Impact of Chlorella vulgaris Bioremediation and Selenium on Genotoxicity, Nephrotoxicity and Oxidative/Antioxidant Imbalance Induced by Polystyrene Nanoplastics in African Catfish (Clarias gariepinus)

Shimaa A. Abdelbaky , Zakaria M. Zaky , Doha Yahia , Mohamed H. Kotob * , Mohammed A. Ali , Mohammed Aufy * , Alaa El-Din H. Sayed

Posted Date: 12 January 2024

doi: 10.20944/preprints202401.0955.v1

Keywords: Polystyrene nanoplastics; Oxidative stress; Single cell gel electrophoresis; Selenium; Chlorella vulgaris

Article

Impact of *Chlorella vulgaris* Bioremediation and Selenium on Genotoxicity, Nephrotoxicity and Oxidative/Antioxidant Imbalance Induced by Polystyrene Nanoplastics in African Catfish (*Clarias gariepinus*)

Shimaa A. Abdelbaky [1], Zakaria M. Zaky [1], Doha Yahia [1], Mohamed H. Kotob [2,3,*], Mohammed A. Ali [1], Mohammed Aufy [3,*] and Alaa El-Din H. Sayed [4]

[1] Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Assiut University, Assiut, 71526, Egypt, shimaa321m0@gmail.com (S.A.A.); zakaria@aun.edu.eg (Z.M.Z.); dohayahia@aun.edu.eg (D.Y.), mohali@aun.edu.eg (M.A.A.)
[2] Department of Pathology, Faculty of Veterinary Medicine, Assiut University, Assiut, 71526, Egypt, kotobm84@univie.ac.at (M.H.K.).
[3] Division of Pharmacology and Toxicology, Department of Pharmaceutical Sciences, University of Vienna, 1090 Vienna, Austria, mohammed.aufy@univie.ac.at.
[4] Zoology Department, Faculty of Science, Assiut University, 71516, Assiut, Egypt, alaasayed@aun.edu.eg.
* Correspondence: mohammed.aufy@univie.ac.at (M.A.); kotobm84@univie.ac.at (M.H.K.)

Abstract: Contamination of the environment with nano- and microplastic particles exerts a threatened impact on the aquatic ecosystems and sustainable catfish aquaculture. The presence of nanoplastics has been found to have a detrimental impact on both aquatic and terrestrial ecosystems. The present study examines the effect of polystyrene nanoplastics on DNA, erythrocytes, oxidative status and renal of catfish in addition to the potential protective effects of *Chlorella vulgaris* bioremeditation and selenium to hinder this effect. Six equal groups of fish were used as follows: Group 1: served as a control group and received water free from PS NPs; Group 2: was exposed to PS NPs at a concentration of 5mg/L; Group 3: was exposed to PS NPs (5mg/L) + selenium (1mg/kg diet); Group 4: was exposed to PS NPs (5mg/L) + *C. vulgaris* (25g/kg diet); Group 5: was supplemented with *C. vulgaris* (25g/kg diet); and Group 6: was supplemented with selenium (1mg/kg diet). The results indicated that PS NPs induced oxidative stress by significantly elevating MDA activities and slightly reducing antioxidant biomarkers, resulting in DNA damage, increased frequency of micronuclei, erythrocyte alterations, and numerous histopathological alterations in kidney tissue. Selenium and *C. vulgaris* significantly ameliorated the oxidative/antioxidant status, reduced DNA damage, micronucleus frequency, erythrocyte alterations, and morphology of kidney tissue. Nevertheless, further research is needed to evaluate in detail the mechanism behind the toxicity with nano-microplatics in aquatic system.

Keywords: Polystyrene nanoplastics; Oxidative stress; Single cell gel electrophoresis; Selenium; *Chlorella vulgaris*

1. Introduction

Polystyrene nanoplastics (PS NPs) are sub-100 nm-sized plastic particles [1]. They can penetrate biological barriers and accumulate along the food chain, posing significant health risks to humans and animals [2].

Microplastics and nanoplastics (M/NPs) are present in various ecosystems such as soils, air, marine, and freshwater environments, as reported by [3]. The issue of water contamination caused by plastics has been recognized as a significant environmental peril in numerous countries worldwide, as stated by [4]. According to research conducted by [5,6], Egypt is the largest plastic consumer in Africa and ranks 7th among 192 countries in producing poorly managed plastic waste. The country generates an annual output of 0.97 million tons of such waste. plastic wastes are

 doi:10.20944/preprints202401.0955.v1

exposed to chemical, biological and environmental elements, and will break down into huge amounts of microplastics and nanoplastics [7].

Recent studies have reported that micro- and nanoplastics reach the human food chain in several ways for example consuming them in their natural environment, food contamination during preparation processes, or through leaching from plastic packaging of food and drinks [8–10].

Polystyrene nanoplastics have the ability to permeate the gut epithelium, leading to systemic exposure in humans. Several *in-vivo* and *in-vitro* studies on animals found that the probable oral bioavailability level of 50 nm polystyrene nanoparticles is ten to one hundred times greater than the level of microplastics [11,12].

There are three routes for exposure to microplastics and nanoplastics: oral, inhalation, and dermal [13,14]. After absorption, nanoplastics will be able to interact with numerous target cells and induce their toxic effects on kidney, gut, liver and other organs [15]. Extensive studies have shown that NPs can lead to different biological adverse effects, such as oxidative stress [16], cytotoxicity, genotoxicity [17], neurotoxicity [18], reproductive and developmental toxicity and immune dysfunction [19]. Furthermore, they have been observed to have detrimental effects on the survival and reproduction of aquatic organisms [20].

Clarias gariepinus inhabits various freshwater habitats, such as ponds, lakes, and pools. In addition, they are evident in rivers, rapids, and near dams. They can survive in water with a pH range of 6.5 to 8.0 and exceedingly low oxygen concentrations [21]**.** Their bodies produce mucous to prevent dehydration [22]. They can tolerate temperatures between 8 and 35 degrees Celsius and persist in extremely murky environments. The optimal temperature for their growth is between 28 and 30 degrees Celsius [23]. *Clarias gariepinus* is widely utilized in aquaculture because it is readily available throughout the year, high food conversion ratio, fast growth rate, and adapts well to laboratory conditions and stress, making it an ideal test organism [24].

Chlorella vulgaris, agreen unicellular microalgae is used in aquatic bioremediation [25]. It exhibits potent antioxidant properties due to the presence of carotenoids and phenolic bioactive compounds responsible for its antioxidant and radical-scavenging activities [26]. On the other hand selenium (Se) is necessary for fish's development and metabolic processes [27]. Selenium can be found in many essential selenoproteins and enzymes that maintain antioxidant status, DNA synthesis, fertility, reproduction, and reduce inflammation [28]. Chromosomal aberration assay, comet assay, and various types of micronucleus (MN) assays, including mammalian erythrocyte MN (EMN), are considered crucial techniques in *In vivo* genotoxicity research in fish [29–31].

The MN tests of fish erythrocytes have been commonly employed to assess genotoxicity in marine and freshwater environments [32]. These tests have also served as an indicator of the exposure of various fish species to mutagenic or genotoxic pollutants caused by numerous substances [33].

There is a dearth of research evaluating the genotoxic effect of PS NPs, the effect on erythrocyte morphology and the patholgical changes in the kidney of catfish. Also, the potential ameliorative effects of selenium and bioremediation by *Chlorella vulgaris*. Therefore, the present study assessed the efficacy of L-Selenomethionine and *Chlorella vulgaris* in mitigating the harmful effects of PS NPs on DNA, RBCs, and kidney of *C. gariepinus*.

2. Materials and Methods

2.1. Chemicals and materials

The Polystyrene nanoplastic particles (PS NPs) were procured from Bangs Laboratories, Inc. The procurement of Seleno-L-methionine powder was carried out from Sigma-Aldrich, USA. The powder was high purity (≥ 98%) and had a molecular weight 196.11. Additionally, the powder was identified by its CAS number, 3211-76-5. The dried green powder of *Chlorella vulgaris* microalgae was procured from the Institute of National Research Center, Cairo, Egypt.

Before conducting the experiments, a stock solution of PS NPs at a concentration of 5 g L−1 was prepared using deionized water. Then, the dimensions and morphology of the nanoplastics were assessed using a JEOL-JEM-100 CX II transmission electron microscope (TEM) located in the electron microscopy unit at Assiut University, Egypt.

2.2. Clarias gariepinus

 doi:10.20944/preprints202401.0955.v1

For the study, a sample of 144 male juvenile catfish (*C. gariepinus*) was obtained from an aquaculture fish farm in Assiut, Egypt. The specimens exhibited an average weight of 160 g and a length of 25 and 30 cm. The age of the specimens was determined to be six months. The conveyance of fish to the Fish Biology Laboratory, situated within the Zoology Department of the Faculty of Science at Assiut University in Egypt, was executed. During a 35-day acclimatization period, the fish were subjected to laboratory conditions where 100 L tanks, dechlorinated tap water, and air pumps were used; this was executed before the initiation of the exposure. The piscine subjects were administered a diet comprising a commercial substrate with a crude protein content of 30%. The nutritional needs of *C. gariepinus* were satisfied by creating a fundamental diet comprising diverse constituents, with the estimated chemical makeup of each component outlined in Table 1.

Table 1. Ingredients and chemical composition of the basal diet (dry weight, %).

Ingredients	**%**
Fish meal (65%)	9
Soybean meal (46%)	36.85
Corn gluten (60%)	12.2
Yellow corn	19.25
Wheat bran	5.7
Fish oil	6.00
Starch	7.00
Mineral premix (without se)	2.00
Vitamin premix	2.00
Total	100
Proximate composition % dry matter (DM)	**%**
Crude protein (CP)	30
Crude fiber (CF)	4.8
Ash	8.2
Ether extract (EE)	6.5
*Nitrogen free extract (NFE)	50.5

*NFE = 100- (CP% + EE% + CF% + Ash%).

2.3. Ethics statement

The research protocol mentioned above has received approval from the veterinary ethical committee of the Faculty of Veterinary Medicine at Assiut University, Egypt, under the reference number 06/2022/0016.

2.4. Experimental design

The fish were randomly put into six groups of 24 each, with three tanks in each group. The first group (C) was used as a control and got water that did not contain PS NPs. According to [34], the second group (NP) was exposed to 5 mg/L PS NPs in water. According to [35], the third group (NPs+Se) was given PS NPs (5 mg/L) and selenium (1 mg/kg food). According to [36], the fourth group (NPs+Ch) was exposed to PS NPs (5 mg/L) and *C. vulgaris* (25 g/kg food). The fifth group (Ch) got only *C. vulgaris* (25 g/kg food). The sixth group (Se) only got selenium (1 mg/kg food).

For 30 days, the animals were kept in tanks with 60 L of naturally dechlorinated water and air blowers for regular aeration. The experiment was done with steady water quality (temperature of 22°C, dissolved oxygen (mg/l) of 6.85, pH of 7.38, and 12:12 light: dark cycle). About 50% of the water was changed daily throughout the experiment, and PS NPs were added at the abovementioned amounts. In addition, waste products like feces were removed daily to keep the ammonia levels in the water as low as possible.

2.5. Sample collection

 doi:10.20944/preprints202401.0955.v1

2.5.1. Blood samples

Following a period of 30 days of exposure, six distinct groups of fish, comprising both control and treatment groups, were captured at random using a hand net and subsequently transferred to ice in order to mitigate any potential stress, as per the study conducted by [37]. The blood collection methodology followed the approach described by [38]. First, EDTA-containing tubes were utilized to obtain blood samples from the caudal vein of *C. gariepinus* to detect DNA damage through the comet assay. Next, additional blood samples were collected using standard vacutainer tubes and subsequently subjected to centrifugation at 3000 revolutions per minute for 15 minutes to procure serum samples, which were then stored at a temperature of -20˚C. These samples assessed Malondialdehyde, catalase enzyme, and glutathione peroxidase enzyme levels. Finally, the blood samples were subjected to the preparation of blood smears, followed by drying at ambient temperature and fixation in absolute methanol to conduct the micronucleus assay and assess other nuclear abnormalities and erythrocyte morphological changes.

2.5.2. Tissue samples

After dissecting the fish, kidney tissues were rapidly removed, cleaned in normal saline to eliminate blood traces, and stored in 10% buffered formalin for histological investigation.

2.6. Oxidative stress biomarkers measurements

2.6.1. Determination of serum malondialdehyde (MDA) activity:

MDA was measured by spectrophotometric method (V-630, JASCO, Japan), using a commercial kit supplied by Bio Diagnostics, Egypt, with a catalog number MD 25 30 and according to the method described by [39].

2.6.2. Determination of serum catalase enzyme activity

Catalase assay was performed by spectrophotometric method (V-630, JASCO, Japan). According to [40] using test kits provided by Bio-diagnostic (Dokki, Giza, Egypt).

2.6.3. Determination of serum glutathione peroxidase (GPx) activity

Glutathione peroxidase activity was measured in serum samples using test kits provided by Bio-diagnostic (Dokki, Giza, Egypt). Providing a spectrophotometric means (V-630, JASCO, Japan) to detect the activity of GPx enzyme [41].

2.7. Genotoxicity assays

2.7.1. Detection of DNA damage by Single cell gel electrophoresis (Comet assay)

DNA damage in the erythrocyte of *C. gariepinus* was estimated using comet assay according to the method of [42], as follows:

a) Preparation of slides

Slides were double coated with 1% standard agarose; 0.3 mL of blood was diluted in 1000 µL PBS; next, ten microliters of cell suspension was mixed with 120 µL of 2 % low melting point agarose at 37 °C, a layer of the mixture was placed on the previously prepared slides, cover-slipped and placed in the refrigerator for 5 min to solidify. Finally, 1 % agarose (100 µL) was layered on top and allowed to gel.

b) Lysis and electrophoresis

All slides were kept in a cold lysis solution (10 mM Trizma, 100 mM Na_4 EDTA, 2.5 M NaCl, 10% dimethyl sulfoxide, 0.1% sodium lauryl sulfate (SDS), and Triton X-100) at 4 °C for 60 minutes. Then transferred to the electrophoresis tank (Cleaver Scientific Ltd., UK) containing chilled alkaline solution (1 mM Na2 EDTA and 300 mM NaOH) at pH 13 for 25 min in the dark for unwinding of the DNA strands. Electrophoresis was run for 25 min at 25 V and approximately 300 mA. Next, slides were neutralized in 400 mM Tris buffer (0.4 M Tris-HCl, pH 7.5) for 7 min. Finally, the neutralized slides were dehydrated in ethanol for a few minutes and then dried at room temperature.

c) Slide examination

The slides were examined through a fluorescence microscope (Olympus BX-43, Japan) with a green filter at a magnification of 200-fold. In addition, 50 µL of ethidium bromide at 20 µg/mL

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 January 2024 doi:10.20944/preprints202401.0955.v1

concentration was used to stain all the slides. In order to assess DNA migration, a minimum of 50 nuclei were examined per slide, with three slides per sample. In addition, the Comet Assay Software Project (CASP) was utilized to measure the diameter of the comet's head and the length of its tail.

2.7.2. Micronucleus and Erythrocyte morphological alterations

Three blood smears were prepared and dried at room temperature. For 10 minutes, the smears were fixed in methanol (100%) and stained with hematoxylin and eosin. The slides were then dehydrated with alcohol with increasing concentrations (30, 50, 70, and 90 percent, absolute) before being cleaned in xylene and permanently mounted with DPX [43]. According to [44], each nuclear abnormality observed during slide examination, including micronuclei formation, was noted. Ten photomicrographs of random fields were obtained for each slide, totaling 30 photomicrographs per experimental group, and one hundred cells were examined in each analysis field, totaling 3000 erythrocytes/group.

$$MN\ (\%) = \frac{Number\ of\ cells\ with\ micronuclei \times 100}{Total\ number\ of\ cells\ counted}$$

2.8. Histopathological examination of the kidney

Kidney specimens were fixed in neutral buffered formalin 10 %, then dehydrated in serial alcohol concentrations, cleared in xylene, and impregnated in paraffin. Five µm paraffin sections were prepared and then stained with the following stains: Hematoxylin and Eosin (H&E) according to [45], Periodic Acid Schiff (PAS) and Hematoxylin for polysaccharides (glycogen) [46] and Picrosirius red stain for collagen fibers [47].

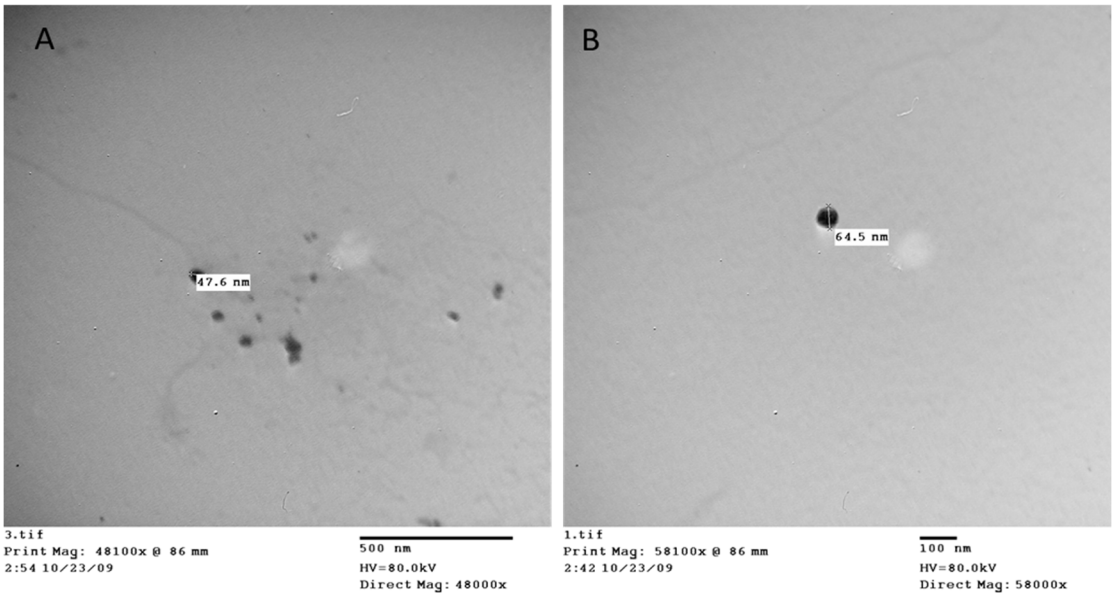
2.9. Statistical analysis

The statistical analysis used SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA). Initially, the statistical method of one-way analysis of variance (ANOVA) was employed, subsequently followed by a post hoc least significant difference (LSD) multiple range test to make comparisons between the control and exposed groups. Finally, the mean ± SE represented all outcomes for the control and experimental groups .The statistical significance level was established at a value of $P < 0.05$.

3. Results

3.1. Polystyrene nanoplastics characterization

The PS nanomaterials were analyzed using transmission electron microscopy (TEM), which revealed that they are made up of spherical particles with a diameter of 50.1 nm ±13.4 Figure 1.



 doi:10.20944/preprints202401.0955.v1

Figure 1. Transmission electron photomicrographs of the PS NPs.

3.2. Mortality rate

The results showed no deaths were recorded in the control group, while the NPs-exposed group showed a high mortality percentage of 36.3%. The administration of selenium reduced the rate of mortality to 14.8%. Similarly, the administration of *C. vulgaris* reduced the percentage of mortalities to 8.3%.

3.3. Oxidative status

3.3.1. Lipid peroxide

Serum MDA activity significantly increased ($P < 0.01$) in *C. gariepinus* exposed only to NPs compared to the control group. Conversely, treatment of fish with Se and *Chlorella* showed a significant ($P < 0.01$) decrease in the MDA activity in comparison with the NPs-exposed group in Table 2.

3.3.2. Catalase and glutathione peroxidase activities:

Catalase and glutathione peroxidase activities in the serum of *C. gariepinus* exposed to NPs showed a mild, non-significant ($P > 0.05$) decrease compared to the control group. While other groups showed no change in the activity of Catalase and GPX (Table 2).

Table 2. MDA (nmol/mL), catalase (U/L) and glutathione (U/L) activities in serum of *C. gariepinus* in control, NPs, (NPs+Se), (NPs+Ch), Ch and Se groups.

Groups / Parameters	Control	NPs	NPs+Se	NPs+Ch	Ch	Se
MDA	3.71± 0.7^{b}	10.22± 2.4 ^{a}	5.56±0.5^{b}	4.98± 0.7^{b}	3.72±0.8^{b}	4.02±0.9^{b}
CAT	225.9±13.6^{b}	201.43±5.2^{b}	235±62.3^{b}	202.43±40.5^{b}	232.93±11.2^{b}	352.9±33.4^{a}
GPX	27.5± 5.6^{a}	19.1± 6.4^{a}	33.7± 10.4^{a}	25.57±4.2^{a}	24.33±2.99^{a}	32.43±8.0^{a}

Data are presented as means ± SE. Different letters a, b and c indicate significant changes among groups. $P < 0.05$. N= 6.

3.4. Genotoxic biomarkers for DNA damage

3.4.1. DNA damage parameters (Comet assay)

The study's findings indicate that the administration of NPs resulted in a notable increase in DNA damage in the blood of *C. gariepinus* compared to the control group. In addition, the study observed a rise in damage indicators, namely tail DNA%, tail length, tail moment, and Olive tail moment, as depicted in (Figure 2 and Table 3). The different levels of DNA damage and the technique employed to evaluate DNA damage using CASP software are presented in Figure 3.

All parameters of DNA damage were significantly increased in the NPs exposed group ($P < 0.01$) compared with the control and other treated groups. Conversely, *Chlorella* (NPs+Ch) and selenium (NPs+SE)-treated groups demonstrated a significant decrease ($P < 0.01$) for all these parameters in comparison with the NPs-exposed group (Figure 2 and Table 3).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 January 2024 doi:10.20944/preprints202401.0955.v1

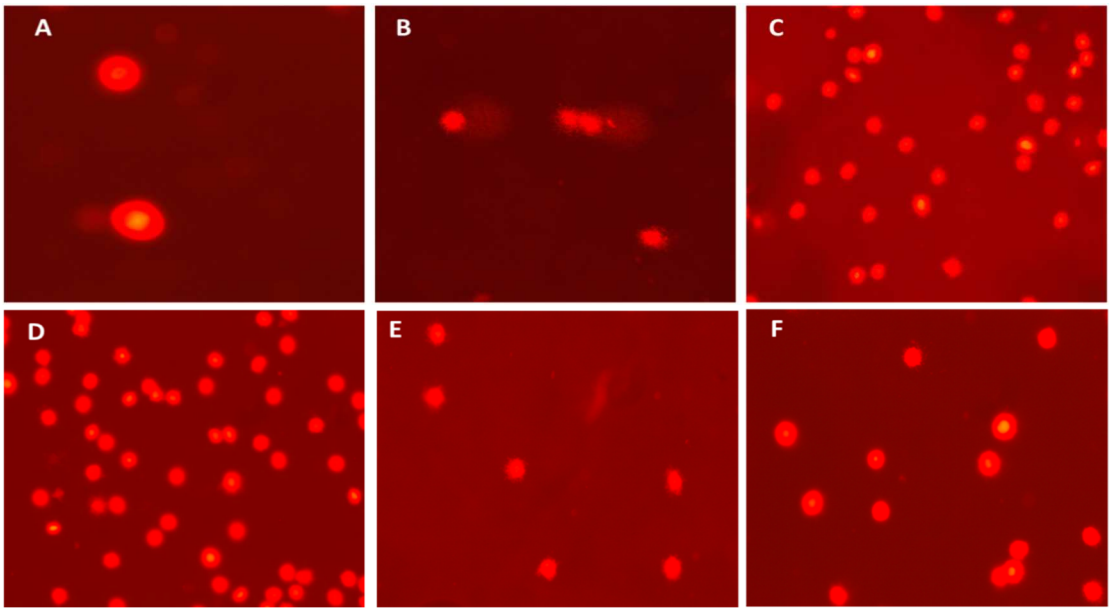


Figure 2. DNA damage in whole blood of *C. gariepinus* in all the experimental groups. A: Control group; B: NPs group; C: (NPs + Se) group; D: (NPs + Ch); E: Ch; F: Se.

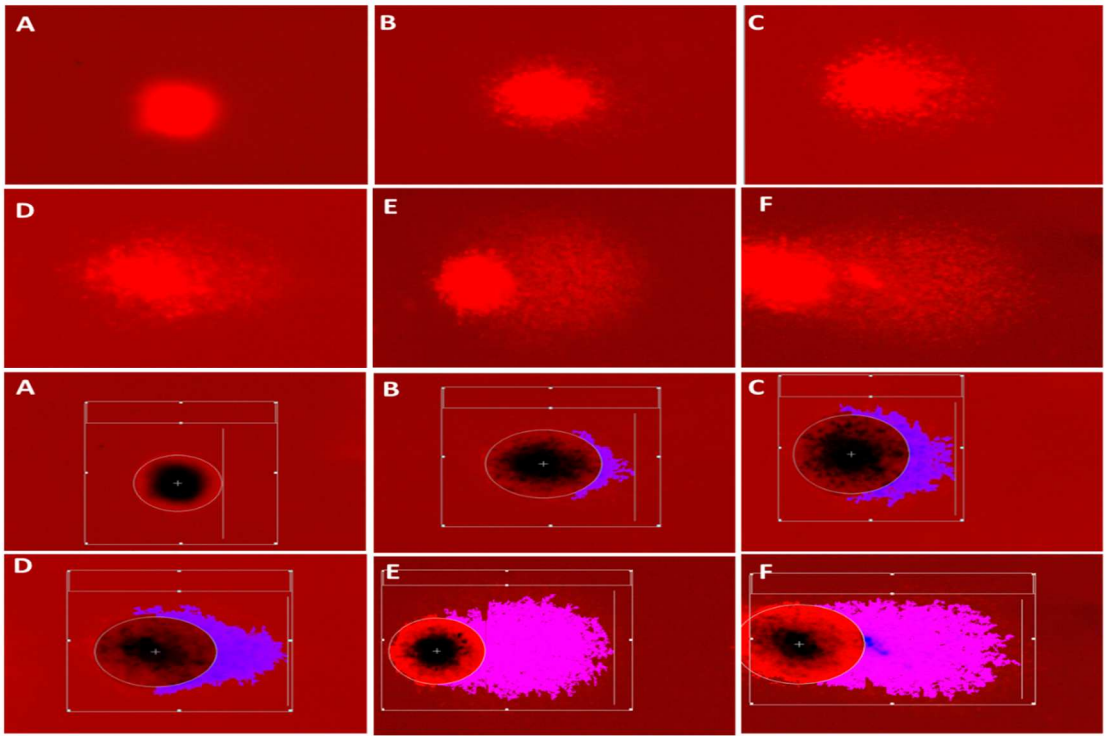


Figure 3. Assessment of different degrees of DNA damage induced by PS NPs using CASP software showed different degrees of DNA migration. A: intact nucleus (no DNA damage). B: mild DNA damage. C: moderate degree of DNA damage. D: severe DNA damage. E&F: extensive DNA damage.

Table 3. DNA damage in the blood of *C. gariepinus* in control, NPs, (NPs+Se), (NPs+Ch), Ch and Se groups.

Parameters	Control	NPs	NP+ Se	NP+ Ch	Ch	Se
Tail DNA%	0.57±0.12bc	3.43 ±0.53a	1.26±0.19b	0.98±0.17b	0.56±0.16b c	0.08±0.03c
Tail Length (μm)	8.24 ±0.62c	32.14 ±2.60a	15.42±1.21 b	9.77±0.60c	7.50 ±0.45cd	4.22 ±0.28 d

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 January 2024 doi:10.20944/preprints202401.0955.v1

Tail moment	0.19±0.05b	3.60±0.88a	0.44±0.07b	0.20±0.06b	0.15±0.03b	0.09±0.01b
Olive tail moment	0.46±0.09b	4.58±0.66a	1.15±0.15b	0.59±0.11b	0.30±0.05b	0.11±0.02b

Data are presented as mean ± SE. Values followed by different letters indicate significant changes among groups. $P < 0.05$. N= 6.

3.4.2. Erythrocyte micronucleus (EMN) and morphological alterations

The normal erythrocytes of *C. gariepinus* are rounded with a centrally located nucleus (Figure 4A). Micronucleus (MN) analysis of erythrocytes of *C. gariepinus* exposed to NPs indicated a significant ($P < 0.01$) increase in comparison with the control group (Figure 4B, 5A and Table 4). In addition, using Se and *C. vulgaris* significantly ($P< 0.01$) reduced the EMN frequency compared with the NPs-exposed group.

Erythrocyte morphological alterations and nuclear abnormalities of RBCs were significantly elevated ($P < 0.01$) in the NPs group compared with the control group.

Both groups exposed to NPs and treated with Se or *Chlorella* showed a significant decrease ($P< 0.01$) in the nuclear abnormalities and erythrocyte alterations compared to those only exposed to NPs.

The Predominant alterations of erythrocytes in NPs exposed group included Micro nucleated cells (Mn); one or more Mn per cell present in most observations, Vacuolated cells (Vc), Acanthocytes (Ac); Crenated cells with fewer surface projections, Eccentric nuclei (Ecn), Spindle-shaped cells (Sp), Crenated cells (echinocytes)(Cr); where the RBCs develop an irregular surface with numerous projections, Sickle cells (Sk), which vary in shape between ellipsoidal, boat-shaped, and genuine sickles; Tear-drop cells (Tr), whose body looks like a tear with pointed apices; bilobed nuclei (Biln); microcytes (Mc); enucleated cells (Enc); and notched nuclei (Non) (Figure 4B-D, 5A-L and Table 4).

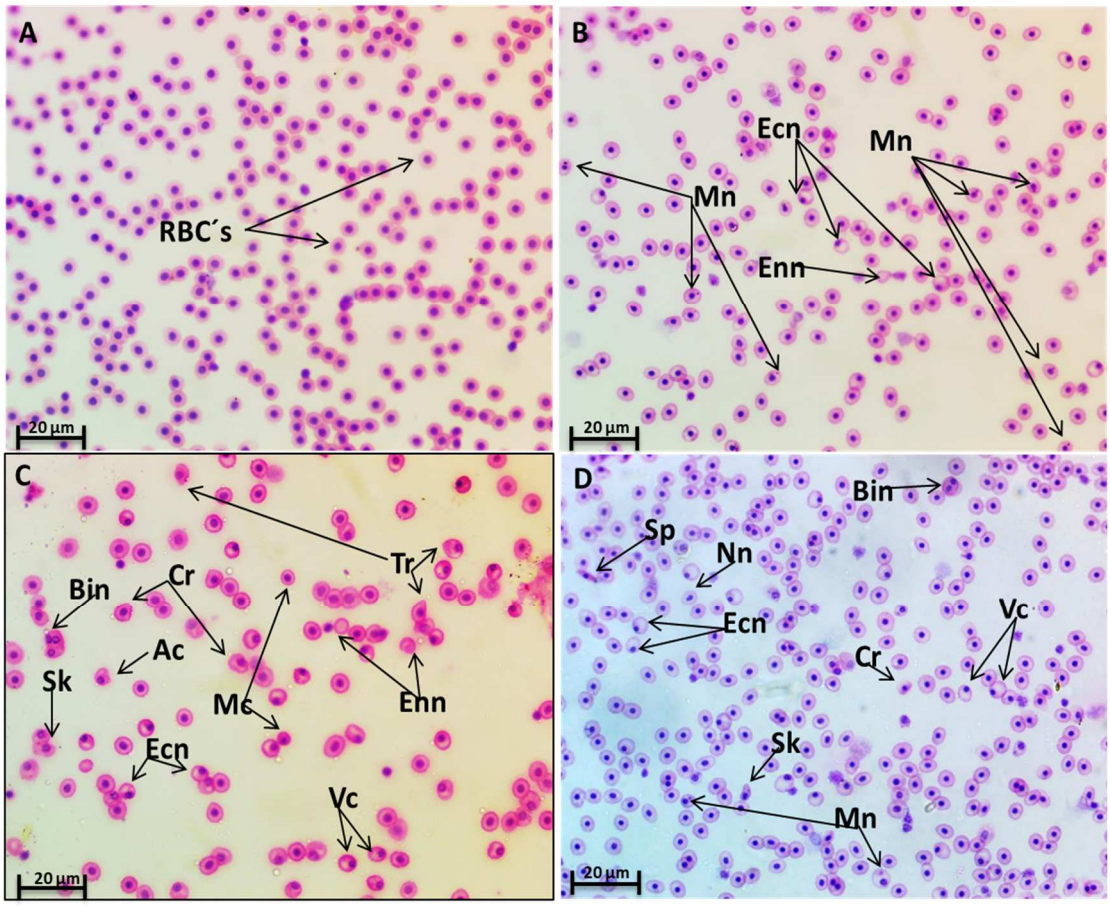


Figure 4. Blood smears stained by H&E showed the morphological alterations and nuclear abnormalities in erythrocytes of *C. gariepiuns* exposed to PS NPs. (a) control fish showing normal red

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 January 2024 doi:10.20944/preprints202401.0955.v1

blood cells (RBCs), (B–D) fish exposed to PS NPs showing Mn; micronucleated cell, Vc: vacuolated cell, Bin; Bilobed nucleus cell, Ac: acanthocyte, Ecn; eccentric nucleus, Sp; spindle-shaped cell, Cr; crenated cell, Sk; sickled cell, Tr; tear-drop cell, En; enucluated cell, Mc; microcyte, Non; notched nucleated cell. Scale bar = 20 µm.

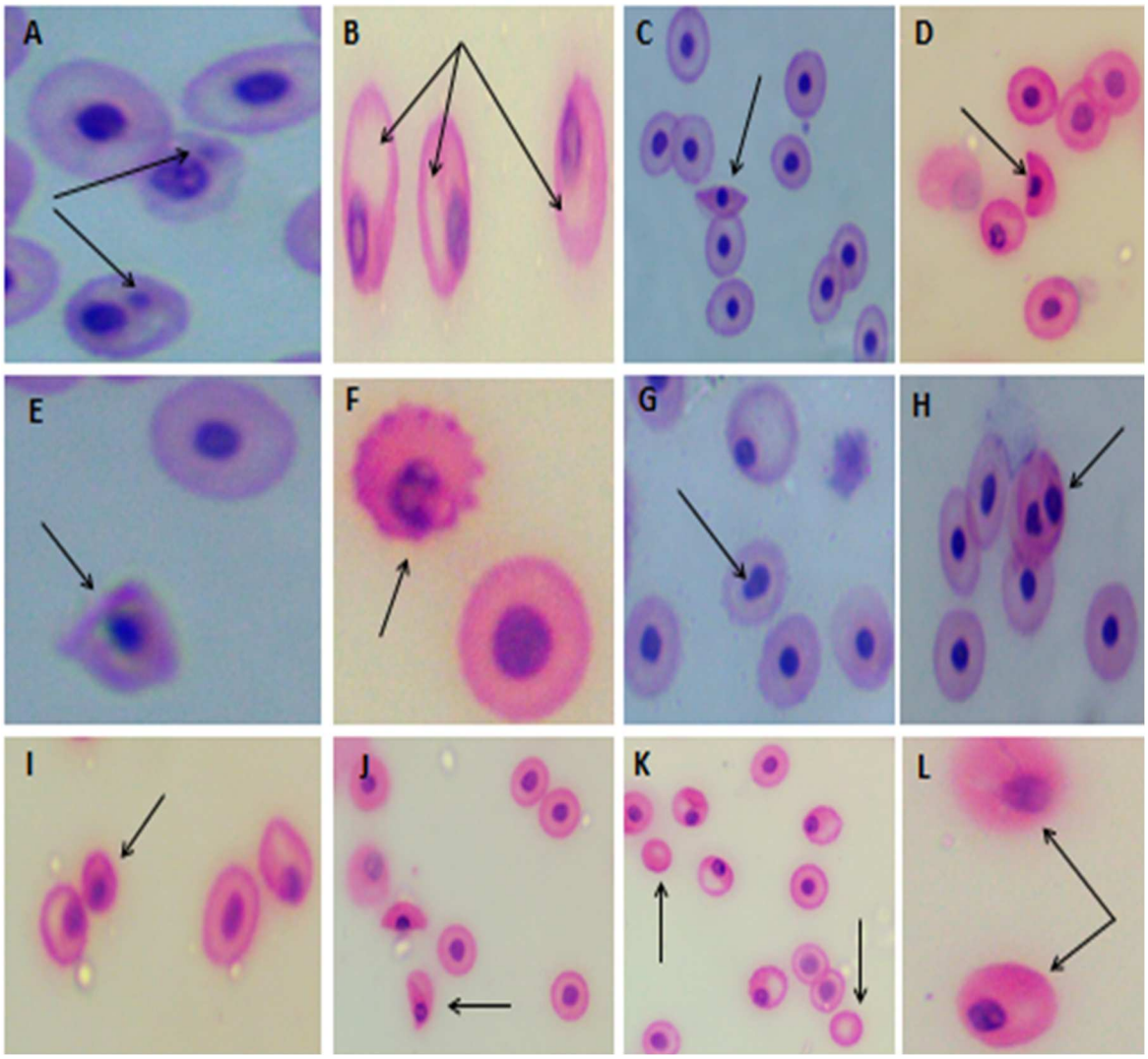


Figure 5. Showing different erythrocyte alterations were recorded in PS NPs exposed group, A; Micronucleated cell, B; Vacuolated cell, C; Spindle cell, D; Sickle cell, E; Acanthocyte, F; crenated cell, G; notched nucleated cell, H; Bilobed nucleus, I; Microcell, J; Tear-drop like cell, K; Enucleated cell, L; Eccentric nucleus.

Table 4. Erythrocytes morphological alterations and nuclear abnormalities in *C.gariepinus* in control, NPs, NPs+Se, NPs+Ch, Ch and Se groups.

Groups / Parameters	Control	NPs	NPs+Se	NPs+Ch	Ch	Se
Mn	2.3±0.88^{b}	22.3±2.00^{a}	4.3±1.20^{b}	3.3±1.2^{b}	2.3±0.33^{b}	3.0±0.57^{b}
Vc	7.0 ±0.57^{b}	47.3±5.78^{a}	9.3±1.45^{b}	8.3±2.6^{b}	6.0±1.52^{b}	5.3±0.88^{b}
Tc	5.0±1.50^{b}	14.3±1.45^{a}	7.6±0.88^{b}	6±0.57^{b}	5.0±1.15^{b}	4.3±1.20^{b}
Ac	4.3±1.20^{b}	11.3±1.45^{a}	5.0±1.15^{b}	4.3±0.88^{b}	3.0±1.00^{b}	3.3±0.88^{b}
Cr	3.6±0.88^{b}	12.6±1.76^{a}	5.6±1.20^{b}	5.3±0.88^{b}	4.3±1.20^{b}	4.0±1.5^{b}
Sk	2.0±0.57^{b}	13.3±0.88^{a}	4.6±0.80^{b}	3.6±1.20^{b}	2.0±0.57^{b}	2.3±0.88^{b}
Sp	1.6±0.33^{b}	7.3±1.45^{a}	2.3±0.80^{b}	2.6±1.20^{b}	1.6±0.66^{b}	2.0±0.57^{b}
Mc	4.6±1.20^{b}	19.0±1.15^{a}	5.3±1.20^{b}	5.6±1.70^{b}	2.6±1.20^{b}	3.6±0.88^{b}

 doi:10.20944/preprints202401.0955.v1

Ecn	7.6±1.76^{bc}	50.0 ±3.20^{a}	12.0±2.50^{b}	7.0±2.00^{bc}	6.3±1.2^{bc}	4.6±0.88^{d}
Bin	3.0±0.57^{b}	10±1.50^{a}	4.3±1.40^{b}	4.0±0.57^{b}	1.3±0.33^{b}	1.3±0.33^{b}
Notn	1.3±0.33^{bc}	8.3±1.20^{a}	3.6±0.80^{b}	2.3±0.88^{bc}	1.0±0.33^{d}	1.6±0.33^{bc}
Enn	2.6±0.88^{b}	9.0±1.15^{a}	3.0±0.57^{b}	2.0±0.57^{b}	1.3±0.33^{b}	2.0±0.57^{b}

Data are presented as mean ± SE. Values followed by different letters indicate significant changes among groups. $P < 0.05$. Mn; micronucleated cell, Vc; vacuolated cell, Tr; tear-drop cell, Ac; acanthocyte, Cr; crenated cell, Sk; sickled cell, Sp; spindle-shaped cell, Mc; microcyte, Ecn; eccentric nucleus, Bin; Bilobed nucleus, Non; notched nucleated cell, En; enucluated cell. N= 6.

3.5. Nephrotoxic effects

Histological examination of transverse kidney sections from control, selenium-treated, and *C. vulgaris*-treated catfish showed typical histological structures of rounded glomeruli, which are lined by the flat epithelium of Bowman's capsule, and proximal and distal convoluted tubules that are lined by simple cuboidal epithelium. The apical part of the proximal tubule cell is covered by a brush border of microvilli (Figures 6A, G, H). PAS and hematoxylin stains demonstrated increased carbohydrate substances in the brush borders of the epithelium of proximal tubules and basement membranes (Figures 7A, E, F). Transverse kidney sections of NPs-intoxicated catfish showed several pathological alterations, mainly in the renal tubules. They consisted of vacuolar degeneration, apoptosis, and coagulative necrosis of cells lining the renal tubules that later dissociated, causing detachment of the tubular epithelium (Figure 6B). The nuclei of the necrotic tubular epithelium showed necrotic changes in the form of pyknosis, karyorrhexis, or complete loss of nuclei. There was also connective tissue proliferation around the renal tubules (peritubular fibrosis), hemorrhages, and tubular dilatation (Figure 6C). Perivascular connective tissue proliferation around the renal blood vessels and thrombosis were also observed (Figure 6D). PAS and hematoxylin-stained transverse kidney sections showed a decline in the carbohydrate materials in the apical brush borders of proximal tubules, confirming the loss of brush borders (Figure 7B). Picrosirius red-stained transverse sections demonstrated the increment of the connective tissue around the renal tubules and blood vessels (Figure 8B) in comparison with the control, selenium-treated, and *C. vulgaris*-treated groups that showed a scarcity of connective tissue surrounding the renal tubules and blood vessels (Figure 8A, E, and F).

Administration of selenium in the NPs+Se-treated group ameliorated and inhibited the nephrotoxic effects of nanoplastics. Minimal alterative changes in the tubular epithelium were noticed. The typical histological appearance of the kidney was nearly restored with less vacuolar degeneration and necrotic changes in the tubular epithelium (Figure 6E). Hematoxylin-PAS-and Hematoxylin stained transverse sections showed increased carbohydrate materials in the apical brush borders of proximal tubules, revealing the restoration of their typical structures (Figure 7C). In addition, transverse kidney sections stained with picrosirius red stain showed a decrease in the amount of connective tissue surrounding renal tubules and blood vessels compared to that found in the NPs-intoxicated group (Figure 8C).

Administration of *Chlorella* in the NPs+Ch treated group restored almost all pathological alterations to near their control levels. Most renal tubules appeared nearly normal, and a few exhibited vacuolar degeneration and tubular necrosis (Figure 6F). PAS and hematoxylin-stained transverse kidney sections showed moderate carbohydrate material localization in the apical brush borders of proximal tubules (Figure 7D). The utilization of picrosirius red stain on transverse kidney sections revealed that the quantity of connective tissue surrounding the renal tubules and blood vessels in the experimental group was lower than that observed in the NPs-intoxicated group. (Figure 8D).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 January 2024 doi:10.20944/preprints202401.0955.v1

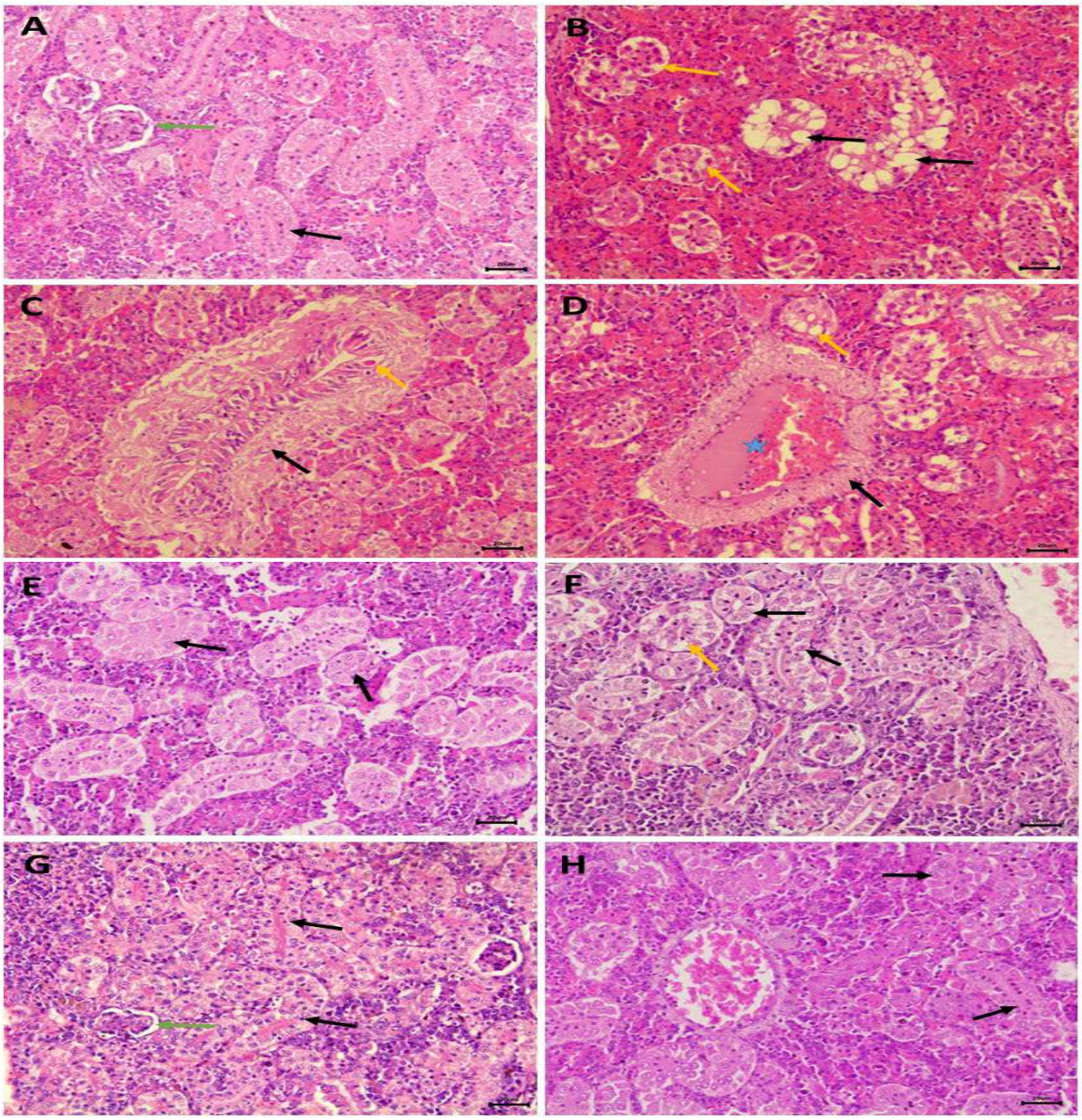


Figure 6. Photomicrographs of kidney transverse sections from control, NPs-intoxicated and treated *C. gariepinus*:A, G, H. Kidney sections of control, selenium and *C. vulgaris* treated catfish showed normal histological structures of the kidney that consisted of normally rounded glomeruli (green arrow) and renal tubules that covered by simple columnar epithelium with apical brush border (black arrow). B, C, D. Kidney transverse sections of NPs-intoxicated catfish in B showed the presence of vacuolar degeneration in the cytoplasm of tubular epithelium with peripherally located pyknotic nuclei (black arrow) and epithelial necrotic changes with nuclear alterations, dissociation of renal cells and epithelium desquamation (yellow arrow), in C showed connective tissue proliferation around the renal tubules (peritubular fibrosis) (black arrow) and necrosis and apoptosis of tubular epithelium (yellow arrow), in D showed perivascular connective tissue proliferation (black arrow), thrombosis (star) and vacuolar degeneration (yellow arrow). E. Kidney transverse section of NPs+Se treated catfish showed minimal vacuolar degenerative changes, necrotic changes of tubular epithelium, their nuclei appeared round, and vesicular centrally located (arrow). F. Kidney transverse section of NPs+Ch treated catfish showed vacuolar degenerative changes, necrotic changes of tubular epithelium and desquamation (yellow arrow), some renal tubules appeared normal with minimal cytoplasmic and nuclear alterations (black arrow). H& E. Scale bar = 20 μm.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 January 2024 doi:10.20944/preprints202401.0955.v1

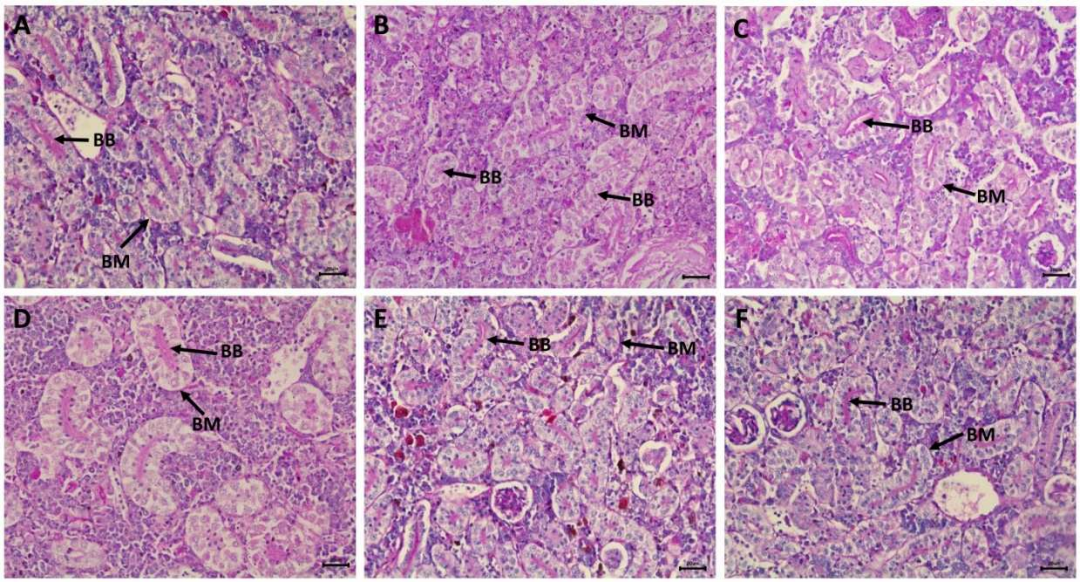


Figure 7. Photomicrographs of kidney transverse sections from control, NPs-intoxicated and treated *C. gariepinus*: **A, E, F.** Kidney transverse sections of control, selenium and *C. vulgaris* treated catfish showed positive PAS reactivity in the brush border (BB) and basement membrane (BM) of the renal tubules. **B**. Kidney transverse sections of NPs-intoxicated catfish showed a decrease in carbohydrate materials in the brush borders and the basement membranes of the renal tubules. **C**. Kidney transverse section of NPs+Se treated catfish showed an increase in the amount of carbohydrate observed in the basement membranes and brush borders. **D**. Kidney transverse section of NPs+Ch treated catfish showed moderate carbohydrate materials localization in the brush border and basement membrane of renal tissue. PAS+Hematoxylin. Scale bar = 20µm.

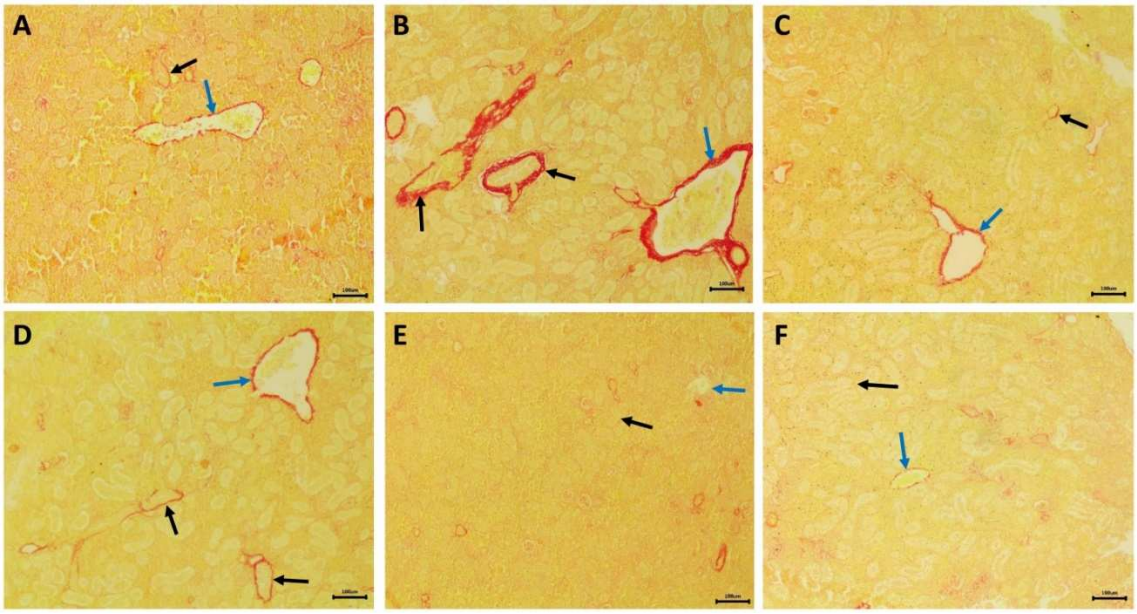


Figure 8. Photomicrographs of kidney transverse sections from control, NPs-intoxicated and treated *C. gariepinus*: A, E, F. Kidney transverse sections of control, selenium and *C. vulgaris* treated catfish showed a scanty amount of connective tissue fibers around the renal tubules and blood vessels. B. Kidney transverse sections of NPs-intoxicated catfish showed an increased amount of connective tissue fibers around the renal tubules and blood vessels with a thickened wall of blood vessels. C, D: Kidney transverse section of NPs+Se and NPs+Ch treated catfish respectively showing a decreased amount of connective tissue fibers around the renal tubules and blood vessels. Renal tubules (black arrow) and blood vessels (blue arrow). Picrosirius red stain. Scale bar = 20 µm.

 doi:10.20944/preprints202401.0955.v1

4. Discussion

Nanoplastics are contaminants of growing worldwide concern that could be hazardous to the aquatic ecosystem and human beings [48].

The current results showed a high mortality rate in the NPs exposed group compared to the control group which demonstrated no deaths. One study [49] demonstrated that microplastics (MPs) induced a lower survival rate in fish. The rise in the mortality rate in NPs exposed group may be attributed to intestinal blockage of the gut or tissue abrasions, which may cause lining epithelium injury, morbidity, and mortality [50,51].

The present study revealed that using selenium reduced fish mortality percentage, which is a similar result to [52], who reported that a diet supplemented with Se (0.21– 0.8 mg Se/kg) increased the survival rate of cobia (*Rachycentron canadum L*) juveniles. Furthermore, this result is similar to the present study as using *Chlorella* reduced the percentage of mortalities of the fish. Also, this result is in harmony with [53], who mentioned that adding *C. vulgaris* to the diet of *rohu Labeo rohita* enhanced the survival rate and phagocytic and antioxidant activity.

The obtained data showed that NPs enhanced oxidative stress by elevating the MDA level in catfish serum. Previous research supports that nanoplastics trigger ROS overproduction and oxidative stress, consistent with the current findings [54–56]. Moreover, [57] reported that exposing *A. franciscana* to PS NPs (50 nm) for 48 h significantly increased lipid peroxidation.

The alkaline comet assay was used in the current study for DNA damage detection in *C. gariepinus* blood. The results revealed that fish exposed to PS NPs showed a significant incline in DNA damage parameters (tail length, tail DNA %, tail moment, and Olive tail moment). This result is similar to [58], who reported that short-term exposure to different concentrations of 100 nm PS NPs (from 0.05 up to 50 mg/L) induced DNA damage in the hemocytes of the Mediterranean mussel (*Mytilus galloprovincialis*).

The findings of this study suggest that DNA damage may have an underlying cause: oxidative stress. These data agree with other studies that showed the association between increased MDA, CAT, and GPX levels and jeopardized DNA integrity [50,59,60]. Furthermore, highly reactive radicals are expected to be the principal cause of oxidative DNA damage and chain breaks of the DNA molecule [61].

Based on the actual data, it has been observed that both microplastics (MPs) and nanoparticles (NPs) can induce DNA strand breaks with the extent of the damage being influenced by their respective size and surface charge. Although the exact mechanism by which MPs/NPs induce DNA damage remains incompletely comprehended, several investigations suggest that oxidative stress and physical interaction are pivotal in instigating DNA strand breaks by NPs [62,63]. Additionally, synthetic polymers may have low-molecular-weight fragments of mono- and oligomers, catalysts, artificial stabilizers, and a variety of particular chemical additives such as (phthalates, bisphenol A, stabilizers, pigments, flame retardants, and polychlorinated biphenyls (PCBs)), each of which is substances with genotoxic properties [64].

The findings of previous studies conducted on mussels [50,65,66], and human blood cells [59,67] were in harmony with the current study findings and emphasizing the idea of PS-NPs are contaminants with genotoxic potential.

On the contrary, some studies reported that exposure to PS microparticles did not cause hemocyte genotoxic damage [68,69]. The presence of inconsistent outcomes is expected as the experiments were conducted under diverse circumstances, encompassing distinct plastic particles varieties, configurations and densities, in addition to variations in temperature and duration of the experiment. The present investigation employed the erythrocyte micronucleus (EMN) assay due to the robust correlation between the micronucleus and comet assays for various nanomaterials. [70]. The results revealed that exposure of catfish to PS NPs increased the frequency of micronucleus and several alterations in RBCs. These findings were similar to the study conducted by [71] on *Sparus aurata* erythrocytes. Guimarães et al. [50] concluded that PS NPs exposure caused erythrocyte morphometric changes in juveniles of *Ctenopharyngodon idella*.

According to Thomas et al. [72], MN happens when the structure or function of the mitotic apparatus is disrupted, failing to properly include chromosomes or fragments of chromosomes in the central nucleus.

 doi:10.20944/preprints202401.0955.v1

The increase in erythrocyte nuclear abnormalities observed after the toxicity with PS NPs may be related to NPs' interaction with the chromosomal material or the oxidative stress induced by the NPs. Multiple studies have reported that oxidative stress is a consequence of exposure to NPs [17,73]. The erythrocyte vacuoles seen could come from an uneven distribution of hemoglobin, as shown by [74]**.** Fish need oxygen to breathe, and erythrocyte deformations lower oxygen levels, which disrupt the circulatory system and impair breathing. This respiratory stress may change the number and form of fish erythrocytes [75]. Catfish and *D. rerio* exposed to microplastics showed these erythrocyte changes, including a considerable increase in the frequency of nuclear abnormalities and morphological RBC changes [76,77].

The current study revealed that selenium and *C. vulgaris* supplementation had improved all measured parameters compared to the NPs exposed group. Where MDA activity, DNA damage, MN, and erythrocyte abnormalities were decreased. These outcomes are consistent with that obtained by [78,79]**,** who reported that Se reduces MDA activity and increases the antioxidant levels in fish.

Bera et al. [80] explained that the protection of selenium against DNA damage might be because it is an essential constituent of selenoproteins as thioredoxin reductases and glutathione peroxidases. These proteins are crucial in antioxidant defense. Selenium therefore, may protect against DNA damage by elevating DNA glycosylase repair enzyme activity and enhancing pathways of DNA damage repair involving p53 gene.

The natural antioxidant components of *C. vulgaris* can scavenge free radicals that are responsible for their protective effect [81]. On the other hand, Bengwayan et., al. [82] explained that *C. vulgaris* has an inhibitory effect on lipid peroxidation. Also, Wu et., al. [83] stated that *C. vulgaris* possesses various therapeutic characteristics, such as anti-inflammatory, immunomodulatory, and antioxidant activities, which may be extremely important for animal health. They increase SOD and CAT activity, guard against DNA oxidation and lipid peroxidation, and activate cellular antioxidant enzymes.

The results revealed that *C. vulgaris* reduced micronucleus frequency and other morphological abnormalities in the erythrocyte. These findings were matched with [76]**,** who demonstrated that treating microplastics-intoxicated *C. gariepinus* with *Chlorella* reduced genotoxicity and cytotoxicity in the blood.

Pathological changes observed in NPs-intoxicated *C. gariepinus* kidney sections were comparable to those demonstrated by [84], who studied the nephrotoxic effect of NPs and MPs in mice and observed that intoxicated kidneys showed necrosis of tubular epithelium, associated with loss of tubular brush border and tubulointerstitial fibrosis. Chen et al. [85] found that PS MPs had an oxidative nephrotoxic effect on HEK293 cells induced by inhibiting the antioxidant haem oxygenase-1. Also they observed that MPs induced apoptosis and autophagy via the depolarization of mitochondrial membranes and the formation of autophagosomes. These results support our hypothesis that PS NPs could induce nephrotoxic effects through oxidative-antioxidant homeostasis disturbance.

Administration of selenium in the NPs+Se group ameliorated and reduced the nephrotoxic effects of nanoplastics. Minimal pathological changes in the tubular epithelium were noticed, and the normal histological appearance was moderately restored. This ameliorative role of Se was also confirmed by [86], who mentions that Se has a protective role against oxidative stress, apoptosis, and kidney damage caused by zearalenone (ZEA) in mice. Zhang et al., [86] found that Se restored alterations in the biochemical and antioxidant indicators of ZEA-induced kidney damage. Moreover, Se reduced the expression of proteins and genes associated with endoplasmic reticulum stress and apoptosis caused by ZEA.

Administration of *C. vulgaris* in the NPs+Ch treated group almost prevents the nephrotoxic effects of nanoplastics and moderately improves the kidney's histology. Consistent with the current results, Latif et al., [87] stated that *C. vulgaris* reduces the adverse effects of paracetamol intoxication on hematological, biochemical, oxidative stress, and histopathological levels. In addition, they found that *C. vulgaris* had antioxidant, nephro-, hepato-, and cardioprotective effects against paracetamol-induced toxic effects in Wister rats.

5. Conclusion

The introduction of nano- and microplastic particles into the environment poses a significant threat to sustainable catfish aquaculture. The current research demonstrated that PS NPs have

deleterious adverse effects on DNA integrity, erythrocytes, oxidative/antioxidant status, and kidneys of African catfish. Administration of selenium and C. vulgaris in NPs exposed fish ameliorated and decrease alterations and to some degree restored the normal kidney morphology. Therefore, in sustainable catfish aquaculture selenium and C. vulgaris can be used as prophylactic agents to decrease the PS NPs toxicity.

Author Contributions: Conceptualization, Zakaria M. Zaky, Doha Yahia and Alaa El-Din H. Sayed; Methodology, Shimaa A. Abdelbaky, Doha Yahia, Mohamed H. Kotob, Mohammed A. Ali, Mohammed Aufy and Alaa El-Din H. Sayed; Validation, Mohamed H. Kotob; Formal analysis, Shimaa A. Abdelbaky, Mohammed A. Ali and Mohammed Aufy; Investigation, Shimaa A. Abdelbaky and Mohamed H. Kotob; Resources, Alaa El-Din H. Sayed; Data curation, Mohammed A. Ali; Writing – original draft, Shimaa A. Abdelbaky and Mohamed H. Kotob; Writing – review & editing, Zakaria M. Zaky, Doha Yahia, Mohamed H. Kotob, Mohammed A. Ali, Mohammed Aufy and Alaa El-Din H. Sayed; Visualization, Mohammed Aufy; Supervision, Zakaria M. Zaky, Doha Yahia and Alaa El-Din H. Sayed; Project administration, Zakaria M. Zaky. All authors agree to publish the current study.

Funding: There is no fund received by the authors to conduct this experiment.

Institutional Review Board Statement: The research protocol mentioned above has received approval from the veterinary ethical committee of the Faculty of Veterinary Medicine at Assiut University, Egypt, under the reference number 06/2022/0016.

Conflict of Interest: The authors report no declaration of competing interest.

References

1. Nag, A.; Baksi, A.; Ghosh, J.; Kumar, V.; Bag, S.; Mondal, B.; Ahuja, T.; Pradeep, T. Tribochemical degradation of polytetrafluoroethylene in water and generation of nanoplastics. ACS Sustain. Chem. Eng. 2019, 7, 17554–17558.
2. Mattsson, K.; Johnson, E.V.; Malmendal, A.; Linse, S.; Hansson, L.A.; Cedervall, T. Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain. Sci. Rep. 2017, 7, 11452.
3. Zhu, F.; Zhu, C.; Wang, C.; Gu, C. Occurrence and ecological impacts of microplastics in soil systems: A review. Bull. Environ. Contam. Toxicol. 2019, 102, 741–749.
4. Álvarez-Ruiz, R.; Picó, Y. Analysis of emerging and related pollutants in aquaticbiota. Trends Environ. Anal. Chem. 2020, 25, 00082.
5. Jambeck, J.R.; Geyer, R.; Wilcox, C.; Siegler, T.R.; Perryman, M.; Andrady, A.; Narayan, R; Law, K.L. Marine pollution. Plastic waste inputs from land into the ocean. Science 2015, 347, 768–771.
6. Babayemi, J. O.; Nnorom, I. C.; Osibanjo, O.; Weber, R. Ensuring sustainability in plastics use in Africa: consumption, waste generation, and projections. Environ. Sci. Europe 2019, 31, 1–20.
7. Song, Y. K.; Hong, S. H.; Jang, M.; Han, G. M.; Jung, S. W.; Shim, W. J. Combined effects of UV exposure duration and mechanical abrasion on microplastic fragmentation by polymer type. Environ. Sci. Technol. 2017, 51, 4368–4376.
8. Santillo, D.; Miller, K.; Johnston, P. Microplastics as contaminants in commercially important seafood species. Integr. Environ. Assess. Manag. 2017, 13, 516–521.
9. Karami, A.; Golieskardi, A.; Keong Choo, C.; Larat, V.; Galloway, T. S.; Salamatinia, B. The presence of microplastics in commercial salts from different countries. Sci. Rep. 2017, 7, 46173.
10. Mason, S. A.; Welch, V. G.; Neratko, J. Synthetic polymer contamination in bottled water. Front. Chem. 2018, 407.
11. Jani, P.; Halbert, G. W.; Langridge, J.; Florence, A. T. Nanoparticle uptake by the rat gastrointestinal mucosa: quantitation and particle size dependency. J. Pharm. Pharmacol. 1990, 42, 821–826.
12. Walczak, A. P.; Kramer, E.; Hendriksen, P. J.; Tromp, P.; Helsper, J. P.; van der Zande, M.; Rietjens I.M.C.M.; Bouwmeester H.; Bouwmeester, H. Translocation of differently sized and charged polystyrene nanoparticles in in vitro intestinal cell models of increasing complexity. Nanotoxicology. 2015, 9, 453–461.
13. Prata, J. C.; da Costa, J. P.; Lopes, I.; Duarte, A. C.; Rocha-Santos, T. Environmental exposure to microplastics: An overview on possible human health effects. Sci. Total Environ. 2020, 702, 134455.
14. Rahman, A.; Sarkar, A.; Yadav, O. P.; Achari, G.; Slobodnik, J. Potential human health risks due to environmental exposure to nano-and microplastics and knowledge gaps: a scoping review. Sci. Total Environ. 2021, 757, 143872.
15. Yee, M. S. L.; Hii, L. W.; Looi, C. K.; Lim, W. M.; Wong, S. F.; Kok, Y. Y.; Tan, B.K.; Wong, C.Y.; Leong, C.O.; Leong, C. O. Impact of microplastics and nanoplastics on human health. Nanomaterials 2021, 11, 496.

16. Jeong, C. B.; Kang, H. M.; Lee, M. C.; Kim, D. H.; Han, J.; Hwang, D. S.; Souissi, S.; Lee, S. J.; Shin, K.H.; Park, H.G.; Lee, J. S. Adverse effects of microplastics and oxidative stress-induced MAPK/Nrf2 pathway-mediated defense mechanisms in the marine copepod Paracyclopina nana. Sci. Rep. 2017, 7, 1–11.
17. Brandts, I.; Cánovas, M.; Tvarijonaviciute, A.; Llorca, M.; Vega, A.; Farré, M.; Teles, M. Nanoplastics are bioaccumulated in fish liver and muscle and cause DNA damage after a chronic exposure. Environ. Res. 2022, 113433.
18. Chen, Q.; Yin, D.; Jia, Y.; Schiwy, S.; Legradi, J.; Yang, S.; Hollert, H., Enhanced uptake of BPA in the presence of nanoplastics can lead to neurotoxic effects in adult zebrafish. Sci. Total Environ. 2017, 609, 1312–1321.
19. Canesi, L.; Ciacci, C.; Fabbri, R.; Balbi, T.; Salis, A.; Damonte, G.; Cortese, K.; Caratto, V.; Monopoli, M.P., Dawson, K., Bergami, E.; Corsi, I. Interactions of cationic polystyrene nanoparticles with marine bivalve hemocytes in a physiological environment: Role of soluble hemolymph proteins. Environ. Res. 2016, 150, 73–81.
20. Han, Y.; Lian, F.; Xiao, Z.; Gu, S.; Cao, X.; Wang, Z.; Xing, B. Potential toxicity of nanoplastics to fish and aquatic invertebrates: Current understanding, mechanistic interpretation, and meta-analysis. J. Hazard Mater. 2021, 127870.
21. Pienaar, U. D. V. The freshwater fished of the Kruger National Park. Koedoe 1968, 11, 1–82.
22. Skelton, P. A Complete Guide to the Freshwater Fishes of Southern Africa. Halfway House: Southern Book Publishers Ltd. 1993.
23. Teugels, G. A systematic revision of the African species of the genus Clarias (Pisces: *Clariidae*). Annales Musee Royal de l'Afrique Centrale 1986, 247, 1–199.
24. Nwani, C. D.; Ifo, C. T.; Nwamba, H. O.; Ejere, V. C.; Onyishi, G. C.; Oluah, S. N.; Ikwuagwu, O.E.; Odo, G. E. Oxidative stress and biochemical responses in the tissues of African catfish *Clarias gariepinus* juvenile following exposure to primextra herbicide. Drug Chem. Toxicol. 2015, 38, 278–285.
25. Brar, A.; Kumar, M.; Vivekanand, V.; Pareek, N. Photoautotrophic microorganisms and bioremediation of industrial effluents: current status and future prospects. 3 Biotech. 2017, 7, 1–8.
26. Safafar, H.; Wagenen, J.V.; Moller, P. and Jacobsen, C. Carotenoids, phenolic compounds and tocopherols contribute to the antioxidative properties of some microalgae species grown on industrial wastewater. Marine Drugs 2015, 13, 7339–7356.
27. Hamilton, S.J. Review of selenium toxicity in the aquatic food chain. Sci. Total Environ. 2004, 326, 1–31.
28. Rayman, M. P. The importance of selenium to human health. The lancet 2000, 356, 233–241.
29. Jackson, S.P.; Bartek, J. The DNA-damage response in human biology and disease, Nature 2009 461, 1071–1078.
30. Mohamed, S. A. K. S.; Upreti, S.; Rajendra, S. V.; Dang, R. Genotoxicity: Mechanisms, testing guidelines and methods. Int. J. Pharm. Pharm. Sci. 2017, 1, 133–138.
31. Gontijo, A.M.M.C.; Barreto, R.E.; Speit, G.; Reyes, V.A.V.; Volpato, G.L.; Salvadori, D.M.F. Anesthesia of fish with benzocaine does not interfere with comet assay results. Mutat Res. 2003, 534, 165–172.
32. Bolognesi, C.; Hayashi, M. Micronucleus assay in aquatic animals. Mutagenesis 2011, 26, 205–213.
33. Braham, R. P.; Blazer, V. S.; Shaw, C. H.; Mazik, P. M. Micronuclei and other erythrocyte nuclear abnormalities in fishes from the Great Lakes Basin, USA. Environ. Mol. Mutagen. 2017, 58, 570–581.
34. Pitt. J.A.; Trevisan, R.; Massarsky, A.; Kozal, J.S.; Levin, E.D.; Di Giulio, R.T. Maternal transfer of nanoplastics to offspring in zebrafish (*Danio rerio*): a case study with nanopolystyrene. Sci. Total Environ. 2018, 643, 324–334.
35. Durigon, E.G.; Kunz, D.F.; Peixoto, N.C.; Uczay, J.; Lazzari, R. Diet selenium improves the antioxidant defense system of juveniles Nile tilapia (Oreochromis niloticus L.). Braz. J. Biol. 2019, 79, 527–532.
36. Bai, S.; Koo, J.; Kim, K.; Kim, S. Effects of *Chlorella* powder as a feed additive on growth performance in juvenile Korean rockfish, Sebastesschlegeli (*Hilgendorf*). Aquacult Res. 2001, 32, 92–98.
37. Wilson, J.M.; Bunte, R.M.; Carty, A.J. Evaluation of rapid cooling and tricaine methanesulfonate (ms222) as methods of euthanasia in zebrafish (*Danio rerio*). J. Am. Assoc. Lab. Anim. Sci. 2009, 48, 785–789.
38. Bello, O. S.; Olaifa, F. E.; Emikpe, B. O. Haematological and blood biochemical changes in African catfish, *Clarias gariepinus* fed walnut (*Tetracarpidium conophorum Mull Arg*) leaf and onion (Allium cepa Linn.) bulb supplemented diets. Am. J. Exp. Agric. 2014, 4, 1593–1603.
39. Kei, S. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica. Chimica. Acta. 1978, 90, 37–43.
40. Aebi, H. Catalase in vitro. In Methods in enzymology. Academic press 1984, 105,121–126.
41. Abd Ellah, M. R.; Niishimori, K.; Goryo, M.; Okada, K.; Yasuda, J. Glutathion peroxidase and glucose-6-phosphate dehydrogenase activities in bovine blood and liver. J. Vet. Med. Sci. 2004, 66, 1219–1221.
42. Villela, I.V.; Oliveira I.M.; Silva, J.; Henriques, J.A.P. DNA damage and repair in haemolynph cells of golden mussel (*Limnoperna fortunei*) exposed to environmental contaminants. Mutat. Res. 2006, 605, 78–86.
43. Pascoe, S.; Gatehouse, D. The use of a simple haematoxylin and eosin staining procedure to demonstrate micronuclei within rodent bone marrow. Mutat. Res. 1986, 164, 237–243.

44. Carrasco, K.R.; Tilbury, K.L.; Myers, M.S. Assessment of the piscine micronucleus test as an in situ biological indicator of chemical contaminant effects. Can. Aquat. Sci. 1990, 47, 2123–2136.
45. Fischer, AH.; Jacobson, KA.; Rose, J.; Zeller, R. Hematoxylin and Eosin Staining of Tissue and Cell Sections. Cold Spring Harbor. Protocol, pdb. Prot. 2008, 4986.
46. McManus, J.F.A. Histological demonstration of mucin after periodic acid. Nature 1946, 158, 202.
47. Bhutda, S.; Surve, M.V.; Anil. A.; Kamath, K.; Singh. N.; Modi, D; Banerjee, A. Histochemical Staining of Collagen and Identification of Its Subtypes by Picrosirius Red Dye in Mouse Reproductive Tissues. Bio-Protocol 2017, 7, 2592–2592.
48. Singh, S.; Naik, T. S. K.; Anil, A. G.; Dhiman, J.; Kumar, V.; Dhanjal, D. S.; Ramamurthy, P. C. Micro (nano) plastics in wastewater: A critical review on toxicity risk assessment, behaviour, environmental impact and challenges. Chemosphere 2022, 290, 133169.
49. Jacob, H.; Besson, M.; Oberhaensli, F.; Taylor, A.; Gillet, B.; Hughes, S.; Melvi, S.D.; Bustamante, P.; Swarzenski, P.W.; Lecchini, D.; Metian, M. A multifaceted assessment of the effects of polyethylene microplastics on juvenile gilthead seabreams (*Sparus aurata*). Aquat. Toxicol. 2021, 241, 106004.
50. Guimarães, A. T. B.; Estrela, F. N.; Pereira, P. S.; de Andrade Vieira, J. E., de Lima Rodrigues, A. S.; Silva, F. G.; Malafaia, G. Toxicity of polystyrene nanoplastics in *Ctenopharyngodon idella* juveniles: a genotoxic, mutagenic and cytotoxic perspective. Science of The Total Environment, 2021, 752, 141937.
51. Lu, Y.F.; Zhang, Y.; Deng; Y.F.; Jiang, W.; Zhao, Y.P.; Geng, J.J.; Ding, L.L.; Zhao, Y. Uptake and accumulation of polystyrene microplastics in zebrafish (Danio rerio) and toxic effects in liver. Environ. Sci. Technol. 2016, 50, 4054–4060.
52. Liu, K.; Wang, X.; Ai, Q.; Mai, K.; Zhang, W. Dietary selenium requirement for juvenile cobia, *Rachycentron canadum* L. Aquac. Res. 2010, 41, e594–e601.
53. Andrews, S.R.; Sahu, N.P.; Pal, A.K.; Kumar, S. Haematological modulation and growth of *Labeo rohita* fingerlings: effect of dietary mannan-oligosaccharide, yeast extract, protein hydrolysate and Chlorella sp. Aquacult. Res. 2009, 41: 61–69.
54. Eom, H.J.; Nam, S.E.; Rhee, J.S. Polystyrene Microplastics Induce Mortality through Acute Cell Stress and Inhibition of Cholinergic Activity in a Brine Shrimp. Mol. Cell. Toxicol. 2020, 16, 233–243.
55. Kim, J.; Rhee, J.S. Biochemospherical and Physiological Responses of the Water Flea Moina Macrocopa to Microplastics: A Multigenerational Study. Mol. Cell. Toxicol. 2021 17, 523–532.
56. Lee, D.H.; Lee, S. Rhee, J.S. Consistent exposure to microplastics induces age-specific physiological and biochemical changes in a marine mysid. Mar. Pollut. Bull. 2021, 162, 111850.
57. Varó, I.; Perini, A.; Torreblanca, A.; Garcia, Y.; Bergami, E.; Vannuccini, M. L.; Corsi, I. Time-dependent effects of polystyrene nanoparticles in brine shrimp *Artemia franciscana* at physiological, biochemical and molecular levels. Sci. Total Environ. 2019, 675, 570–580.
58. Brandts, I.; Teles, M.; Gonçalves, A.P.; Barreto, A.; Franco-Martinez, L.; Tvarijonaviciute, A. Oliveira, M. Effects of nanoplastics on Mytilus galloprovincialis after individual and combined exposure with carbamazepine. Sci. Total Environ. 2018, 643, 775–784.
59. Gopinath, P.M.; Saranya, V.; Vijayakumar, S.; Mythili Meera, M.; Ruprekha, S.; Kunal, R.; Pranay, A.; Thomas, J.; Mukherjee, A.; Chandrasekaran, N. Assessment on interactive prospectives of nanoplastics with plasma proteins and the toxicological impacts of virgin, coronated and environmentally released-nanoplastics. Sci. Rep. 2019, 9, 1–15.
60. Shah, N.; Khan, A.; Habib Khan, N. and Khisroon, M. 2020, Genotoxic consequences in common grass carp (*Ctenopharyngodon idella* Valenciennes, 1844) exposed to selected toxic metals. Biol. Trace Elem. Res. 199, 305–314.
61. Ribeiro, F.; Garcia, A. R.; Pereira, B. P.; Fonseca, M.; Mestre, N. C.; Fonseca, T. G.; Bebianno, M. J. Microplastics effects in *Scrobicularia plana*. Mar. Pollut. Bull. 2017, 122 , 379–391.
62. Hu, M.; Palić, D. Micro-and nano-plastics activation of oxidative and inflammatory adverse outcome pathways. Redox Biol. 2020, 37, 101620.
63. Mazur, A. A.; Chelomin, V. P.; Zhuravel, E. V.; Kukla, S. P.; Slobodskova, V. V.; Dovzhenko, N. V. Genotoxicity of polystyrene (PS) microspheres in short-term exposure to gametes of the sand dollar *Scaphechinus mirabilis* (Agassiz, 1864)(Echinodermata, Echinoidea). J. mar. sci. eng. 2021, 9, 1088.
64. Hamlin, H.J.; Marciano, K.; Downs, C.A. Migration of nonylphenol from food-grade plastic is toxic to the coralreef fish species Pseudochromis fridmani. Chemosphere 2015, 139, 223–228.
65. Gonçalves, J.M.; Sousa, V.S.; Teixeira, M.R.; Bebianno, M.J. Chronic toxicity of polystyrene nanoparticles in the marine mussel *Mytilus galloprovincialis*. Chemosphere 2022, 287, 132356.
66. Estrela, F.N.; Batista Guimarães, A.T.; Silva, F.G.; Marinho da Luz, T.; Silva, A.M.; Pereira, P.S.; Malafaia, G. Effects of polystyrene nanoplastics on *Ctenopharyngodon idella* (grass carp) after individual and combined exposure with zinc oxide nanoparticles. J. Hazard Mater. 2021, 403, 123879.
67. Ballesteros, S.; Domenech, J.; Barguilla, I.; Cortés, C.; Marcos, R.; Hernández, A. Genotoxic and immunomodulatory effects in human white blood cells after ex vivo exposure to polystyrene nanoplastics. Environ. Sci Nano. 2020,7, 3431– 3446.

68. Magni, S.; Gagné, F.; André, C.; Della Torre, C.; Auclair, J.; Hanana, H.; Parenti, C.C.; Bonasoro, F.; Binelli, A. Evaluation of uptake and chronic toxicity of virgin polystyrene microbeads in freshwater zebra mussel Dreissena polymorpha (*Mollusca: Bivalvia*). Sci. Total Environ. 2018, 631–632, 778–788.
69. Xia, B.; Zhang, J.; Zhao, X.; Feng, J.; Teng, Y.; Chen, B.; Sun, X.; Zhu, L.; Sun, X.; Qu, K. Polystyrene microplastics increase uptake, elimination and cytotoxicity of decabromodiphenyl ether (BDE-209) in the marine scallop *Chlamys farreri*. Environ. Pollut. 2020, 258, 113657.
70. Karlsson, H.L. The comet assay in nanotoxicology research. Anal Bioanal Chemosphere 2010, 398, 651–666.
71. Brandts, I.; Barría, C.; Martins, M.A.; Franco-Martínez, L., Barreto, A.; Tvarijonaviciute, A.; Tort, L.; Oliveira, M.; Teles, M. Waterborne exposure of gilthead seabream (*Sparus aurata*) to polymethylmethacrylate nanoplastics causes effects at cellular and molecular levels. J. Hazard. Mater. 2021, 644; 403, 123590.
72. Thomas, P.; Holland, N.; Bolognesi, C.; Kirsch-Volders, M.; Bonassi, S.; Zeiger, E.; Knasmueller, S.; Fenech, M. Buccal micronucleus cytome assay. Nat. Protoc. 2009, 4, 825–837.
73. Jacob, H.; Besson, M.; Swarzenski, P.W.; Lecchini, D.; Metian, M. Effects of virgin micro- and nanoplastics on fish: trends, meta-analysis, and perspectives. Environ. Sci. Technol. 2020, 54, 4733–4745.
74. Ateeq, B.; Ali, M. N.; Ahmad, W. Induction of micronuclei and erythrocyte alterations in the catfish Clarias batrachus by 2, 4-dichlorophenoxyacetic acid and butachlor. Mutat. Res. 2002, 518, 135–144.
75. Brown, L. Aquaculture for veterinarians: fish husbandry and medicine Pergamon press, Oxford, North Chicago, USA 1993, 440 pp.
76. Sayed, A. H.; Hamed, M.; Badrey, A. E.A.; Soliman, H.A.M. Bioremediation of hemotoxic and oxidative stress induced by polyethylene microplastic in *Clarias gariepinus* using lycopene, citric acid, and *Chlorella*. Comp. Biochem. Physiol. 2021, Part C 250, 109189.
77. Da Costa Araujo, A.P.; de Andrade Vieira, J.E; Malafaia, G. Toxicity and trophic transfer of polyethylene microplastics from Poecilia reticulata to Danio rerio. Sci. Total Environ. 2020, 742, 140217.
78. Ashouri, S.; Keyvanshokooh, S.; Salati, A. P.; Johari, S. A.; Pasha-Zanoosi, H. Effects of different levels of dietary selenium nanoparticles on growth performance, muscle composition, blood biochemical profiles and antioxidant status of common carp (*Cyprinus carpio*). Aquaculture 2015, 446, 25–29.
79. Saffari, S.; Keyvanshokooh, S.; Zakeri, M.; Johari, S. A.; Pasha-Zanoosi, H. J. A. N. Effects of different dietary selenium sources (sodium selenite, selenomethionine and nanoselenium) on growth performance, muscle composition, blood enzymes and antioxidant status of common carp (*Cyprinus carpio*). Aquac. Nutr. 2017, 23, 611–617.
80. Bera, S.; Rosa, V. D.; Rachidi, W.; Diamond, A. M. Does a role for selenium in DNA damage repair explain apparent controversies in its use in chemoprevention?. Mutagenesis 2013, 28, 127–134.
81. Abdelhamid, F. M.; Elshopakey, G. E.; Aziza, A. E. Ameliorative effects of dietary *Chlorella vulgaris* and β-glucan against diazinon-induced toxicity in Nile tilapia (*Oreochromis niloticus*). Fish Shellfish Immunol. 2020, 96, 213–222.
82. Bengwayan, P.T.; Laygo, J.C.; Pacio, A.E.; Poyaoan, J.L.Z.; Rebugio, J.F.; Yuson, A.L.L. A comparative study on the antioxidant property of *Chlorella* (*Chlorella sp*.) tablet and glutathione tablet. Int. J. Sci. Res. 2010, 2, 25–35.
83. Wu, Q.; Liu, L.; Miron, A.; Klímová, B.; Wan, D.; Kuča, K. The antioxidant, immunomodulatory, and anti-inflammatory activities of *Spirulina*: an overview. Arch. Toxicol. 2016, 90, 1817–1840.
84. Meng, X.; Zhang, J.; Wang, W.; Gonzalez-Gil, G.; Vrouwenvelder, J. S.; Li, Z. Effects of nano-and microplastics on kidney: Physicochemospherical properties, bioaccumulation, oxidative stress and immunoreaction. Chemosphere 2022, 288, 132631.
85. Chen, Y.C.; Chen, K.F.; Lin, K.Y.A.; Chen, J.K.; Jiang, X.Y.; Lin, C.H. The nephrotoxic potential of polystyrene microplastics at realistic environmental concentrations. J. Hazard. Mat. 2022, 427, 127871.
86. Zhang, Y.; Hu, B.; Wang, M.; Tong, J.; Pan, J.; Wang, N.; Gong, P.; Long, M. Selenium protects against zearalenone-induced oxidative stress and apoptosis in the mouse kidney by inhibiting endoplasmic reticulum stress. Oxidative Med. Cell. Longev, 2020.
87. Latif, A. A. E.; Assar, D. H. Elkaw, E. M.; Hamza, H. A.; Alkhalifah, D. H. M.; Hozzein, W. N.; Hamouda, R. A. 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. 2021, 11, 3911.