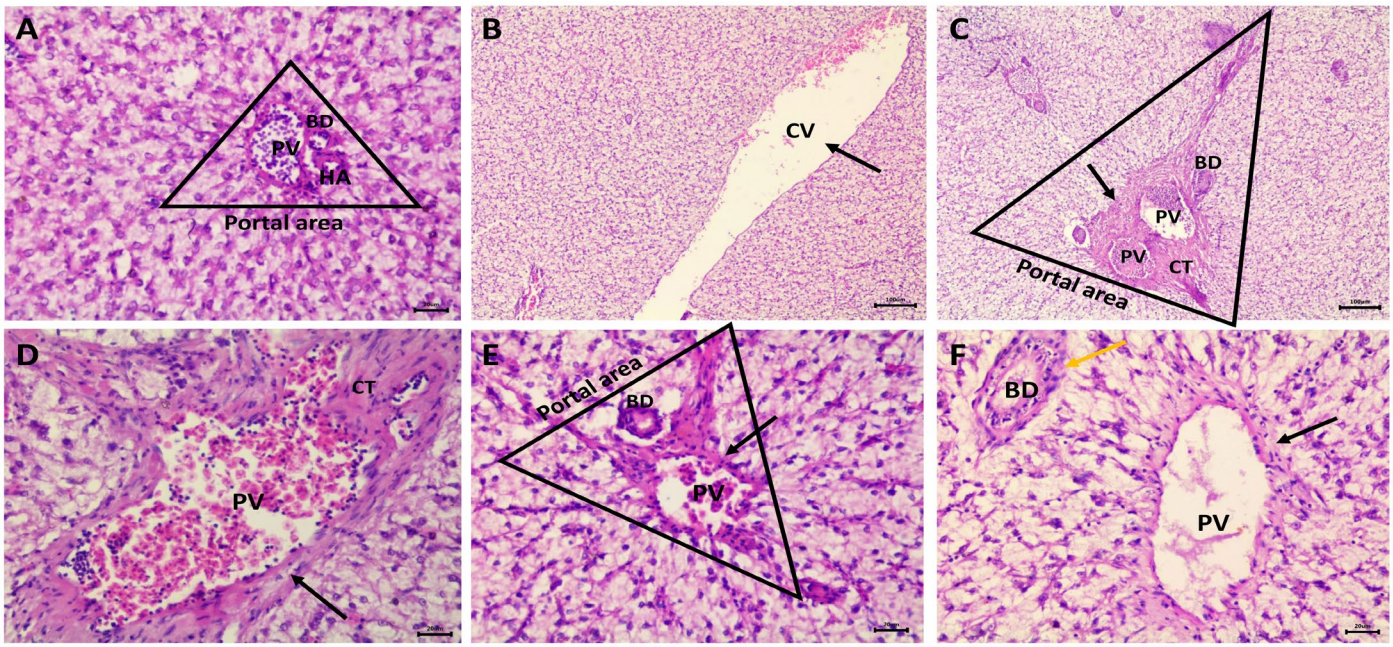

Fig. 3. Photomicrographs of paraffin sections in the liver of *C. gariepinus* exposed to NPs and NPs with *Chlorella* and selenium: A. Liver section of control catfish showed the portal area that appeared as a triangular area contained a branch of the portal vein (PV), hepatic artery (HA) and bile ductule (BD). B, C, D. Liver sections of NPs-intoxicated catfish showed in B dilatation of central vein with blood (CV), in C proliferation of connective tissue (CT) around the triangular portal area (periportal fibrosis) that extend into the hepatic parenchyma (arrow), in D higher magnification of portal area showed an increase in the amount of connective tissue (CT) around the portal area (arrow). E. Liver section of NPs+*Chlorella* treated catfish showed less amount of connective tissue surrounding the portal area and bile ductules and portal vein appeared normal (arrow). F. Liver section of NPs+selenium treated catfish showed connective tissue proliferation surrounding branches of portal veins (black arrow) and bile ductile (yellow arrow). Hematoxylin and Eosin stain, scale bar = 100 µm in B, C and = 20 µm in A, D, E, F.

portal areas (periportal fibrosis) were seen in H&E stained sections (Fig. 3C, D). Pericentral and periportal fibrosis were confirmed with Picrosirius red (Figs. 4B, C, D) and Gomori's trichrome stains (Fig. 5C, D) compared to the scanty amount of connective tissue that appeared around the central veins and portal areas in the liver of control group (Figs. 4A, 5A, 5B).

PAS stained sections showed areas of glycogen depletion and a decrease of PAS-positive glycogen granules in the hepatocytes in NPs-intoxicated group (Fig. 6B) compared to control, selenium treated and *Chlorella* treated groups that exhibited the presence of more PAS-positive pink glycogen granules in the cytoplasm (Figs. 6A, E, F).

Histological examination of H&E stained hepatic tissues from NPs+*Chlorella* treated group showed moderately improving and restoring the normal histological structures of hepatic tissues. The cytoplasm of hepatocytes appeared eosinophilic stained and most of the hepatocytes contained round vesicular centrally located nuclei (Fig. 2C). Histological sections stained with H&E, Picrosirius red and Gomori's trichrome stains demonstrated the decrease of the amount of connective tissue around the central

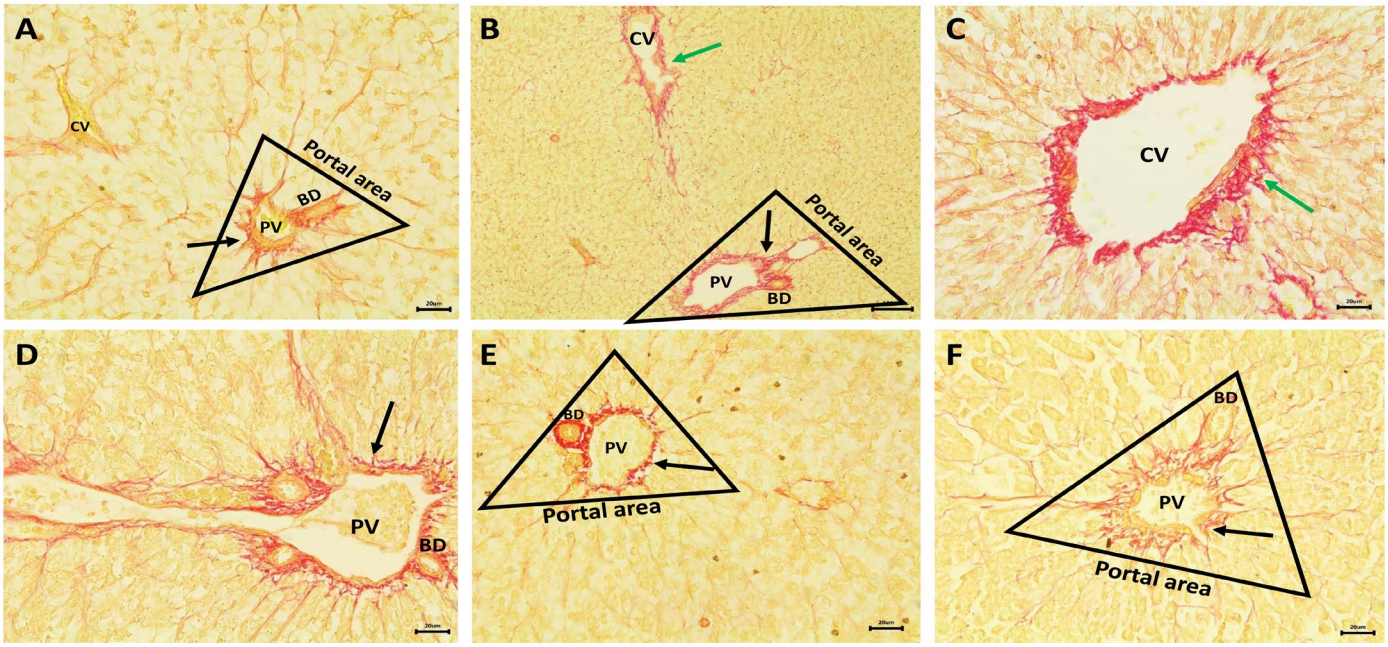

Fig. 4. Photomicrographs of paraffin sections stained with Picrosirius red in the liver of *C. gariepinus* exposed to NPs and NPs with *Chlorella* and selenium: A. Liver section of control catfish showed a scanty amount of connective tissue around the blood vessels and bile ductules in the portal area and central vein (arrow). B, C, D. Liver sections of NPs-intoxicated catfish showed an increase in the amount of connective tissue around the central veins (green arrow) and around the blood vessels and bile ductules in the portal areas (black arrow) and the connective tissue extended between the hepatic cords. E. Liver section of NPs+*Chlorella* treated catfish showed a decline in the amount of connective tissue around the branches of portal veins and bile ductules in the portal area (arrow). F. Liver section of NPs+selenium treated catfish showed a moderate amount of connective tissue surrounding the branches of portal veins and bile ductules (arrow). Scale bar = 100 µm in B, and = 20 µm in A, C, D, E, F.

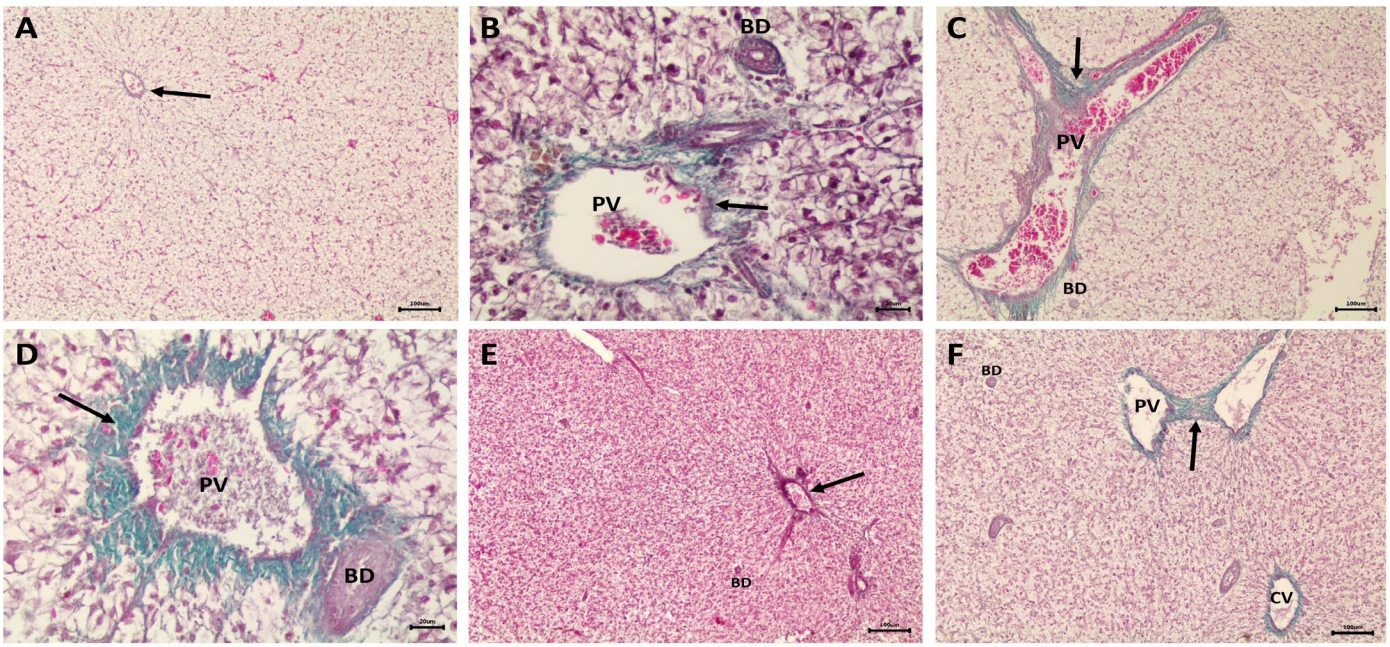

Fig. 5. Photomicrographs of paraffin sections stained with Gomori's trichrome in the liver of *C. gariepinus* exposed to NPs and NPs with *Chlorella* and selenium: A, B. Liver sections of control catfish showed a scanty amount of connective tissue around the central vein (arrow) in A and around the portal area (arrow) in B. C, D. Liver sections of NPs-intoxicated catfish showed dilatation of portal veins and an increase in the amount of connective tissue around the branches of portal veins and bile ductules (arrow). E. Liver section of NPs-*Chlorella* treated catfish showed a scanty amount of connective tissue around central veins and the branches of portal veins and bile ductules in the portal area (arrow). F. Liver section of NPs-Selenium treated catfish showed a moderate amount of connective tissue around central veins and portal veins. Scale bar = 100 µm in A, C, E, F and = 20 µm in B, D.

veins and in the portal areas around the portal veins and branches of bile ductile (Figs. 3E, 4E, 5E). PAS stained sections showed the presence of more PAS-positive pink glycogen granules in the cytoplasm of hepatocytes (Fig. 6C).

Histological examination of H&E stained hepatic tissues from NPs+selenium treated group showed moderate improvement in the histological appearance of hepatic tissue. Fatty degeneration and changes (Micro and macrovesicular steatosis) with peripherally located nuclei, coagulative necrosis with nuclear alterations were still present (Fig. 2D). A moderate amount of connective tissue was around the central veins (pericentral fibrosis) and in the portal areas around the portal vein and branches of bile ductules (periportal fibrosis) (Fig. 3F) and this was confirmed with Picrosirius red (Fig. 4F) and Gomori's trichrome stains (Fig. 5F). PAS stained sections showed fewer PAS-positive pink glycogen granules in the cytoplasm of hepatocytes (Fig. 6D). Lesions scoring was made to compare the pathological lesions between groups and to show the improvement in treated groups after *Chlorella* and selenium administration (Table 2).

Semithin sections examination

Semithin sections of liver of control, selenium treated and *Chlorella* treated groups demonstrated the normal structures of the liver that consisted of dark stained fine granular cytoplasm and round central or eccentric vesicular nuclei containing one or two nucleoli (Fig. 7A, E, F). Semithin sections of liver of NPs-intoxicated group showed most of the hepatocytes exhibited necrotic changes in form of pale stained cytoplasm, large lipid vacuolation and uncleared cell bounders. The nuclear alterations were involved pyknosis and karyorrhexis, karyolysis and complete loss of nuclei in most of the affected hepatocytes (Fig. 7B). NPs+*Chlorella* treated group showed most of the hepatocytes appeared normal with darkly stained cytoplasm and round central and peripherally located nuclei (Fig. 7C). NPs+selenium treated group showed some hepatocytes with darkly stained cytoplasm, and central and peripherally located nuclei, and some necrotic cells with faintly stained cytoplasm and with pyknotic or loss of nuclei (Fig. 7D).

Transmission electron microscopy

Transmission electron microscopy of the liver of *C. gariepinus* showed that the hepatocyte of the control group had euchromatic nucleus with distinct nucleolus, well-developed rough endoplasmic reticulum, well-developed smooth endoplasmic reticulum, abundant mitochondria and many lysosomes (Fig. 8A). Whereas the hepatocyte of the NPs-intoxicated group showed some cytotoxicity; nucleus had increasing amount of heterochromatin peripherally and around the nucleolus, degenerated rough

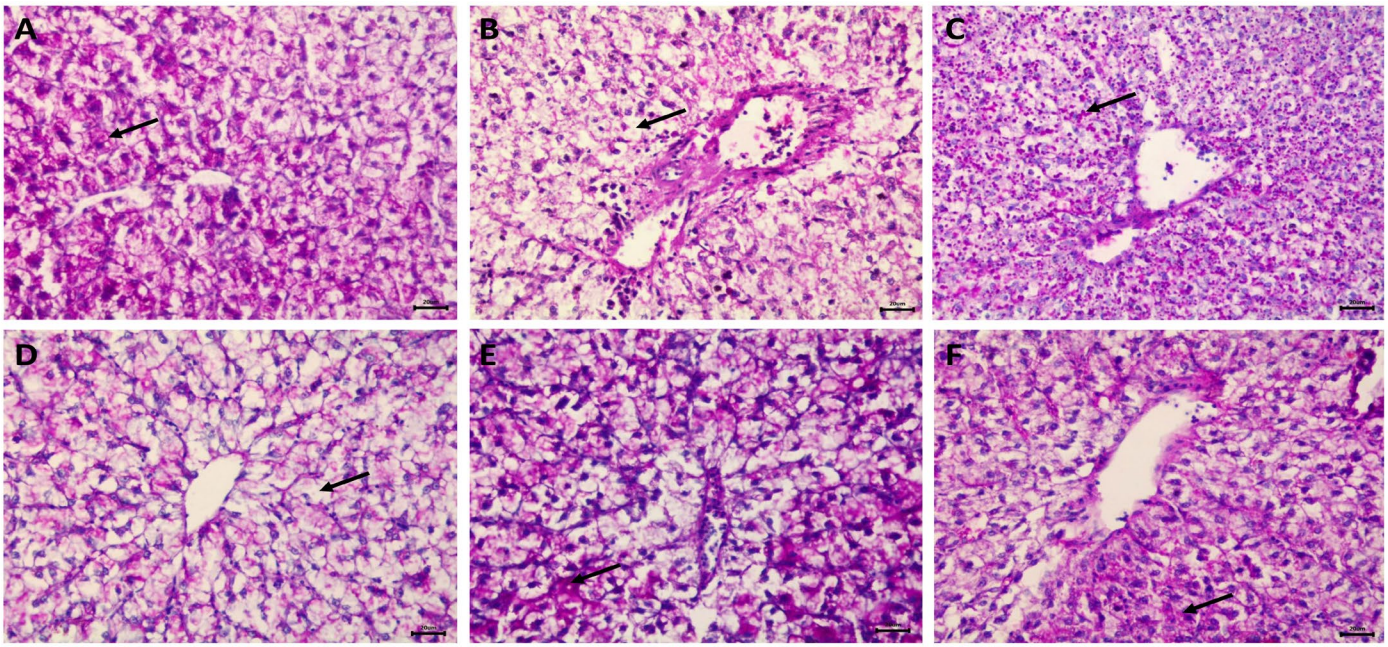

Fig. 6. Photomicrographs of paraffin sections stained with Periodic acid Schiff reagent (PAS) in the liver of *C. gariepinus* exposed to NPs and NPs with *Chlorella* and selenium: A, E, F. Liver sections of control, selenium and *Chlorella* treated catfish showing an increased amount of PAS-positive glycogen in the cytoplasm of hepatocytes (arrow). B. Liver section of NPs-intoxicated catfish showed depletion of PAS-positive glycogen granules in the cytoplasm of hepatocytes that appeared with macrovesicular steatosis (fatty change) with necrotic nuclear changes (arrow). C. Liver section of NPs-*Chlorella* treated catfish showed the presence of many fine PAS-positive glycogen granules in the cytoplasm of hepatocytes (arrow). D. Liver section of NPs-Selenium treated catfish showed less fine PAS-positive glycogen granules in the cytoplasm of hepatocytes (arrow). Scale bar = 20 µm.

Table 2. Lesion scoring of the pathological changes in the different groups.

Hepatic lesions	Control	NPs	NPs+Ch	NPs+Se	Ch	Se
Fatty degeneration and change	-	+++	+	++	-	-
Congestion of central and portal vines	-	+++	+	+	-	-
Pericentral fibrosis	-	+++	+	+	-	-
Periportal Fibrosis	-	+++	+	++	-	-
Depletion of intrahepatic glycogen granules	-	+++	+	++	-	-
Degenerative changes of the ultrastructure of hepatocytes	-	+++	+	+	-	-

NPs: Nanoplastics; NPs+Se: Nanoplastics with selenium; NPs+Ch: Nanoplastics with *Chlorella*; Ch: *Chlorella*; Se: Selenium.

endoplasmic reticulum, degenerated smooth endoplasmic reticulum, degenerated mitochondria, many lipid droplets of different sizes, lysosomal activity, and myelin figure (Fig. 8B). While the hepatocyte of the NPs-*Chlorella* treated group showed euchromatic nucleus with distinct nucleolus, nearly healthy rough endoplasmic reticulum, smooth endoplasmic reticulum, mitochondria and phagocytic vacuole. Phagocytic kupffer cell could be demonstrated in the wall of the hepatic sinusoid (Fig. 8C). Hepatocyte

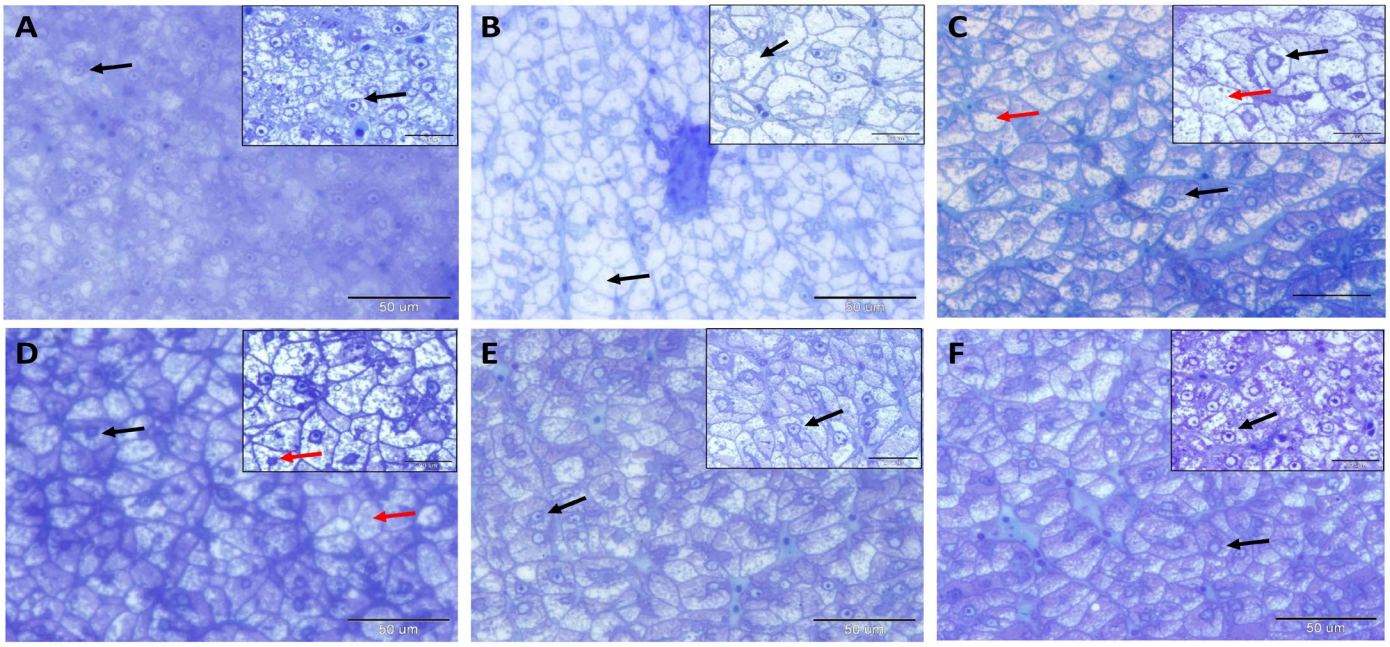

Fig. 7. Photomicrographs of paraffin sections stained with Toluidine blue in the liver of *C. gariepinus* exposed to NPs and NPs with *Chlorella* and selenium: A, E, F. Semithin sections of control, selenium and *Chlorella* treated catfish showed normal hepatic structures dark stained fine granular cytoplasm and round central or eccentric vesicular nuclei contain one or two nucleoli (arrow). B. Semithin section of NPs-intoxicated catfish showed necrotic hepatocytes with faint stained vacuolated cytoplasm with peripherally small pyknotic, fragmented and in most cells loss of nuclei (arrow) C. Semithin section of NPs-*Chlorella* treated catfish showed most of the hepatocytes appeared normal with darkly stained cytoplasm and round central and peripherally located nuclei (black arrow), few necrotic hepatocytes with fainted cytoplasm and without nuclei also present (red arrow). D. Semithin section of NPs-Selenium treated catfish showed some hepatocytes with darkly stained cytoplasm, and central and peripherally located nuclei (black arrow), and some necrotic cells with faintly stained cytoplasm and with pyknotic or loss of nuclei (red arrow). Scale bar = 50 µm in A-F, small insets scale bar = 20 µm.

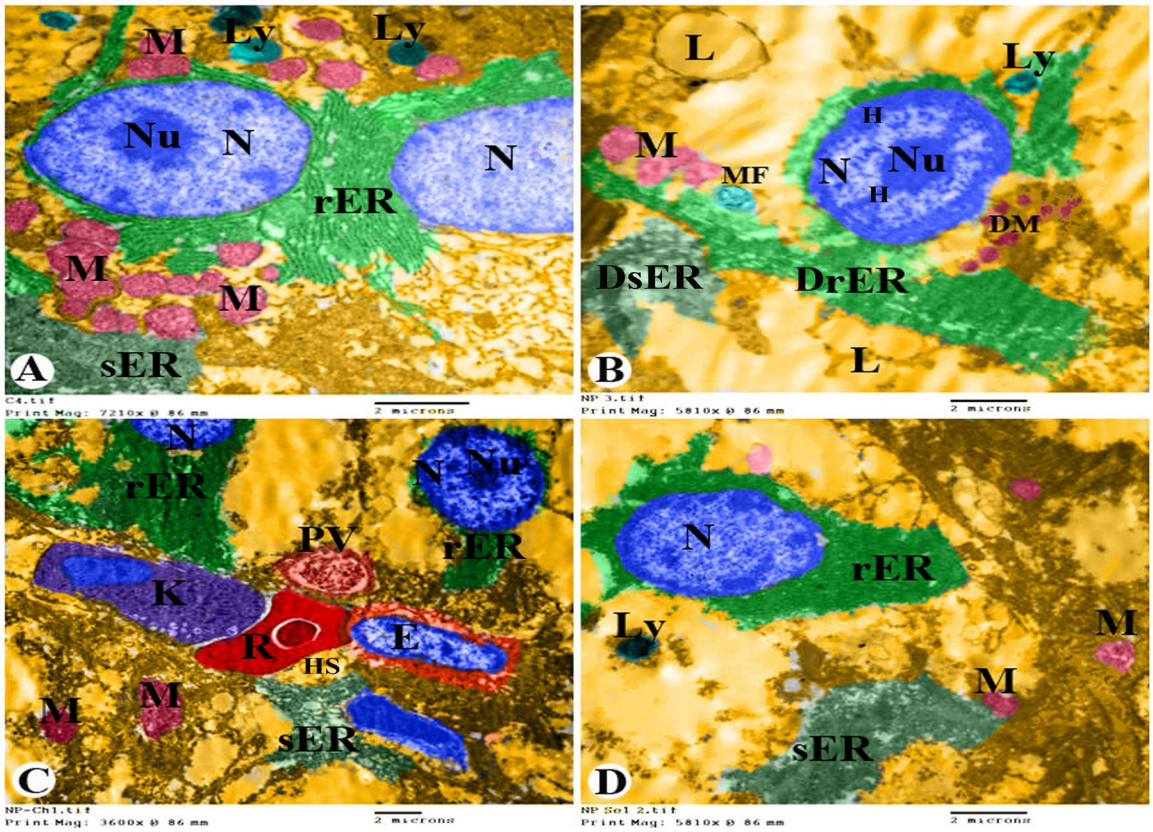

Fig. 8. Colored transmission electron photomicrographs of sections of the liver of *C. gariepinus* in different treatment groups, showing the ultrastructure of the hepatocytes. A: Hepatocyte of the control group showing euchromatic nucleus (N) with distinct nucleolus (Nu), well-developed rough endoplasmic reticulum (rER), well-developed smooth endoplasmic reticulum (sER), abundant mitochondria (M) and many lysosomes (Ly). B: Hepatocyte of the NPs-intoxicated group showing nucleus (N) with increasing amount of heterochromatin (H) peripherally and around the nucleolus (Nu), mitochondria (M), degenerated rough endoplasmic reticulum (DrER), degenerated smooth endoplasmic reticulum (DsER), degenerated mitochondria (DM) and many lipid droplets of different sizes (L), lysosomes (Ly) and myelin figure (MF). C: Hepatocyte of the NPs-*Chlorella* treated group showing euchromatic nucleus (N) with distinct nucleolus (Nu), nearly healthy rough endoplasmic reticulum (rER), smooth endoplasmic reticulum (sER), mitochondria (M) and phagocytic vacuole (PV). Note the hepatic sinusoid contain red blood corpuscle (R) and formed of kupffer cell (K) and endothelial cells (E). D: Hepatocyte of the NPs-selenium treated group showing euchromatic nucleus (N), nearly healthy rough endoplasmic reticulum (rER), smooth endoplasmic reticulum (sER), mitochondria (M) and lysosomes (Ly).

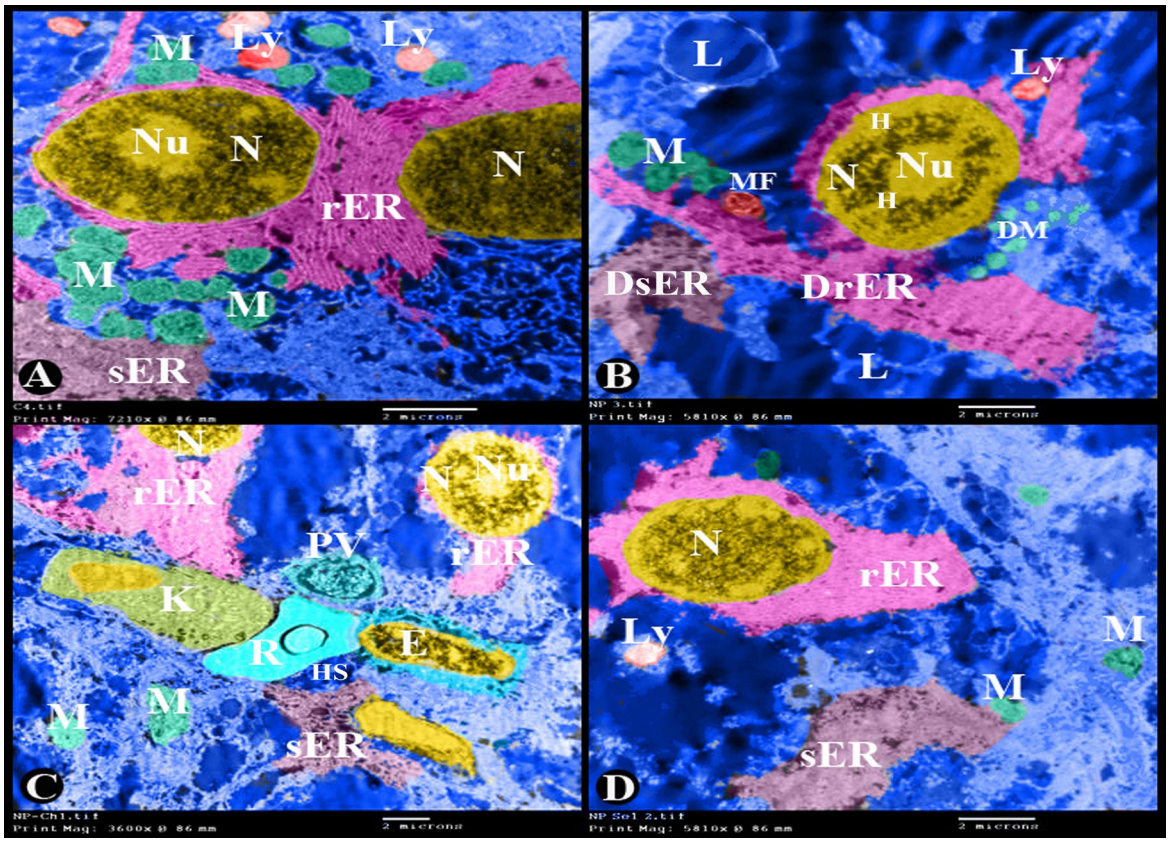

Fig. 9. Negative images of the photomicrographs shown in Figure 8.

of the NPs-selenium treated group showed euchromatic nucleus, nearly healthy rough endoplasmic reticulum, smooth endoplasmic reticulum, mitochondria and lysosomes (Fig. 8D).

DISCUSSION

Nowadays, NPs pollution is an important matter due to its direct impact on environmental health and public safety. The continuous discharge of microplastics (MPs) and nanoplastics wastes into the rivers and other water sources, and their environmental accumulation attracted more intention to study their toxicological effect on people and other Mamalis especially aquatic inhabitants (Lau *et al*., 2020).

ALT and AST activities in fish serum are used as markers of their hepatic functioning (Abdel-Latif *et al*., 2020). These enzymes were mostly found in the hepatocyte's cytoplasm, but when the liver was damaged, they were released into the bloodstream (Amacher 1998). In this study, after exposure to NPs, the activities of serum AST and ALT significantly elevated compared with control referring to liver damage. The obtained findings agree with those found in Wistar rats exposed to pristine polystyrene nanoplastics (Amereh *et al*., 2019).

In marine fish large yellow croaker, NPs supplementation significantly raised plasma ALT and AST activities as compared to the control group (Lai *et al*., 2021). After exposure to MPs, *C. gariepinus* showed a significant elevation in AST and ALT as reported by Sayed *et al*. (2021). The common goby P. microps demonstrated a considerable raising in AST and ALT activities after exposure to MPs (Oliveira *et al*., 2013; Dos santos Norberto, 2014). Increased serum ALT and AST activities were also observed in common carp (Cyprinus carpio) subjected to sublethal microplastics concentrations (Haghi and Banaee, 2017).

In the present study, the activities of ALT and AST in Catfish exposed to PS-NPs and fed OS-supplemented diets were found to be significantly lower compared to PS-NPs exposed group, and there were no significant changes in comparison to the control group. In a study by Naiel *et al*. (2021), the authors discovered a significant drop in Nile tilapia ALT and AST levels fed on selenium diet. Furthermore, serum ALT and AST values were significantly higher in African catfish fed on high level of OS-supplemented diets (0.5 g Se/kg), (Abdel-Tawwab *et al*., 2007), which may be related to high concentration of supplemented Selenium.

Results from the current study, demonstrated that, ALT, AST activities were significantly lowered ($P < 0.01$) in group exposed to PS-NPs and treated with *Chlorella* supplemented diets, these results are harmonious with Zahran *et al*. (2019) who found a significant reduction in serum ALT and AST activities in Nile tilapia (Oreochromis niloticus) supplemented with *Chlorella vulgaris* against the chronic toxicity in liver induced by sodium arsenite. Moreover, Sayed *et al*. (2021) found an improvement in the AST activity in *C. gariepinus* exposed to MPs and *Chlorella vulgaris*.

Histological findings of H&E stained hepatic tissues of NPs-intoxicated catfish demonstrated the hepatotoxic effects of NPs that consisted of fatty degeneration and change (micro and macrovesciular steatosis) of heptocytes. Other Hepatic cells showed coagulative necrosis with nuclear changes. Disorganization of the hepatic cord was also observed (Karami *et al*., 2016). Pericentral fibrosis and periportal fibrosis were also detected and an increased amount of connective tissue around the biliary ductiles and portal veins were also seen. These connective tissue proliferations were confirmed by the specific stains Picrosirius red and Gomori's trichrome. PAS-stained paraffin liver sections showed a depletion and a decrease of PAS-positive glycogen granules in the affected liver cells as a response of nanoplastics intoxication.

Administration of *Chlorella* to the diet of NPs+*Chlorella* treated catfish moderately improved the general state of catfish, the liver function tests and moderately restored the normal histological structures of hepatic tissues with a decrease in the amount of connective tissue around veins and more PAS-positive pink glycogen granules in the cytoplasm of hepatocytes. These findings were in accordance with the results obtained by Zahran *et al*., (2019) who found that supplementation of *Chlorella vulgaris* in the diet ameliorate the long-term liver damage induced by sodium arsenite in *Nile tilapia* (*O. niloticus*) as shown by morphological, biochemical and immunological gene expression analysis.

Administration of selenium to the diet of NPs+selenium treated catfish also showed a moderate improvement in the general

state of catfish, the liver function tests. The histological findings of liver tissue were moderately improved with still presence of fatty degeneration and changes, moderate amount of connective tissue was found around the veins and less PAS-positive glycogen granules were detected in the cytoplasm of hepatocytes. These findings agreed with Kothari and Choughule (2014).

The obtained results revealed that the hepatocyte of the NPs-intoxicated group showed some cytotoxicity as; an increasing amount of heterochromatin inside the nucleus, degenerated rough endoplasmic reticulum, degenerated smooth endoplasmic reticulum, degenerated mitochondria, many lipid droplets of different sizes, lysosomal activity, and myelin figure. NPs enhance oxidative stress and induced liver lipid deposition by inhibiting lipolysis, (Lai *et al*., 2021; Li *et al*., 2021). The distribution and the generation of reactive oxygen species may be the plausible mechanisms of these degenerated cytotoxic effects of NPs. In addition, NPs induced some inflammatory and adverse immunotoxicity effects in the liver (Cheng *et al*., 2022).

The present work demonstrated that the hepatocyte of the NPs+*Chlorella* treated group showed euchromatic nucleus with a distinct nucleolus, nearly healthy rough endoplasmic reticulum, smooth endoplasmic reticulum, mitochondria and phagocytic vacuole. This indicated that *Chlorella* had antioxidant and hepatoprotective effects (Wu, 2020; Latif *et al*., 2021). The hepatic sinusoid was lined by active endothelial cells and phagocytic Kupffer cells. It was noticed that the Kupffer cells were responsible for the endocytosis of most foreign material from the circulation (Ferri and Sesso, 1981). While scavenger endothelial cells were geared to endocytosis of soluble macromolecules (Seternes *et al*., 2021). Whereas hepatocyte of the NPs+selenium treated group showed euchromatic nucleus, nearly healthy rough endoplasmic reticulum, smooth endoplasmic reticulum, mitochondria and lysosomes this due to the antioxidant properties of selenium (Ge *et al*., 2021).

CONCLUSION

Supplementation of *Chlorella vulgaris* and selenium to the diets might be an effective approach for alleviating the toxic effects induced by nanoplastics toxicity and minimizes NPs-induced hepatic cytotoxic damages.

CONFLICT OF INTEREST

The authors have declared that no competing interest exist.

REFERENCES

Abd-Elkareem, M., 2017. Cell-specific immuno-localization of progesterone receptor alpha in the rabbit ovary during pregnancy and after parturition. Anim. Reprod. Sci. 180, 100-120.

Abd-Elkareem, M., AbouKhalil, N.S., Sayed, A.E.D.H., 2020. Cytoprotective effect of *Nigella sativa* seed on 4-nonylphenol-induced renal damage in the African catfish (*Clarias gariepinus*). Chemosphere 259, 127379.

Abdel-Tawwab, M., Mousa, M.A.A., Abbass, F.E., 2007. Growth performance and physiological response of African catfish, *Clarias gariepinus* (B.) fed organic selenium prior to the exposure to environmental copper toxicity. Aquaculture 272, 335–345.

Abdel-Latif, H.M.R., Abdel-Tawwab, M., Khafaga, A.F., Dawood, M.A.O., 2020. Dietary oregano essential oil improved the growth performance via enhancing the intestinal morphometry and hepato-renal functions of common carp (*Cyprinus carpio* L.) fingerlings. Aquaculture 526, 735432.

Amacher, D.E., 1998. Serum transaminase elevations as indicators of liver injury following the administration of drugs. Regul. Toxicol. Pharm. 27, 119-130.

Amereh, F., Eslami, A., Fazelipour, S., Rafiee, M., Zibaii, M.I., Babaei, M., 2019. Thyroid endocrine status and biochemical stress responses in adult male Wistar rats chronically exposed to pristine polystyrene nanoplastics. Toxicol. Res. 8, 953–963.

Bai, S., Koo, J., Kim, K., Kim, S. 2001. Effects of *Chlorella* powder as a feed additive on growth performance in juvenile Korean rockfish, *Sebastes schlegelii* (Hilgendorf). Aquaculture Research 32, pp. 92-98.

Berghe, G.V.D., 1991. The role of the liver in metabolic homeostasis: implications for inborn errors of metabolism. J. Inherit. Metab. Dis. 14, 407–420.

Bhutda, S., Surve, MV., Anil, A., Kamath, K., Singh, N., Modi, D., Banerjee, A., 2017. Histochemical Staining of Collagen and Identification of Its Subtypes by Picrosirius Red Dye in Mouse Reproductive Tissues. Bio-Protocol 7, e2592-e2592.

Brandts, I., Teles, M., Tvarijonaviciute, A., Pereira, M.L., Martins, M.A., Tort, L., Oliveira, M., 2018. Effects of polymethylmethacrylate nanoplastics on Dicentrarchus labrax. Genomics 110, 435–441.

Cheng, H., Duan, Z., Wu, Y., Wang, Y., Zhang, Haihong, Shi, Y., Zhang, Huajing, Wei, Y., Sun, H., 2022. Immunotoxicity responses to polystyrene nanoplastics and their related mechanisms in the liver of zebrafish (*Danio rerio*) larvae. Environ. Int. 161, 107128.

Cheville, N.F., Stasko, J., 2014. Techniques in Electron Microscopy of Animal Tissue. Vet. Pathol. 51, 28–41.

Daniel, N., Sivaramakrishnan, T., Saravanan, K., Shalini, B., Arunjyoti, B., Sankar, R., Dann Roy, S., 2016. A review on microalgae as potential fish feed ingredient. J. Andeman Sci. Assoc. 140-144.

Dos Santos Norberto, R.S., 2014. Toxic effects of nickel alone and in combination with microplastics on early juveniles of the common goby (*Pomatoschistus microps*). Master's Thesis, School of Medicine and Biomedical Sciecnes, Buffalo, NY, USA.

Durigon, E.G., Kunz, D.F., Peixoto, N.C., Uczay, J., Lazzari, R., 2019. Diet selenium improves the antioxidant defense system of juveniles Nile tilapia (*Oreochromis niloticus* L.). Braz. J. Biol. 79, 527–532.

Ferri, S., Sesso, A., 1981. Ultrastructural study of Kupffer cells in teleost liver under normal and experimental conditions. Cell Tissue Res. 220, 387-391.

Fischer, AH., Jacobson, KA., Rose, J., Zeller, R., 2008. Hematoxylin and Eosin Staining of Tissue and Cell Sections. Cold Spring Harbor. Protocol, pdb. prot4986.

Ge, J., Liu, L.L., Cui, Z.G., Talukder, M., Lv, M.W., Li, J.Y., Li, J.L., 2021. Comparative study on protective effect of different selenium sources against cadmium-induced nephrotoxicity via regulating the transcriptions of selenoproteome. Ecotoxicol. Environ. Saf. 215, 112135.

Gomori, G., 1950. A rapid one-step trichrome stain. Am. J. Clin. Pathol. 20, 661-664.

Haghi, B.N., Banaee, M., 2017. Effects of micro-plastic particles on paraquat toxicity to common carp (*Cyprinus carpio*): Biochemical changes. Int. J. Environ. Sci. Technol. 14, 521-530.

Holmes, L.A., Turner, A., Thompson, R.C., 2012 Adsorption of trace metals to plastic resin pellets in the marine environment. Environ. Pollut. 160, 42-48.

Hsin-yi, P., Yu-chan, C., Shu-ju, C., Su-tze, C., 2009. Hepatoprotection of *Chlorella* against carbon tetrachloride-induced oxidative damage in rats. *In vitro* 23, 747-754.

Iheanacho, S.C., Ogueji, E., Igberi, C., Avwemoya, F., Amadi-Eke, A., Yaji, A., Mbah, C., 2019. Suitability of discarded Cashewnut (*Anacardium occidentale*) meal as replacement of soybean meal (Glycine max) in the diet of juvenile African catfish *Clarias gariepinus* (Burchell, 1822). Indian J. Fish. 66, 78-86.

Ilham, I., Hapsari, F., Fotedar, R., 2018. Growth, enzymatic glutathione peroxidase activity and biochemical status of juvenile barramundi (*Lates calcarifer*) fed dietary fermented lupin meal supplemented with organic selenium. Aquacult. Res. 49, 151-164.

Jiang, Z.Y., Shan, K.Z., Song, J., Liu, J.B., Rajendran, S., Pugazhendhi, A., Jacob, J.A., Chen, B., 2019. Toxic effects of magnetic nanoparticles on normal cells and organs. Life Sci. 220, 156-161.

Karami, A., Teh, S.J., Zakaria, M.P., Courtenay, S.C., 2015. Ploidy-, gender-,and dose-dependent alteration of selected biomarkers in *Clarias gariepinus* treated with benzo[a]pyrene. J. Environ.Sci. 38, 95-102.

Karami, A., Romano, N., Galloway, T., Hamzah, H. 2016. Virgin microplastics cause toxicity and modulate the impacts of phenanthrene on biomarker responses in African catfish (*Clarias gariepinus*). Environ. Res. 151, 58-70.

Kihara, S., Köper, I., Mata, J.P., McGillivray, D.J., 2021. Reviewing nanoplastic toxicology: It's an interface problem. Adv. Colloid. Interface 288, 102337.

Bhattacharya, P., Lin, S., Turner, J.P., Ke, P.C., 2010 Physical adsorption of charged plastic nanoparticles affects algal photosynthesis. J. Phys. Chem. C. 114, 16556-16561.

Kothari, S., Choughule, N., 2015. Ameliorative stroke of selenium against toxicological effects of mercuric chloride in liver of freshwater catfish Heteropneustes fossilis (Bloch). Environ. Toxicol. 30, 927-936.

Küçükbay, F.Z., Yazlak, H., Karaca, I., Sahin, N., Tuzcu, M., Cakmak, M.N.,

Sahin, K., 2009. The effects of dietary organic or inorganic selenium in rainbow trout (*Oncorhynchus mykiss*) under crowding conditions. Aquacult. Nutr. 15, 569-576.

Lai, W., Xu, D., Li, J., Wang, Z., Ding, Y., Wang, X., Li, X., Xu, N., Mai, K., Ai, Q., 2021. Dietary polystyrene nanoplastics exposure alters liver lipid metabolism and muscle nutritional quality in carnivorous marine fish large yellow croaker (*Larimichthys crocea*). J. Hazard. Mater. 419, 126454.

Latif, A.A.E., Assar, D.H., Elkaw, E.M., Hamza, H.A., Alkhalifah, D.H.M., Hozzein, W.N., Hamouda, R.A., 2021. Protective role of *Chlorella vulgaris* with Thiamine against Paracetamol induced toxic effects on haematological, biochemical, oxidative stress parameters and histopathological changes in Wistar rats. Sci. Rep. 11, 3911.

Lau, W.W.Y., Shiran, Y., Bailey, R.M., Cook, E., Stuchtey, M.R., Koskella, J., Velis, C.A., Godfrey, L., Boucher, J., Murphy, M.B., Thompson, R.C., Jankowska, E., Castillo Castillo, A., Pilditch, T.D., Dixon, B., Koerselman, L., Kosior, E., Favoino, E., Gutberlet, J., Baulch, S., Atreya, M.E., Fischer, D., He, K.K., Petit, M.M., Sumaila, U.R., Neil, E., Bernhofen, M.V., Lawrence, K., Palardy, J.E., 2020. Evaluating scenarios toward zero plastic pollution. Science 369, 1455-1461.

Lemly, A.D., 1993. Metabolic stress during winter increases the toxicity of selenium to fish. Aquat. Toxicol. 27, 133-158.

Li, L., Gu, H., Chang, X., Huang, W., Sokolova, I.M., Wei, S., Sun, L., Li, S., Wang, X., Hu, M., Zeng, J., Wang, Y., 2021. Oxidative stress induced by nanoplastics in the liver of juvenile large yellow croaker *Larimichthys crocea.* Mar. Pollut. Bull. 170, 112661.

Lu, Y.F., Zhang, Y., Deng, Y.F., Jiang, W., Zhao, Y.P., Geng, J.J., Ding, L.L., Zhao, Y., 2016. Uptake and accumulation of polystyrene microplastics in zebrafish (*Danio rerio*) and toxic effects in liver. Environ. Sci. Technol. 50, 4054-4060.

Manabe, M., Tatarazako, N., Kinoshita, M., 2011. Uptake, excretion and toxicity of nano-sized latex particles on medaka (*Oryzias latipes*) embryos and larvae. Aquat. Toxicol. 105, 576-581.

McManus, J.F.A., 1946. Histological demonstration of mucin after periodic acid. Nature. 158, 202.

Mechlaoui, M., Dominguez, D., Robaina, L., Geraert, P.A., Kaushik, S., Saleh, R., Briens, M., Montero, D., Izquierdo, M., 2019. Effects of different dietary selenium sources on growth performance, liver and muscle composition, antioxidant status, stress response and expression of related genes in gilthead seabream (*Sparus aurata*). Aquaculture 507, 251-259.

Naiel, M.A.E., Negm, S.S., Abd El-hameed, S.A.A., Abdel-Latif, H. M. R., 2021. Dietary organic selenium improves growth, serum biochemical indices, immune responses, antioxidative capacity, and modulates transcription of stress-related genes in *Nile tilapia* reared under sub-optimal temperature. J. Therm. Biol. 99, 102999.

Nicula, M., Pacala, N., Stef, L., Pet, I., Dronca, D., Ahmadi, M., Gherbon, A., 2018. Garlic and *Chlorella* biomodulate Lead toxicity on manganese homeostasis in *Carassius gibelio* Bloch. Rev. Chim. 69, 986-989.

Oliveira, M., Ribeiro, A., Hylland, K., Guilhermino, L., 2013. Single and combined effects of microplastics and pyrene on juveniles (0+group) of the common goby Pomatoschistus microps (Teleostei, Gobiidae). Ecol. Indic. 34, 641-647.

Parenti, C.C., Ghilardi, A., Torre, C.D., Magni, S., Del Giacco, L., Binelli, A., 2019. Evaluation of the infiltration of polystyrene nanobeads in zebrafish embryo tissues after short-term exposure and the related biochemical and behavioural effects. Environ. Pollut. 254, 112947.

Patterson, J.M.M., Paige, G.B., Reddy, K.J., 2010. Selenium in surface and irrigation water in the Kendrick irrigation district, Wyoming. Environ. Monit. Assess. 171, 267-280.

Pitt. J.A., Trevisan, R., Massarsky, A., Kozal, J.S., Levin, E.D., Di Giulio, R.T., 2018. Maternal transfer of nanoplastics to offspring in zebrafish (*Danio rerio*): a case study with nanopolystyrene. Sci. Total Environ. 643, 324-334.

Reitman, S., Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28, 56-63.

Rider, S.A., Davies, S.J., Jha, A.N., Fisher, A.A., Knight, J., Sweetman, J.W., 2009. Supra nutritional dietary intake of selenite and selenium yeast in normal and stressed rainbow trout (*Oncorhynchus mykiss*): implications on selenium status and health responses. Aquaculture 295, 282-291.

Roest Crollius, H., Weissenbach, J., 2005. Fish genomics and biology. Genome Res. 15, 1675-1682.

Rossi, G., Barnoud, J., Monticelli, L., 2014. Polystyrene nanoparticles perturb lipid membranes. J. Phys. Chem. Lett. 5, 241-246.

Sarasamma, S., Audira, G., Siregar, P., Malhotra, N., Lai, Y.H., Liang, S.T., Chen, J.R., Chen, K.H.C., Hsiao, C.D., 2020. Nanoplastics cause neurobehavioral impairments, reproductive and oxidative damages, and biomarker responses in zebrafish: throwing up alarms of wide spread health risk of exposure. Int. J. Mol. Sci. 21, 1410.

Sayed, A. H., Hamed, M., Badrey, A. E.A., Soliman, H.A.M., 2021. Bioremediation of hemotoxic and oxidative stress induced by polyethylene microplastic in *Clarias gariepinus* using lycopene, citric acid, and *Chlorella*. Comparative Biochemistry and Physiology, Part C 250, 109189.

Seternes, T., Bøgwald, J., Dalmo, R.A., 2021. Scavenger endothelial cells of fish, a review. J. Fish Dis. 44, 1385-1397.

Wu, J.P., 2020. *Chlorella* sp. Protective Effect on Acetaminophen-Induced Liver Toxicity in ICR Mice. Int. J. Prev. Med. 11, 111.

Sokmen, T.O., Sulukan, E., Turkoglu, M., Baran, A., Ozkaraca, M., Ceyhun, S.B., 2020. Polystyrene nanoplastics (20nm) are able to bioaccumulate and cause oxidative DNA damages in the brain tissue of zebrafish embryo (Danio rerio). Neurotoxicology 77, 51-59.

Soontornchaiboon, W., Joo, S.S., Kim, S.M., 2012. Anti-inflamatory Effects of Violaxanthin Isolated from Microalga *Chlorella ellipsoidea* in RAW 264.7 Macrophages. Biological and Phamaceutical Bulletin 35, 1137-1144.

Sunde, R.A., 2006. Selenium. In: Present Knowledge in Nutrition (Bowman, B., Russell, R., Eds.), 9th ed. International Life Sciences Institute, Washington DC, pp. 480-497.

Tao, T., Peng, J., 2009. Liver development in zebrafish (*Danio rerio*). J. Genet. Genomics 36, 325-334.

Tomaselli, L., 2004. The microalgal cell. In: Handbook of Microalgal Culture: Biotechnology and Applied Phycology (Richmond, A., Ed.), Oxford, UK: Blackwell Science Ltd.

Triebskorn, R., Braunbeck, T., Grummt, T., Hanslik, L., Huppertsberg, S., Jekel, M., Knepper, T.P., Krais, S., Müller, Y.K., Pittroff, M., Ruhl, A.S., Schmieg, H., Schür, C., Strobel, C., Wagner, M., Zumbülte, N., Köhler, H.-R., 2019. Relevance of nano- and microplastics for freshwater ecosystems: a critical review. TrAC Trends Anal. Chem. 110, 375-392.

Wang, Y., Han, J., Li, W., Xu, Z., 2007. Effect of different selenium source on growth performances, glutathione peroxidase activities, muscle composition and selenium concentration of allogynogenetic crucian carp (*Carassius auratus gibelio*). Anim. Feed Sci. Technol. 134, 243-251.

Wang, Z.Y., Yin, L.Y., Zhao, J., Xing, B.S., 2016. Trophic transfer and accumulation of TiO2 nanoparticles from clamworm (*Perinereis aibuhitensis*) to juvenile turbot (*Scophthalmus maximus*) along a marine benthic food chain. Water Res. 95, 250-259.

Wilson, J.M., Bunte, R.M., Carty, A.J., 2009. Evaluation of rapid cooling and tricaine methanesulfonate (ms222) as methods of euthanasia in zebrafish (*Danio rerio*). Journal of the American Association for Laboratory Animal Science 48, 785-789.

Yin, L.Y., Liu, H.Y., Cui, H.W., Chen, B.J., Li, L.L., Wu, F., 2019. Impacts of polystyrene microplastics on the behavior and metabolism in a marine demersal teleost, black rockfish (*Sebastes schlegelii*). J. Hazard. Mater. 380, 120861.

Zahran, E., Awadin, W., Risha, E., Khaled, A. A., Wang, T., 2019. Dietary supplementation of *Chlorella vulgaris* ameliorates chronic sodium arsenite toxicity in Nile tilapia *Oreochromis niloticus* as revealed by histopathological, biochemical and immune gene expression analysis. Fisheries Science 85, 199-215.