



OPEN A multi-biomarker approach to assess the exposure effects of MPs and/or sodium lauryl sulfate on *Clarias Gariepinus*

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Pollutants impact fish health, leading to environmental diseases. Anionic surfactant detergents and MPs (MPs) consist serious threat in freshwater environments either lonely or in combination. However, their combined effects are not well studied. Our research therefore focuses on studying the harmful effects of the anionic surfactant sodium lauryl sulfate (SLS) and polyethylene MPs (PMPs) on the biology of freshwater African catfish (*Clarias gariepinus*), either alone or in combination. A 15-day exposure trial to PMPs (10 mg/L), SLS (4 mg/L), or their combination was conducted. Hematological, biochemical, antioxidant, and immunological markers were estimated. The erythrocytic morphology was investigated. The pathological harms were monitored, and the histological abnormalities were scored. In addition, histochemical appraisals of fibrosis and hypoglycemia in the liver and spleen were estimated. This was achieved by semi-quantification of polysaccharide deposits and the fibrotic collagen density and distribution pattern in the tissue micro-sections. On the one hand, our findings revealed deteriorated biological markers in *C. gariepinus* exposed to PMPs (10 mg/L) or SLS (4 mg/L) by close levels. Significant decreases in the hematological indices, while substantial increases in the biochemical markers were recorded. As well, significant decreases were recorded in the total antioxidant capacity and superoxide dismutase, while an elevation was recorded in the level of IL-1 β and IL-6 cytokines. Poikilocytosis of erythrocytes and severe hepatic and splenic pathological lesions were observed. Furthermore, high levels of fibrosis and hypoglycemia were detected. On the other hand, our findings showed antagonistic effects upon the combined exposure to PMPs (10 mg/L) and SLS (4 mg/L). Fluctuated non-significant differences were observed in hematology and biochemical markers. Mild erythrocytic poikilocytosis and moderate pathological lesions were scored in liver as well as spleen. In addition, moderate quantitative fibrosis, and hypoglycemia were estimated. Exposure to PMPs and SLS deteriorate the biology and pathology of *C. gariepinus* by severe effects. Interestingly, ameliorated biological alterations were recorded evidenced a surprising antagonistic effect of PMPs + SLS. Possibly, a chemical chelation between both reagents counteracted their singular effect inside the biological system, which retorted their harm. Therefore, further investigations by chemists on the probable chemical interaction between PMPs and SLS inside biological systems, which might change their physical or chemical characteristics, may explain the case.

Keywords PMPs, Superoxide dismutase, SLS, Liver, Spleen, fibrosis, Hypoglycemia

Aquatic pollutants produced by anthropogenic activities are the primary cause of fish's disorders. They alter the biology, physiology, and immune defense status of exposed fish, which in turn deteriorates their resistance to infections^{1,2}. Additionally, many environmental pollutants are toxicants and act, at once, as hidden carriers for fish pathogens transferring them deeply inside the living tissues^{3,4}. Physiologically, pollutants in the aquatic environment can alter fish hematological, biochemical, and molecular markers. Therefore, hematological and biochemical indices were employed as bioindicators of abnormalities since the blood is the primary pathway via

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which a material enters the fish^{5,6}. In addition, sensitive indicators of structural changes within cells and tissues of some organs are provided by the histopathological and histochemical testing techniques⁷.

Plastics are widely utilized in manufacturing to improve strength, lower product weight, and provide transparency for applications such as displays⁸. Polymerizing monomers like ethylene and propylene create plastics known as synthetic and semisynthetic materials⁹. In greater demand and several uses, they have raised humankind's standard of living¹⁰. Non-biodegradable plastics are widely used, have contaminated the environment, and progressively accumulated and transmitted through the food chains from animals to humans. Microplastics (MPs) are recently described pollutants that have gained international notice and are now a topic of study in aquatic environmental science. While MPs come from several sources, primary and secondary sources are the frequent pathways they reach the environment that are strongly linked to the widespread use of MPs and their existence^{11,12}. The primary sources are the microbeads of the micro-sized plastic particles added to personal care products like toothpaste, face and hand cleaners, etc^{11,13}. The secondary sources are those micro-sized plastic particles resulting from the physical, chemical, or biological fragmentation of macro- and meso-sized plastic products¹⁴. The enormous input of plastics is considered the primary reason for the contamination of aquatic farms with MPs¹⁵. These MPs are abundantly accumulated in the farmed aquatic species, including fish, which construct the most suppliers of high-quality protein in Egypt's aquaculture that produce > 50% of commercial supply^{16–18}. The bioaccumulation of MPs is more easily due to exogenous factors, such as plastic fishing tools, industrial farming facilities, and equipment, or endogenous factors, such as animal health fortifiers, and feed additives.

Owing to their bioaccumulation, several studies have reported that MPs can elicit eco-toxicological effects in fish, such as anemia, biochemical disturbance, and oxidative stress¹⁹. The histopathological hazards of MPs on fish tissues have been extensively documented and primarily cause inflammations and oxidative stress^{20–23}. Furthermore, fish residues from MPs can pose a variety of risks on aquaculture safety and intimately linked to human health as it has been discovered in human feces^{24,25}. Moreover, because MPs have a strong affinity for hydrophobic chemicals persist in water, they can not only directly harm aquatic organisms, but they can also act as concentrators and transporters of harmful substances^{22,26}.

Surfactants are classified as hazardous toxicants among all other types of pollutants based on their hydrophilicity, which produces anionic, cationic, or non-ionic substances²⁷. They are extensively utilized in a wide range of products, including detergents, personal care products, and household cleaners, paints and dyes, textiles, herbicides, medicines, oils, foods, cosmetics, papers, rubbers, and metal processing²⁸. Usually, surfactants persist in aquatic ecosystems in relatively low quantities, ranging from a few $\mu\text{g L}^{-1}$ to mg L^{-1} ²⁹. One common anionic surfactant is sodium lauryl sulfate (SLS) or sodium dodecyl sulfate (SDS), which is a primary alkyl sulfate belonging to the alcohol phosphate family³⁰. It is highly biodegradable and has a low bioaccumulation rate and does not linger in the environment for long period, therefore it is formally categorized as "environmentally friendly"³¹. Nonetheless, some research has indicated that SLS may be fatal in short-term exposures³². It has been shown that SLS is toxic to fish, echinoderms, bacteria, and microalgae³³.

Noteworthy, the different chemical substrates in the same ecosystem might interact antagonistic or synergistic³⁴. Interactions between MPs and ambient chemical contaminants were reported in natural water systems³⁵. Therefore, combined effects of chemically hazardous pollutants, such as metal ions, detergents, agrochemical pesticides, and MPs on a variety of aquatic animals, mostly fish, have been the subject of various studies conducted more recently^{36,37}.

Recently, our group conducted deep investigations on the nephrotoxicity of PMPs and/or SLS on the African catfish (*Clarias gariepinus*)³⁸. *C. gariepinus* is a common freshwater fish species mostly desirable for aquaculture, and it is a suitable fish model frequently utilized in the biological investigations of toxicological research³⁹. Therefore, the current study employed *C. gariepinus* as a fish model to evaluate the biological and physiological alterations caused by singular and/or combined exposure to nonlethal doses of PMPs and SLS. Several biological indicators of hematological, biochemical, and antioxidant indices were estimated. In addition, some histopathological and histochemical biomarkers were investigated. Here, semi-quantification of pathological traits, fibrosis, and hypoglycemia in fish liver and spleen highlighted the antagonism between MPs and SLS inside the biological tissues. This opened new horizons for further in-deep investigations of the possible chemical chelation between MPs and SLS under water ecosystems in general and inside the biological systems specifically. This antagonistic interaction between MPs and SLS summarizes the novelty of the current investigation.

Materials and methods

Chemicals

PMPs raw powder was purchased from the Toxmerge Pty Ltd. Company (Melbourne, Australia). SLS (> 99% purity) was purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Kits for the analysis were bought from Bio-Diagnostic Co., Cairo, Egypt.

Stock preparation and characterization of MPs (MPs)

The manufacturer's protocol was followed in preparing the stock solution (1 g PMPs/L), which was then kept in the dark at 4 °C, and sonicated using Milli-Q purified water before use. Just before starting each experiment, this stock was further diluted for test concentrations. Scanning electron microscopy (SEM, JEOL JEM-1200 EX II) was used to characterize the morphology of PMPs at Assiut University.

Experimental design

Ethics statement

The in vivo experimental protocol (CSRE-37-24) and the fish handling procedures were approved by the Research and Ethical Committee of the Faculty of Science, Sohag University, Sohag, Egypt. All methods were

carried out in accordance with relevant guidelines and regulations. All methods are reported in accordance with ARRIVE guidelines.

Fish exposure

Purchased from an authorized private farm, healthy *C. gariepinus* with an average body weight of 250–300 g and an average length of 25–30 cm was delivered to the wet Laboratory of Fish Biology and Pollution, Faculty of Science, Assiut University. The fish were checked for pathogen-free status to ensure their healthy condition⁴⁰.

Before starting the experiment, the fish were examined to ensure they were healthy and clear of pathogens. They also spent four weeks becoming used to the lab environment. Fish were reared in 100-liter fiberglass tanks with pH = 7.4, dissolved oxygen = 6.9 mg/L, and a temperature of 20 °C in dechlorinated tap water. After 2 weeks of acclimation to a 12:12 h light/dark photoperiod, fish were given a commercial basal diet at 3% body weight twice daily.

For a 15-day toxicant exposure testing, 64 *C. gariepinus* were divided into four groups in duplicate (8 fish/tank; 16 fish/group). Fish that had not been exposed to toxins were in the first group, called Control, while the fish that had been exposed to toxicants comprised the other three categories. The second group, known as PMPs-exposed, was exposed to 10 mg/L of PMPs; the third group, known as SLS-exposed, was subjected to 4 mg/L of SLS; and the fourth group, known as PMPs + SLS-exposed, was exposed to 10 mg/L of PMPs + 4 mg/L of SLS. The hazardous doses reported in earlier research guides our selection of the exposure dosages of the current study. Besides, our selected doses ensure fish safety throughout the 15-day acute exposure period^{41,42}. As directed by their manufacturer, stock solutions comprising 1 g/L of PMPs or SLS in ultrapure water (Milli-Q) were designated and maintained in the dark at 4 °C for our investigations. To adjust the measured exposure doses, the stock solutions were quickly diluted in the new rearing water and mixed before each rearing water exchange⁴³. After two days of the prior exposure, the exposure regimen was repeated by replacing half of the water in the tanks and doing the PMPs and/or SLS again. All groups were maintained in the same acclimation setup and fed according to the same regimen (3% body weight) for 15 days of exposure.

Blood biological and immunological indices

I. Hematological Parameters.

The following hematological parameters were measured following⁴⁴, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hemoglobin (Hb), Hematocrit (HCT), red blood cell count (RBCs), and the differential count of white blood cells (WBC).

II. Biochemical Parameters.

Using kits from SG Mitalia Company (U.S.A.) and a spectrophotometer (T80 + UV/VIS, Bioanalytic Diagnostic Industry, Co.), total protein, glucose, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were measured in fish serum^{43,45}. *Antioxidant Parameters.*

Serum samples were used to evaluate the total antioxidant capacity (TAC) and SOD activities using previously published techniques^{46–48}. The thiobarbituric acid reaction was used to calculate the malondialdehyde (MDA) level⁴⁹.

III. Inflammatory Signals (Cytokines).

According to Soliman et al.⁵⁰, blood cytokines (interleukin-6 “IL-6” and interleukin-1 β “IL-1 β ”) were quantified in the fish sera commercially using a highly sensitive ELISA set (Human Ultrasensitive, BioSource International Inc.).

IV. Erythrocyte Morphology.

Blood smears of all groups were prepared, fixed, and air-dried. Erythrocytic differentiation was achieved using Hematoxylin & Eosin (H&E) staining. Visual observations of cell morphology under a light microscope (VE-T2) were documented using an attached camera (14 MP OMAX) (A35140U3)^{43,45}.

Histopathology and histochemistry

Small pieces of liver and spleen were meticulously gathered, cleaned, and fixed in 10% neutral buffered formalin for 48 h. They were then dehydrated in ascending concentrations of ethanol, cleared in xylene, wax embedded, and sectioned at 5 μ m. Dewaxing in xylene, and staining for the microscopic examination using Harris's H&E counter stain was conducted to differentiate tissue degenerations and/or cell death⁵¹. The semi-quantitative pathological comparison was conducted as described by⁵² with minor modification. Triplicate tissue sections of each group were examined under an Olympus microscope (BX50F4, Olympus Optical Co., LTP, Japan) and the collective structural damages and abnormalities observed under 40x magnification power in three fields per section ($n = 9$) were monitored and graded as 0 for the normal structure or 1–3 for the gradual severity appraisals (mild, moderate, or severe). The obtained data were manipulated statistically and represented in bar histograms as means \pm SE. In addition, a differential lesion severity grading was assessed for each organ according to Fernandes protocols with some modifications⁵³. The differential severity levels represent the percent of existing trait of a lesion in the nine fields examined per organ. The individual lesion severity was graded in 3 levels according to its frequency. The obtained data were expressed as mild (+), moderate (++), or severe (+++).

Special staining with Sirius Red (SR) stain was used to detect fibrosis⁵⁴, and with Periodic acid Schiff reaction (PAS) was used for the detection of carbohydrate content, primarily glycogen, was carried out following the routine protocol⁵⁵. With slight modifications to Saleh et al.'s semi-quantitative assessments⁵⁶, collagen type I and III fibers in SR-stained sections of the liver, and spleen were statistically quantified to estimate tissue fibrosis using ImageJ software (version 1.41o, Public Domain, BSD-2, <https://imagej.net/Ops>). The software processes were applied to eighteen ($n = 18$) triplicate randomly selected digital images (taken at 40 \times) of the sections from each dissected fish. For every group, two characteristics were displayed in semi-quantification: (i) the growing pattern of these fibers in the whole area (%) and (ii) the density of fibers per 0.5 mm² area of each image that was chosen. The acquired information was represented as Means \pm standard error and shown in histograms. Similarly, the total positively stained area (%) of collagen deposits in randomly chosen digital pictures of PAS-stained sections of the liver from each group (acquired at 40 \times , $n = 18$) was semi-quantified.

Statistical analysis

The obtained data were checked using Pearson's chi-squared test for normality, and the values clustered around the mean, indicating a homogeneous distribution suitable for statistical comparison. Simple descriptive statistics were used to obtain the means and standard error (SE). Duncan's multiple range test (MRT) and a one-way analysis of variance (ANOVA) were used to assess the significance of the mean and standard error comparison. All statistical analyses were conducted using the statistical package for social sciences (SPSS) software (version 17.0 for Windows 10), with a significance threshold of 0.05. P-values of less than 0.01 (**) are regarded as highly significant, and less than 0.001 (***) as very highly statistically significant⁵⁷.

Results

Surface characteristics of PMPs

The PMPs have uneven shape and size as shown by the SEM micrography in Fig. 1.

Hematological indices

Hematological parameters

The hematological indices of RBCs, blood hemoglobin concentration (Hb), hematocrit (Ht), Platelets, and total (WBC), showed a significant ($P < 0.05$) decrease after exposure to PMPs, or SLS, or their combination for 15 days in comparison with the control group. Reversely, MCV level showed a significant increase ($P < 0.05$) in the SLS-exposed group while the levels of MCHC and MCH showed no significant change after exposure to PMPs, or SLS, or their combination for 15 days.

The differential count of WBC showed a significant decrease ($P < 0.05$) in lymphocyte and monocyte percent, while a significant ($P < 0.05$) increase in eosinophil and neutrophil percent after exposure to PMPs, or SLS, or their combination for 15 days compared to the control group (Table 1).

Biochemical parameters

The liver functions showed no significant change in aspartate aminotransferase (AST) level, and a significant ($P < 0.05$) increase in alanine aminotransferase (ALT) level, while a significant ($P < 0.05$) decrease in alkaline phosphatase (ALP) level. The other biochemical parameters of glucose, cholesterol, and total protein levels

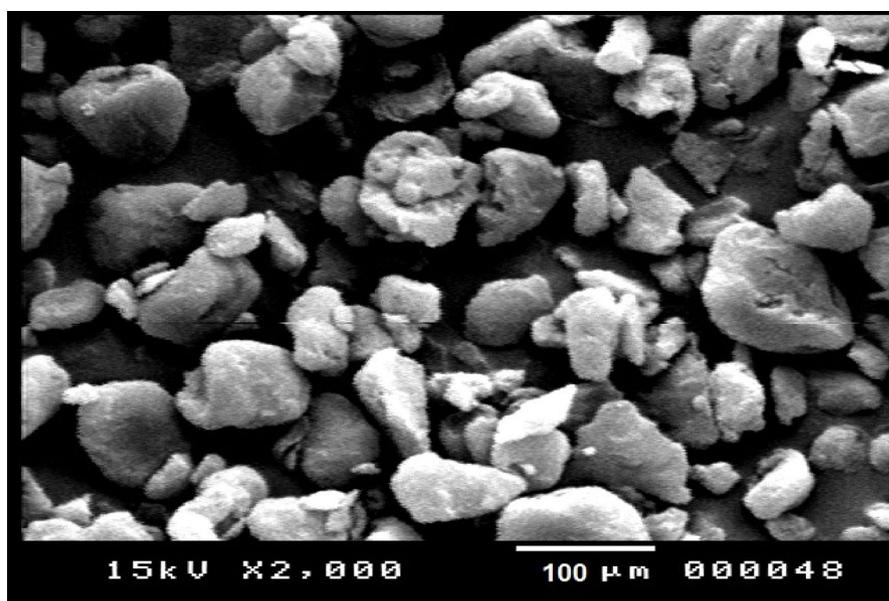


Fig. 1. Photomicrograph displays the irregular-shaped particles of the PMPs in water medium under a scanning electron microscope (JEOL JEM-1200 EX II).

Parameter	Control	PMPs	SLS	PMPs + SLS
RBC's (Million/mm ³)	3.04 ± 0.06 ^a	2.61 ± 0.04 ^b	2.60 ± 0.04 ^b	2.54 ± 0.04 ^b
Hb (g/dL)	8.81 ± 0.4 ^a	7.3 ± 0.3 ^b	7.6 ± 0.2 ^b	7.1 ± 0.3 ^b
Ht (%)	33.96 ± 0.2 ^a	30.91 ± 0.21 ^b	30.91 ± 0.2 ^b	30.19 ± 0.2 ^c
MCV (µm ³)	106 ± 1.4 ^a	113 ± 1.7 ^b	113 ± 1.7 ^b	111 ± 1.7 ^b
MCH (Pg)	27.4 ± 0.7 ^a	26.6 ± 1.0 ^a	26.6 ± 1.02 ^a	26.03 ± 0.9 ^a
MCHC (%)	24.6 ± 0.9 ^a	22.4 ± 0.95 ^a	22.4 ± 0.5 ^a	21.9 ± 0.9 ^a
Thrombocytes (Thousands/mm ³)	211 ± 3.9 ^a	198 ± 1.7 ^b	198 ± 1.7 ^b	193 ± 1.6 ^b
WBC's (Thousands/mm ³)	11.2 ± 0.2 ^a	10.4 ± 0.1 ^b	10.4 ± 0.1 ^b	10.1 ± 0.1 ^b
Neutrophils (%)	11.5 ± 0.2 ^a	13.5 ± 0.2 ^b	13.3 ± 0.3 ^b	13.8 ± 0.2 ^b
Large lymphocyte (%)	58.8 ± 0.2 ^a	55 ± 0.4 ^b	54 ± 0.5 ^{bc}	53.8 ± 0.4 ^c
Small lymphocyte (%)	25.2 ± 0.2 ^a	21.8 ± 0.3 ^b	22.3 ± 0.3 ^b	22.3 ± 0.3 ^b
Monocyte (%)	3.7 ± 0.2 ^a	2.5 ± 0.2 ^b	2.7 ± 0.3 ^b	3.3 ± 0.2 ^a
Eosinophils (%)	2.8 ± 0.2 ^a	7.1 ± 0.4 ^b	7.3 ± 0.2 ^b	6.8 ± 0.2 ^b

Table 1. Single and combined effect of 14-days exposure to 10 mg/L polyethylene MPs (PMPs), and 4 mg/L sodium lauryl sulfate (SLS) on the hematological parameters of the African catfish (*Clarias gariepinus*). * Data are represented as means ± SE (n = 3). Values with different superscript letters in the same row for each parameter are significantly different ($P < 0.05$). Hb: Hemoglobin, Ht: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, and MCHC: Percentage of mean corpuscular hemoglobin concentration.

Parameter	Control	PMPs	SLS	PMPs + SLS
AST (U/L)	32.4 ± 0.8 ^a	33.4 ± 0.6 ^a	33.4 ± 0.6 ^a	32.6 ± 0.6 ^a
ALT (U/L)	16.01 ± 0.4 ^a	17.2 ± 0.3 ^b	17.2 ± 0.3 ^b	16.8 ± 0.3 ^{ab}
ALP (U/L)	44.5 ± 1.5 ^a	40.7 ± 0.99 ^b	40.7 ± 0.99 ^b	39.8 ± 1.0 ^b
Glucose (mg/dL)	69.2 ± 1.0 ^a	74.99 ± 0.4 ^b	74.99 ± 0.41 ^b	73.3 ± 0.5 ^b
Total protein (TP) (mg/dL)	3.89 ± 0.1 ^a	4.5 ± 0.2 ^b	4.5 ± 0.2 ^b	4.4 ± 0.2 ^{ab}
Total Cholesterol (mg/dL)	201 ± 0.9 ^a	215 ± 2.4 ^b	215 ± 2.4 ^b	210 ± 2.3 ^b

Table 2. Single and combined effect of 14-days exposure to 10 mg/L polyethylene MPs (PMPs), and 4 mg/L sodium lauryl sulfate (SLS) on the biochemical parameters of African catfish (*Clarias gariepinus*) blood. * Data are represented as means ± SE (n = 3). Values in the same row with different superscripts are significantly different ($P < 0.05$). AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, and ALP: Alkaline phosphatase.

Parameter	Control	PMPs	SLS	PMPs + SLS
Superoxide dismutase (SOD) (U/mL)	2.6 ± 0.1 ^a	1.9 ± 0.1 ^b	1.9 ± 0.1 ^b	1.8 ± 0.1 ^b
Total antioxidant capacity (nmol/L)	52.1 ± 3.1 ^a	41.2 ± 0.89 ^b	41.2 ± 0.89 ^b	40.2 ± 0.86 ^b
Malondialdehyde (nmol/mL)	15 ± 1.0 ^a	28.6 ± 2.2 ^b	28.6 ± 2.110 ^b	27.9 ± 2.1 ^b

Table 3. Single and combined effect of 14-days exposure to 10 mg/L polyethylene MPs (PMPs), and 4 mg/L sodium lauryl sulfate (SLS) on the antioxidant enzymes and lipid peroxidation in African catfish (*Clarias gariepinus*) blood. * Data are represented as means ± SE (n = 3). Values with different superscripts in the same row are significantly different ($P < 0.05$).

showed a significant ($P < 0.05$) increase after exposure to PMPs, or SLS, or their combination for 15 days in comparison with the control group (Table 2).

Oxidative stress biomarkers

The data presented in Table (3) showed the results of the investigated serum oxidant and antioxidant status of *C. gariepinus*. The total antioxidant capacity and SOD showed significant ($P < 0.05$) decreases in the SLS-exposed group while the Malondialdehyde level showed significant increases ($P < 0.05$) after the exposure to PMPs, or SLS, or their combination for 15 days compared to the control group.

Erythron profile

Blood smears from the control fish revealed normal erythrocytic morphology with a central nucleus and leucocytes (Fig. 2, A). Alterations of erythrocyte shape (poikilocytosis) were observed in the blood smears of fish exposed to PMPs (10 mg/L) in the form of schistocytes, sickle cells, and elliptocytes, tear drop-like cells, vacuolated cells, peripheral nucleus. Smears from fish exposed to SLS (4 mg/L) showed poikilocytosis of erythrocytes including swollen cells, hemolyzed cells, teardrop cells, and vacuolated cells. The blood from fish exposed to combined PMPs + SLS-exposed group showed erythrocyte poikilocytosis including elliptocytes, tear drop-like cells, vacuolated cells, peripheral nucleus, and sickle cells (Figs. 2 B–D).

Histological analyses

Tissue integrity

I. Hepatic Pathology.

As shown in Fig. 3, A liver the control group showed polygonal hepatocytes (H) containing fine-granulated acidophilic cytoplasm with vesicular nuclei (VN), central vein (CV), blood sinusoids (BS), and hepatic veins (HVs). Liver sections from PMPs-exposed fish (Fig. 3, B) showed hydropic degeneration (HD) of whole H, enormous necrotic area (NA), many degenerated or necrotic cells (NC), many pyknotic nuclei (PN) fatty deposition (FD), foci of inflammatory cells (IC), increased BS, and dilated blood vessels (DBV). Liver sections from SLS-exposed fish (Fig. 3, C) showed severe degeneration in hepatic structure, degenerated or necrotic hepatocytes (NC), increased NA, HD, PN, unclear BS, and melanomacrophage centers (MMC) beside congested central vein (CCV). In addition, full red blood corpuscles (RBC) which are surrounded by thick connective tissues (CT) and few FD were also observed. Liver sections from fish exposed to combined doses of MP (10 mg/L) + SLS (4 mg/L) (Fig. 3, D) showed HD, NA, NC, degenerated hepatocytes (DH), PN, FD, and increased BS. The scoring of the general pathological lesions observed in fish liver is represented in a column histogram (Fig. 3, E). In addition, the differential scoring of the observed pathological lesions is collected in Table 4.

II. Splenic pathology.

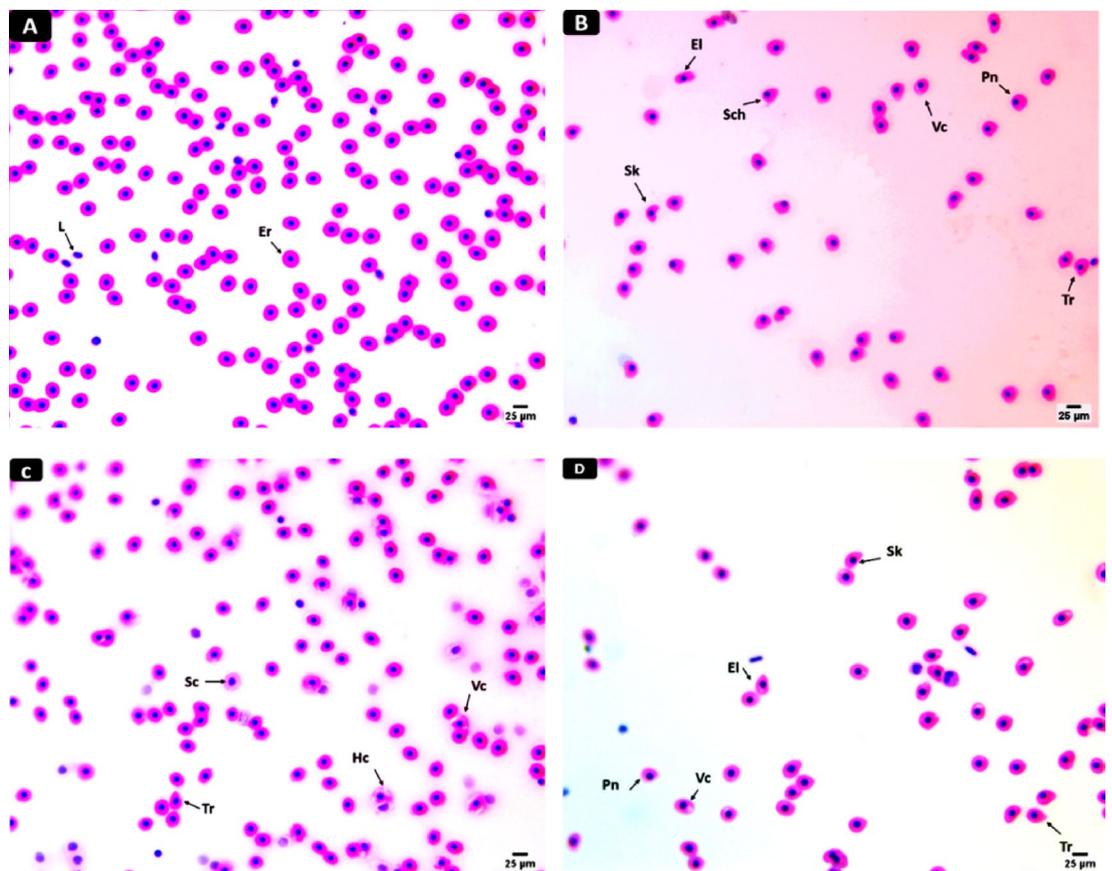


Fig. 2. Micrographs of blood smears stained with H & E ($\times 400$; Scale bar = 25 μm) displaying normal and deformed erythrocytes following exposing *C. gariepinus* to PMPs, SLS or their combination for 15 days. A) Normal erythrocytes, B – D) deformed erythrocytes containing (Er) erythrocytes, (L) leucocytes, (Tr) teardrop cell, (Pn) peripheral nucleus, (Hc) hemolyzed cell, (El) elliptocyte, (Sch) schistocyte, (Vc) vacuolated cells, (Sc) swollen cells, and (Sk) sickle cell.

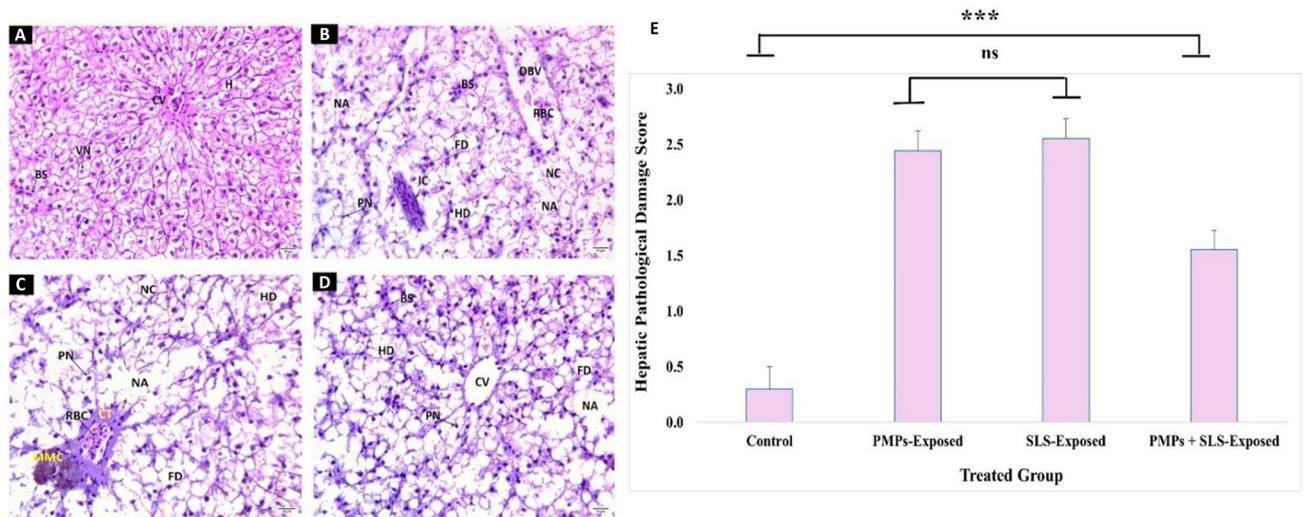


Fig. 3. Photomicrographs of H & E-counterstained sections (×400; scale bar = 25 μm) of *C. gariepinus* liver after 15 days of exposure to PMPs, SLS, or their combination. (A) A micrograph from a control fish demonstrating typical hepatic integrity of H, VN, CV, and BS. (B) A micrograph from a fish subjected to PMPs demonstrates complete HD of hepatic cells with the appearance of IC, RBC, PN, NC, FD, congested BS, NA, and DBV. (C) A micrograph from a fish exposed to SLS displays severe deterioration in hepatic tissue appearing as HD, NA, RBC, PN, and FD. Wide MMC were obvious in CT surrounding the HVs rich in RBC. (D) A micrograph from a fish subjected to PMPs + SLS shows modest histological assessments of the hepatic tissue. HD, NA, PN, FD, and congested BS were noticed around the CV. (E) A bar graph displaying the severity score of the general hepatic histopathological lesion ranging from 0 to 3. The data were estimated at 40x magnification and displayed as means ± SE of nine replicates for each group (n = 9). The statistically significant differences in values between groups (P < 0.05) are indicated by superscript symbols on the bars.

Lesion	Group/Score			
	Control	PMPs-Treated	SLS-Treated	PMPs + SLS-Treated
Hepatic cell degeneration (HD)	-	+++	+++	+++
Enriched inflammatory cells (IC)	-	+++	++	+
Enriched red blood corpuscles (RBC)	+	+++	+++	+
Enriched pyknotic nuclei (PN)	-	+++	+++	++
Enriched fatty deposition (FD)	+	+++	+++	++
Congested blood sinusoids (BS)	-	+++	++	++
Dilated blood vessel (DBV)	-	+++	++	+
Necrotic areas (NA)	-	+++	+++	++
Enriched melano-macrophage centers (MMC)	+	++	+++	++

Table 4. The differential severity scoring of the observed hepato-pathological lesions after the exposure to PMPs and/or SLS. * Symbols indicate the total number of the lesion discovered in 9 examined fields; - no; + mild, ++ moderate, and +++ severe lesion.

Figure 4, A displays the normal architecture of spleen tissue from the control group with white pulp (WP) consisting of aggregated lymphocytes and red pulp (RP) containing hemopoietic tissues mainly of RBC, MMC, and ellipsoid structures (ES), which lined by cubic cells. Spleen sections from the MP-exposed fish (10 mg/kg) (Fig. 4, B) show deformation of the splenic structure, and depletion of lymphocytes (LC). Increased patches of ES, which are surrounded by sinus-containing blood cells, and MMC were increased and distributed among the hemopoietic tissue (HT). Heavily drainage of hemopoietic tissues, mainly RBC, and acidophilic network fibers (F) were markedly increasing. The spleen sections from the SLS-exposed fish (4 mg/kg) (Fig. 4, C) showed dilated and congested blood vessels (DCBV) and small shape ES, depletion of LC, and increased HT, mainly RBCs. MMC were increased, distributed among the HT, and were observed surrounding the ES or near DBV. Spleen sections from the PMPs- and SLS-exposed fish (Fig. 4, D) show increased patches of ES surrounded by sinus-containing blood cells. MMC were increased and distributed among the HT and were observed surrounding the ES associated with depletion of LC, and increased HT, mainly RBCs. The scoring of the general pathological lesions observed in fish spleen is represented in a column histogram (Fig. 4, E). In addition, the differential scoring of the observed pathological lesions is collected in Table 5.

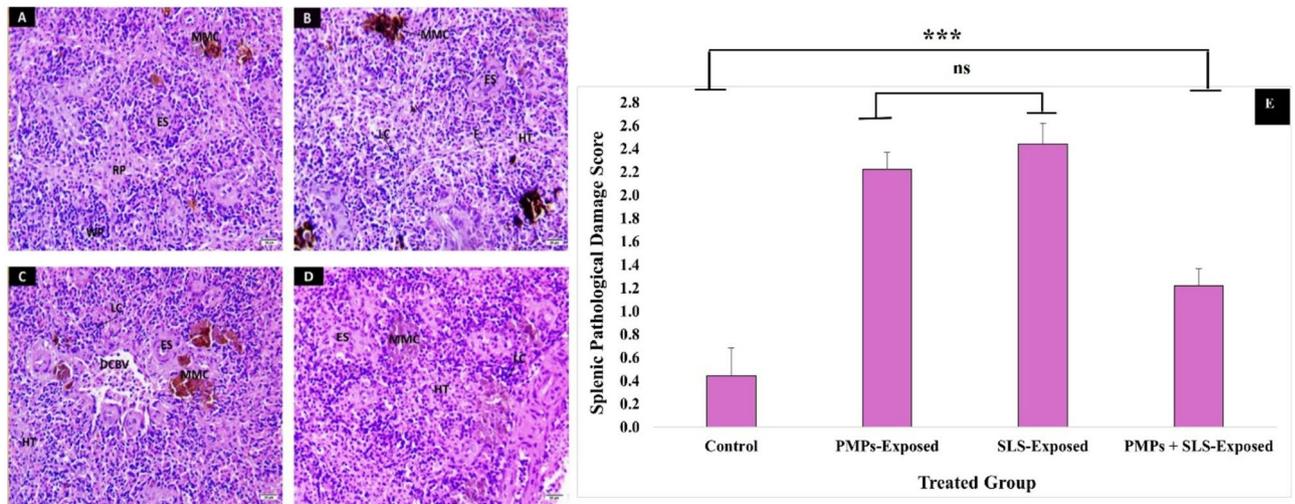


Fig. 4. Photomicrographs of H & E-counterstained sections (×400; scale bar = 25 μm) of *C. gariepinus* spleen after 15 days of exposure to PMPs, SLS, or their combination. (A) A micrograph from a control fish demonstrates typical splenic tissue integrity with intact RP and WP. Limited MMC and ES patch were observed. (B) A micrograph from a fish subjected to PMPs demonstrates an increase in ES patch encircled by an RBC-containing sinus, MMC dispersed throughout the HT, and F. In addition, LC was noticeable. (C) A micrograph from a fish exposed to SLS displays severe deterioration in splenic tissue integrity. An increase in ES, HT, primarily RBC, and MMC, and a decrease in LC were obvious. In addition, DCBV were seen. (D) A micrograph from a fish subjected to PMPs + SLS shows minor histological assessments of MMC scattered throughout the HT around the ES, along with a depletion of LC and ES. (E) A bar graph displaying the severity score of the general splenic histopathological lesion ranging from 0 to 3. The data were estimated at 40x magnification and displayed as means ± SE of nine replicates for each group (n = 9). The statistically significant differences in values between groups (P < 0.05) are indicated by superscript symbols on the bars.

Lesion	Group/score			
	Control	PMPs-treated	SLS-treated	PMPs + SLS-treated
Enriched ellipsoid structure (ES)	-	+++	+++	+
Encircled melano-macrophage centers (MMC)	+	+++	+++	++
Lymphocytic depletion (LC)	-	+++	+++	+++
Enriched hemopoietic tissues (HT) and RBCS	++	++	+++	+
Dilated and congested blood vessels (DCBV)	-	++	+++	+

Table 5. The differential severity scoring of the observed spleen-pathological lesions after the exposure to PMPs and/or SLS. * Symbols indicate the total number of the lesion discovered in 9 examined fields; - no; + mild, ++ moderate, and +++ severe lesion.

Histochemical analyses

I. Localization and quantification of fibrosis.

The liver sections of the control fish showed a normal distribution of collagen types I and III colocalized around the central vein with fine fibers between the H (Figs. 5, A). The intensity of collagen deposition was increased in fish exposed to PMPs (10 mg/L) and/or SLS (4 mg/L) (Figs. 5, B, and C). Compared to the other exposed groups, decreased intensity of collagen deposition was noticed in the fish exposed to the combined PMPs + SLS-exposed (Fig. 5, D).

Using ImageJ software, semi-quantifications of the microscopic observations of the hepatic fibrotic depositions from collagen fibers (types I and III) in the exposed groups were set up. Figure 5, E displays the statistical estimates of the density per 0.5 mm² and distribution pattern (total area expansion) of hepatic collagen fibers. All exposed groups showed very high statistically significant levels of hepatic fibrosis (P < 0.001) when compared to the control group. Whereas the combination of PMPs + SLS-exposure revealed a significant reduction (P < 0.05) in fibrosis.

Similarly, exposure to PMPs and/or SLS affects the spleen tissue integrity and shows signs of fibrosis. ES in the spleen tissue had a red tinge, as shown by the SR stain on collagen fibers in the CT of control fish (Fig. 6, A). Vascular walls and the splenic tissue matrix (BV) were shown to be covered in fine collagen fibers (arrow). Every exposed group showed increased fibrosis; most of the increases were seen in the groups exposed to SLS

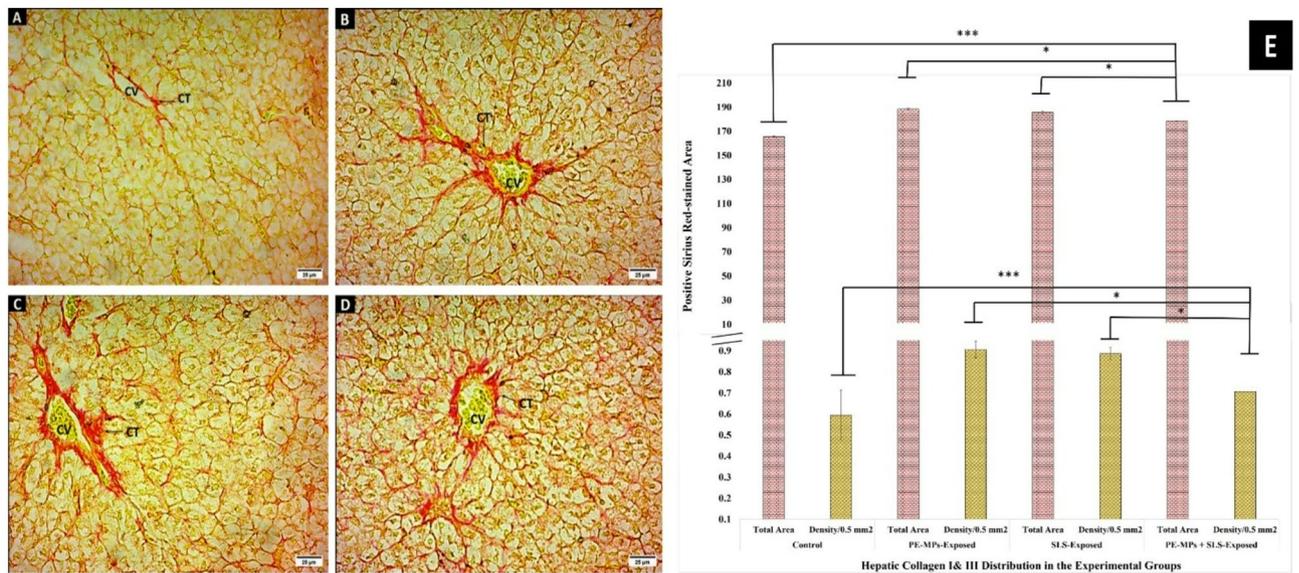


Fig. 5. SR-staining findings of *C. gariepinus* liver after 15 days of exposure to PMPs, SLS, or their combination. **A–D**) Photomicrographs of SR-stained sections from *C. gariepinus* ($\times 400$; scale bars = 25 μm) showing: **(A)** A micrograph from the control group displaying the normal distribution of collagen types I & III colocalized around the CV with fine fibers between H. **(B)** A micrograph from a fish exposed to PMPs, and **(C)** a micrograph from a fish exposed to SLS show increasing levels of collagen depositions in the CT of the organ framework and around CV. **(D)** A micrograph from a fish exposed to PMPs + SLS demonstrates a decreased intensity of collagen I & III depositions in the CT surrounding the CV and between H in BS. **(E)** Column histogram displaying the density per 0.5 mm^2 area and the expanding pattern (% area) of the hepatic collagen fibers (types I & III) in SR-stained liver sections from control, PMPs, SLS, or PMPs + SLS-exposed *C. gariepinus*. Using ImageJ software, the data were acquired and displayed as means \pm SE of each group's eighteen parts ($n = 18$). The statistically significant differences in values between groups ($P < 0.05$) are indicated by superscript symbols on the bars.

or PMPs (Figs. 6, B, and C). The group exposed to the combination of SLS and PMPs showed steadily decreased fibrosis (Fig. 6, D).

Using ImageJ software, the fibrotic depositions of collagen fibers (types I and III) in the exposed groups' spleen were semi-quantified. Figure 6, E shows the statistically estimated total area expansion and density per 0.5 mm^2 of the splenic collagen fibers. Like the liver, all exposed groups had significantly higher levels of splenic fibrosis ($P < 0.05$) compared to the control group, with the combined PMPs + SLS-exposed group showing a significant diminution in fibrosis ($P < 0.05$).

II. Localization and quantification of hepatic polysaccharide depositions.

Sections stained by PAS reagent displayed the polysaccharide localization in the liver (Fig. 7). In the control group, extensive reactivity of polysaccharides in H was observed (Fig. 7, A). A substantial reduction in the hepatocellular polysaccharide content was observed in PAS staining liver sections of the PMPs or SLS-exposed fish (Figs. 7, B, C). In liver sections from the combined PMPs + SLS-exposed group, partially reversed glycogen content was seen (Fig. 7, D).

In a bar histogram (Fig. 7, E), the semi-quantification of hepatic polysaccharide depositions in the experimental groups is statistically represented. The groups exposed to PMPs or SLS showed a significant decrease ($P < 0.001$) in the amount of glycogen deposits in the liver than the control group. Once more, the SLS-exposed group had the substantially lowest ($P < 0.001$) glycogen level whereas the PMPs + SLS-exposed group had the significantly highest ($P < 0.05$) glycogen level in comparison to the other groups. The glycogen depositions of the PMPs- and SLS-exposed groups did not differ statistically significantly ($P > 0.05$).

Discussion

The current study was set out to evaluate the biological and immunological alterations in *C. gariepinus* exposed to PMPs and SLS, either singly or in combination. Physiological, immunological and haemato-biochemical characteristics were evaluated. In addition, tissue integrity biomarkers were investigated in liver and spleen.

Hematological indices are considerable indicators of the fish's health condition⁵⁸. According to Cole et al.¹² MP's sharp surfaces can harm RBC membranes physically, resulting in cell lysis. This directly affects Hb levels and Hct values, especially with higher concentrations in blood, which accelerates hemolysis, disrupts erythropoiesis, destroys RBCs in the hematopoietic organ, or develops anemia⁵⁹. Therefore, PMPs, SLS, and PMPs + SLS-exposed fish groups of our study showed a substantial decrease in RBC account, Hct, and Hb concentration. In

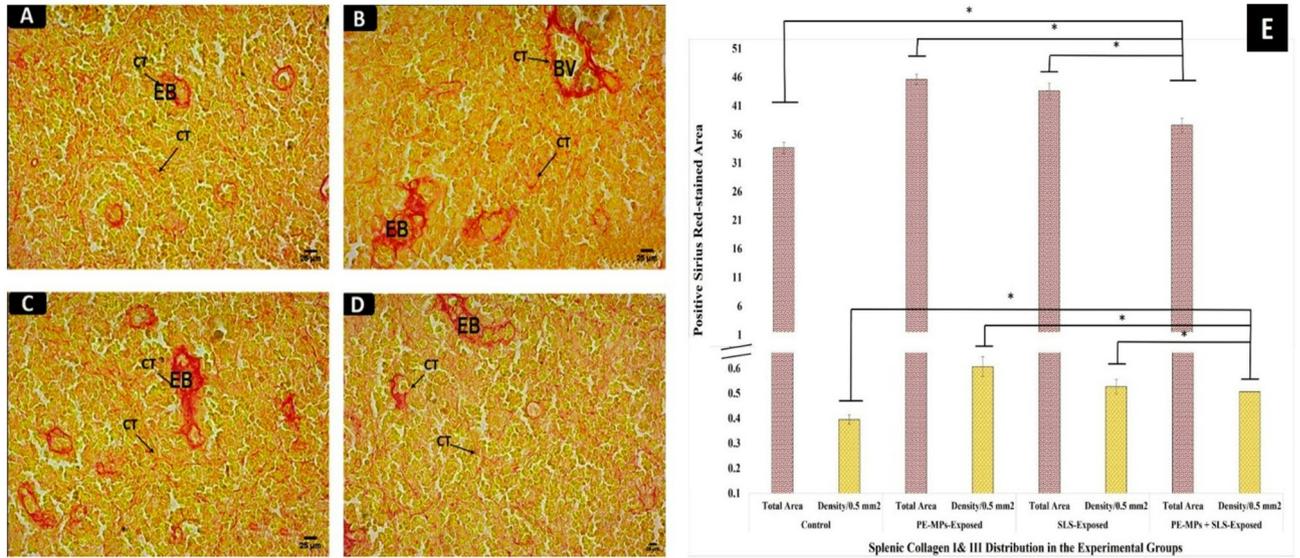


Fig. 6. SR-staining findings of *C. gariepinus* spleen after 15 days of exposure to PMPs, SLS, or their combination. **A – D**) Photomicrographs of SR-stained sections from *C. gariepinus* (×400; scale bars = 25 μm) showing: **A**) A portion of a control spleen demonstrates the fine network of subterranean substances, blood vessels, and ES surrounded by scant CT fibers. **B, and C**) Sections of the spleen from fish subjected to PMPs or SLS demonstrate CT fiber accumulations around blood arteries, and ES. Fine collagenous networks are obvious in subterranean materials. **D**) A segment of a spleen from a fish exposed to PMPs + SLS demonstrates a visible decrease in the accumulation of collagen I and III fibers. **E**) Column histogram displaying the density per 0.5 mm² area and the expanding pattern (% area) of the splenic collagen fibers (types I & III) in SR-stained spleen sections from control, PMPs, SLS, or PMPs + SLS-exposed *C. gariepinus*. Using ImageJ software, the data were acquired and displayed as means ± SE of each group’s eighteen parts (*n* = 18). The statistically significant differences in values between groups (*P* < 0.05) are indicated by superscript symbols on the bars.

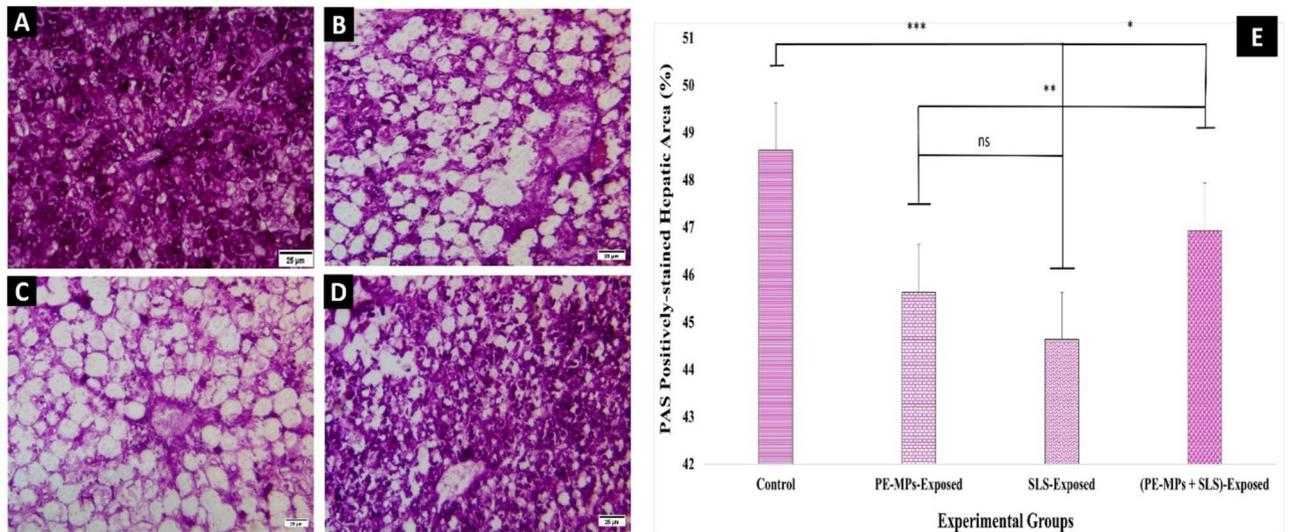


Fig. 7. Photomicrographs (×400; scale bar 25 μm) of liver tissue from *C. gariepinus* stained by the PAS for polysaccharides showing: **A**) A micrograph from control fish showing the normal distribution of carbohydrates in H with the extensive glycogen. **B - C**) A micrographs from fish exposed to PMPs or SLS, showing glycogen depletion in H. **D**) A micrograph from combined PMPs + SLS-exposed group showing partially reversed glycogen content PAS reaction. **E**) A column histogram shows the growing pattern (% area) of the hepatic polysaccharides’ depositions in PAS-stained sections using ImageJ software. The data were acquired and displayed as means ± SE (*n* = 18). Superscript asterisks on bars denote the statistically significant different values of groups (*P* < 0.05).

agreement with our findings, a decrease in erythropoiesis and an increase in the rate of erythrocyte breakdown in hematopoietic organs, which could account for a notable reduction in RBCs, Hb, and HCT were reported in SLS-exposed catfish^{60,61}. High level of MCV could be caused by an increase in the immature RBCs or by the presence of a significant number of older or larger RBCs⁶². According to the results of the current study, the MCH and MCHC levels in the PMPs, SLS, and PMPs + SLS-exposed groups are not significantly different compared to those of the control group. The MCHC level is an indicator of RBC shrinkage (increased MCHC) or swelling (decreased MCHC). Ultraviolet exposure induced marked RBC swelling and non-significant decreases in MCH⁶³. The reduction in WBC count is suggestive of a weakened immune response and less stress after exposure to toxic substances. Therefore, the decrease in WBC count in our findings could be the consequence of MPs and SLS bioaccumulation inside tissues. According to Alkaladi et al.⁶⁴, the decline in total WBC counts may be due to the deleterious impact of nanoparticles on the lymphoid tissues of the exposed fish. In addition, the physical blockage of the digestive tract by PMPs might lower nutrition absorption and hampers energy allocation has the potential to affect fish immune system⁶⁵.

After 15 days of exposure to PMPs, SLS, or PMPs + SLS, the fish's biochemical response increased the AST and ALT levels in serum. Many fish species have elevated AST and ALT in their circulation due to the hepatic potency of chemical contaminants, which typically results in cellular damage and necrosis in the hepatic tissues^{19,66}. Alternatively, this elevation of ALT and AST activities can be an effort to supply the energy required to endure stress⁶⁷. In our investigation, all exposed groups had low levels of ALP activity after 15 days of exposure to MPs, SLS, or both. Since ALP is an alkaline transphosphorylase that is necessary for biological membrane transport activities and skeleton mineralization, the reduced activity of the enzyme in fish serum caused by exposure to PMPs or SLS in the current study suggests disruptions in physiological and functional mechanisms linked to a defect in the membrane transport system⁶⁸. In similar line to our findings, ALP activity was decreased in serum and other tissues of Nile tilapia in response to metal exposure in different ways^{68–70}.

Here, the blood glucose levels significantly increased in all the exposed groups of *C. gariepinus*, which indicates disturbances in glucose metabolism or uptake. This was further indicated by a reduction in the liver glycogen depositions. In agreement with our study, Hamed et al.¹⁹ reported a significant elevation of blood glucose of Nile tilapia (*Oreochromis niloticus*) exposed to MPs. In addition, the blood of exposed fish showed a considerable increase in the total protein level. This implies that the fish's physical health, ability to function as an antioxidant, and immunological response were impacted by upsetting the overall protein influential balance. Furthermore, fish need to provide more energy in the blood to deal with stress, eliminate harm, and recover; therefore, serum protein levels rise and their liver proteins deteriorate⁷¹.

A significant rise in blood cholesterol levels was recorded in the current study. This may be because fish steroidogenesis utilizes cholesterol as primarily energy source since MPs and SLS may alter the circulation of fatty acids in the hepatic tissue, hence altering cholesterol levels. In a similar context, Hamed et al.¹⁹ reported that toxicants such as herbicides and MPs may alter the hepatic tissue's fatty acid circulation, and perhaps affect cholesterol levels. Reversely, SOD and total antioxidant capacity activities in catfish serum showed a significant decrease indicating the negative reactivity for the fish's antioxidant defense system following toxicant exposure, which increases the free radicals and reduces the overall antioxidant capacity. Comparable outcomes were documented utilizing antioxidant markers, which are extensively employed to assess the influence of distinct polyethylene plastic sizes (nano, micro, and macro) on juvenile *C. carpio* L.⁴⁵. Similarly, co-exposure of zebrafish to Cd and MPs revealed a reduction in SOD activity⁷². The reduce levels of antioxidant enzymes like SOD and CAT in blood indicates the oxidative stress attributed to increased lipid peroxidation in the liver⁷³.

Increased activities of MDA were recorded in the current study, which indicate high lipid peroxidation. Oxidative stress and lipid peroxidation may be attributed to either malnutrition or inhibition of food digestion due to the ingested MPs by fish⁷². In addition, the release, absorption, or containment of potentially harmful compounds by MPs might also affect the immune system or the energy allocation and limit nutrient absorption¹². In agreement to our finding, the marine juvenile *Pomatoschistus microps* exhibited elevated levels of lipid peroxidation after being exposed to PMPs⁷⁴.

Here, fish exposure to SLS and PMPs upset homeostasis and had pro-inflammatory effects, based on elevated blood cytokine levels. According to Wang et al.⁷⁵, this is a common immune reaction to inflammation and cellular damage. Tissue injury impairs cellular homeostasis, and trigger interleukins as an instant immunological response to offset the increasing stressor⁷⁵. In addition, the increased interleukin production is an adverse consequence of immunological defense and marked systemic inflammatory response^{75,76}.

Notably, the morphology of erythrocytes was altered under a microscope, displaying a range of changes such as enlarged, sickle-shaped, teardrop-shaped, and visible vacuoles, as well as RBCs with a karyolysis signal. Fish erythrocyte changes have been noticed during hypoxic conditions⁷⁷ and in response to agents such as radiation that cause death of the blood cells⁷⁸. In addition, the existence of vacuoles in erythrocytes may be caused by an uneven distribution of hemoglobin, as noted by Ateeq et al.⁷⁹. The observation of large blood cells was a common modification of their necrotic cellular boundaries. Because erythrocytes are essential for oxygen delivery, deformations of the erythrocytes can lead to low oxygen levels in the blood, which can aggravate or even induce respiratory disorders by affecting the circulatory system. Changes in the quantity and makeup of fish erythrocytes could result from this respiratory stress⁸⁰. Additionally, pathological circumstances leading to the breakdown of RBCs may be displayed by fish exposed to toxins^{81,19}. concluded that the interaction of PMPs with erythrocytes limits the dehydrogenase of delta-aminolevulinic acid, which induces plasma membrane interruptions and produces poikilocytosis. Araújo et al.⁸² suggested that the increased generation of relative oxygen species (ROS) in erythrocytes may result from a direct interaction of their plasma membranes with the MPs.

Smaller-sized MPs are more likely to amass inside the fish body organs because they can transit past the digestive tract and into the blood circulation, but larger-sized MPs typically congregate in the digestive tract

and are easily evacuated because of differences in size⁸³. According to our investigation, toxic effects following exposure to PMPs, SLS, or both extend to the hepatic tissue integrity. As the primary organ involved in numerous metabolic processes, fish liver alterations in histology are now commonly employed as indicators of exposure to toxins and carcinogens. Hepatocytic injury may be associated with reduced energy and metabolic disruptions, disintegration of microtubules, inhibition of protein synthesis, and increased hepatic RBC formation⁸⁴. Furthermore, physical blockage of hepatic capillaries by Polyethylene polymers translocated to the liver may cause sinusoidal dilatation and inflammatory cell infiltration, particularly when they are present in micro or nano sizes⁸⁵. MPs that have accumulated and circulated inside fish tissues may have caused inflammatory responses, and the adherent immune cells would have produced more ROS as defense reactions to combat oxidation⁸⁶. The oxidative stress revealed substantial reduction in SOD, CAT, GSH, and GST levels, which in turn caused kidneys and liver damage of toxified rats⁷³. Our observation suggests that PMPs and SLS secreted pro-inflammatory chemokines and cytokines that boosted oxidative stress in fish's immune-relevant organs and changed their immunological state by facilitating phagocytosis's recognition and elimination of foreign antigens. The spleen experienced macrophage aggregation. As far as we know, the spleen is the only organ in the bony fish lymphoid organs that has a sizable number of macrophages and lymphocytes⁸⁷. Here, histological analysis revealed large areas of fibrosis in the spleen's trabeculae, indicating that the exposed fish had less parenchyma and more interstitial stroma. In addition, the tissues of fish exposed to these conditions displayed increased CT and pigmented macrophages associated with blood vessels. Previous studies have shown that fish exposed to heavy metals in various habitats also displayed significant levels of fibrosis, suggesting a relationship between toxicant exposure and the occurrence of fibrosis^{88,89}.

Histochemical investigations revealed that the exposed groups showed a decrease in hepatic glycogen content, as indicated by PAS staining. This suggests either a decrease in the intestinal absorption of carbohydrates or an increase in the glycolytic activity required by a stressed-out, increased metabolism. This reduction in the liver glycogen might indicate disturbance of glucose metabolism, energy balance, or other glycogen-related processes. Moreover, SR-staining revealed that the groups exposed to PMPs or SLS had detrimental effects on the fibrotic texture of the splenic and hepatic tissues integrity. They revealed high levels of fibrosis either singly or in combination. However, the combined exposure had lower effect on liver glycogen and tissue fibrosis. Similarly, after the exposure to PMPs, a dose-dependently higher collagen depositions were displayed by Masson's trichrome staining of fish liver^{90,91}. In a similar line, Japanese medaka (*Oryzias latipes*) exposed to the toxicant "dimethyl nitrosamine" developed hepatic fibrosis and neoplasia⁹².

MPs and chemicals have a complicated relationship that probably depends on the chemical makeup, plastic quality, and the environmental factors. There are several pathways of the interaction between chemicals and MPs in water. In seawater, the sorption capacity of chemicals onto MPs is affected by the water ions, which enable the interaction of various surface groups on chemicals with MPs and modify their overall charge and/or characteristics⁹³. The toxicity of the insecticide on rainbow trout (*Oncorhynchus mykiss*) was increased when it was sorbed onto polystyrene MPs⁹⁴. Additionally, their muscle contents of protein, fatty acids, and amino acids were significantly altered⁹⁵. Similarly, PMPs reduced the toxicity of chlorpyrifos insecticide on the microalga *Isochrysis galbana*⁹⁶. However, in common carp (*Cyprinus carpio*) PMPs increased the toxicity of paraquat herbicide and changed the blood's biochemical properties [98]. Hydrophobic partitioning is a key mechanism that has been the subject of numerous published studies. Chemical solubility plays a major role in the process of hydrophobic partitioning, which occurs when substances separate between an aqueous phase and a hydrophobic solvent, in this case, PMPs⁹⁷. In the aquatic environments, chemical's hydrophobicity hinders their dissolution, and therefore, they tend to be sorb onto other organic particles such as MPs³⁵. Recently, SLS solution has been proven to interact with PMPs relative to their concentrations and medium acidity⁹⁸.

Conclusion

PMPs and SLS are hazards for fish altering their biology and pathology either singularly or in combination. Fish exposure to non-lethal doses of 10 mg/L PMPs and 4 mg/L SLS for 15 days deteriorated their biomarkers in blood and tissue. Unexpectedly, the severity of their harm was mitigated upon their combination, which indicate the antagonistic interaction between both reagents inside the biological systems. This was clear in the reduced hematologic and biochemical alterations, mild pathological appraisals, and the moderate fibrosis as well as hypoglycemia. Our findings indicate that excessive residues of plastics and detergents are considered a precursor for environmental hazards in aquaculture systems. Therefore, social processes, laws, and technological developments for the reduction of pollution caused by these reagents should be considered.

Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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References

1. Van Muiswinkel, W. B., Wiegertjes, G. F. & Stet, R. J. The influence of environmental and genetic factors on the disease resistance of fish. *%J Aquaculture*. **172** (1–2), 103–110 (1999).
2. Mustafa, S. A., Al-Rudainy, A. J. & Salman, N. M. *Effect of Environmental Pollutants on Fish Health: an Overview* (%) Egyptian Journal of Aquatic Research, (2024).
3. Guerrero, M. C. et al. Micro and nano plastics distribution in fish as model organisms: histopathology, blood response and bioaccumulation in different organs. *%J Appl. Sci.* **11** (13), 5768 (2021).

4. Zhong, H. et al. *The Hidden Risk of microplastic-associated Pathogens in Aquatic Environments* (%J Eco-EnvironmentHealth, 2023).
5. Nwani, C. D. et al. Genotoxicity assessment and oxidative stress responses in freshwater African catfish *Clarias Gariepinus* exposed to fenthion formulations. *Drug Chem. Toxicol.* **40** (3), 273–280 (2017).
6. Ogueji, E. O. et al. Acute toxicity of ibuprofen on selected biochemical and oxidative stress parameters of liver in *Clarias gariepinus* juveniles (Burchell). *J. Entomol. Zool. Stud.* **5** (4), 1060–1068 (2017).
7. Haredi, A. M. M. et al. Lake Edku pollutants induced biochemical and histopathological alterations in muscle tissues of Nile tilapia (*Oreochromis niloticus*). *J. Toxicol. Environ. Health Sci.* **12**, 247–255 (2020).
8. Joseph, T. M. et al. *Polyethylene terephthalate (PET) recycling: A review*. 9: p. 100673. (2024).
9. Abarghouei et al. Size-dependent effects of microplastic on uptake, immune system, related gene expression and histopathology of goldfish (*Carassius auratus*). *Chemosphere* **276**, 129977 (2021).
10. Abarghouei, S. et al. Size-dependent effects of microplastic on uptake, immune system, related gene expression and histopathology of goldfish (*Carassius auratus*). *Chemosphere* **276**, 129977 (2021).
11. Browne, M. A. et al. Accumulation of microplastic on shorelines worldwide: sources and sinks. *Environ. Sci. Technol.* **45** (21), 9175–9179 (2011).
12. Cole, M. et al. Microplastics as contaminants in the marine environment: a review. *Mar. Pollut. Bull.* **62** (12), 2588–2597 (2011).
13. Napper, I. E. et al. Characterisation, quantity and sorptive properties of microplastics extracted from cosmetics. *Mar. Pollut. Bull.* **99** (1–2), 178–185 (2015).
14. Cole, M. et al. Microplastics as contaminants in the marine environment: a review. *Mar. Pollut. Bull.* **62**(12), 2588–2597 (2011).
15. Lakshari, I. A. et al. Quantitative analysis of selected plastics in high-commercial-value Australian seafood by pyrolysis gas chromatography mass spectrometry. *Agronomy*. **14** (3), p. 548. (2024).
16. Ribeiro, F. et al. Plastic pollution in agriculture as a threat to food security, the ecosystem, and the environment: an overview. *Environmental Science & Technology*. **54** (15), 9408–9417 (2020).
17. Yang, D. et al. Microplastic pollution in table salts from China. *Environ. Sci. Technol.* **49** (22), 13622–13627 (2015).
18. Kaleem, O. & Sabi, A. F. B. S. Overview of aquaculture systems in Egypt and Nigeria, prospects, potentials, and constraints. *%J Aquaculture Fisheries*. **6** (6), 535–547 (2021).
19. Hamed, M. et al. Assessment of the effect of exposure to microplastics in Nile tilapia (*Oreochromis niloticus*) early juvenile: I. blood biomarkers. *Chemosphere* **228**, 345–350 (2019).
20. da Costa Araújo, A. P. et al. *How much are microplastics harmful to the health of amphibians? A study with pristine polyethylene microplastics and *Physalaemus cuvieri**. 382: p. 121066. (2020).
21. González-Doncel, M. et al. *Effects of life cycle exposure to polystyrene microplastics on medaka fish (*Oryzias latipes*)*. 311: p. 120001. (2022).
22. Jabeen, K. et al. Effects of Virgin microplastics on goldfish (*Carassius auratus*). *Chemosphere* **213**, 323–332 (2018).
23. Hoseini et al. Hepatic transcriptomic and histopathological responses of common carp, *Cyprinus carpio*, to copper and microplastic exposure. *Mar. Pollut. Bull.* **175**, 113401 (2022).
24. Schwabl, P. et al. Detection of various microplastics in human stool: A prospective case series. *Ann. Intern. Med.* **171** (7), 453–457 (2019).
25. Zhou, A. et al. Microplastics and their potential effects on the aquaculture systems: a critical review. *Rev. Aquac.* **13** (1), 719–733 (2021).
26. Barboza, L. G. A. et al. Microplastics cause neurotoxicity, oxidative damage and energy-related changes and interact with the bioaccumulation of mercury in the European seabass, *dicentrarchus labrax* (Linnaeus, 1758). *Aquat. Toxicol.* **195**, 49–57 (2018).
27. Wang, Y. et al. Exploring the effects of different types of surfactants on zebrafish embryos and larvae. *Sci. Rep.* **5** (1), 10107 (2015).
28. Shukla, A. & Trivedi, S. P. *Anionic surfactant, linear alkyl benzene sulphonate induced oxidative stress and hepatic impairments in fish *Channa punctatus**. in *Proceedings of the zoological society*. Springer. (2018).
29. Barra Caracciolo, A. et al. Characteristics and environmental fate of the anionic surfactant sodium Lauryl ether sulphate (SLES) used as the main component in foaming agents for mechanized tunnelling. *Environ. Pollut.* **226**, 94–103 (2017).
30. Bondi, C. A. et al. Human and environmental toxicity of sodium Lauryl sulfate (SLS): evidence for safe use in household cleaning products. *Environ. Health Insights*. **9**, 27–32 (2015).
31. Belanger, S. E. et al. Responses of periphyton and invertebrates to a tetradecyl-pentadecyl sulfate mixture in stream mesocosms. *Environ. Toxicol. Chem.* **23** (9), 2202–2213 (2004).
32. JeenJose, P. & Johnson, M. K. *Histological Study on Gills and Liver of Fresh water Fish *Rasbora dandia* Exposed to Sodium Lauryl Sulfate (SLS)*. (2018).
33. Messina, C. M. et al. Effect of sodium Dodecyl sulfate (SDS) on stress response in the Mediterranean mussel (*Mytilus galloprovincialis*): regulatory volume decrease (Rvd) and modulation of biochemical markers related to oxidative stress. *Aquat. Toxicol.* **157**, 94–100 (2014).
34. Cedergreen, N. Quantifying synergy: a systematic review of mixture toxicity studies within environmental toxicology. *PLoS One*. **9** (5), e96580 (2014).
35. Marchant, D. J. et al. *Do microplastics mediate the effects of chemicals on aquatic organisms?* 242: p. 106037. (2022).
36. Eder, M. L. et al. *Microplastics as a vehicle of exposure to chemical contamination in freshwater systems: Current research status and way forward*. 417: p. 125980. (2021).
37. Impellitteri, F. et al. Hemocytes: A useful tool for assessing the toxicity of Microplastics, heavy Metals, and pesticides on aquatic invertebrates. *Int. J. Environ. Res. Public Health*. **19** (24), 16830 (2022).
38. El-Sayed, A. et al. Nephrotoxicity of single or combined exposure to microplastics (MPs) and sodium Lauryl sulfate (SLS) in African catfish (*Clarias gariepinus*). *%J Sohag J. Sci.* **9** (3), 342–349 (2024).
39. Sayed Ael, D. et al. Antioxidant and antiapoptotic activities of *Calotropis procera* latex on catfish (*Clarias gariepinus*) exposed to toxic 4-nonylphenol. *Ecotoxicol. Environ. Saf.* **128**, 189–194 (2016).
40. Ahmed, F. et al. Dietary Chitosan nanoparticles: potential role in modulation of rainbow trout (*Oncorhynchus mykiss*) antibacterial defense and intestinal immunity against enteric Redmouth disease. *%J Mar. Drugs*. **19** (2), 72 (2021).
41. Hamed, M. et al. *Assessment of the effect of exposure to microplastics in Nile Tilapia (*Oreochromis niloticus*) early juvenile: I. blood biomarkers*. 228: pp. 345–350. (2019).
42. Freitas, R. et al. *Toxic impacts induced by Sodium lauryl sulfate in *Mytilus galloprovincialis**. 242: p. 110656. (2020).
43. Sayed, A. E. H. et al. Haemato-biochemical, mutagenic, and histopathological changes in *Oreochromis niloticus* exposed to BTX. *Environ. Sci. Pollut. Res. Int.* **30** (21), 59301–59315 (2023).
44. Mekkawy, I. A., Mahmoud, U. M. & Sayed, A. E. D. H. *Effects of 4-nonylphenol on blood cells of the African catfish *Clarias gariepinus* (Burchell, 1822)*. *%J Tissue cell*, **43**(4): pp. 223–229. (2011).
45. Hamed, M., Monteiro, C. E. & Sayed, A. E. H. Investigation of the impact caused by different sizes of polyethylene plastics (nano, micro, and macro) in common carp juveniles, *Cyprinus Carpio* L., using multi-biomarkers. *Sci. Total Environ.* **803**, 149921 (2022).
46. Koracevic, D. et al. Method for the measurement of antioxidant activity in human fluids. *J. Clin. Pathol.* **54** (5), 356–361 (2001).
47. Nishikimi, M., Appaji, N. & Yagi, K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem. Biophys. Res. Commun.* **46** (2), 849–854 (1972).
48. Sayed, A. E. H. & Soliman, H. A. M. Modulatory effects of green tea extract against the hepatotoxic effects of 4-nonylphenol in catfish (*Clarias gariepinus*). *Ecotoxicol. Environ. Saf.* **149**, 159–165 (2018).

49. Ohkawa, H., Ohishi, N. & Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95** (2), 351–358 (1979).
50. Soliman, H. A. et al. Toxicity of co-exposure of microplastics and lead in African catfish (*Clarias gariepinus*). *%J Front. Veterinary Sci.* **10**, 1279382 (2023).
51. Shu, C. C. et al. A histopathological scheme for the quantitative scoring of intervertebral disc degeneration and the therapeutic utility of adult mesenchymal stem cells for intervertebral disc regeneration. *%J Int. J. Mol. Sci.* **18** (5), 1049 (2017).
52. Ibrahim, K. E. et al. Histopathology of the liver, kidney, and spleen of mice exposed to gold nanoparticles. *Molecules* **23** (8), 1848 (2018).
53. Fernandes, C. et al. Bioaccumulation of heavy metals in *Liza saliens* from the Esmoriz–Paramos coastal lagoon, Portugal. *Ecotoxicol. Environ. Saf.* **66** (3), 426–431 (2007).
54. Huang, Y. et al. Image analysis of liver collagen using Sirius red is more accurate and correlates better with serum fibrosis markers than trichrome. *%J Liver Int.* **33** (8), 1249–1256 (2013).
55. Shafiei, M. T. et al. Detecting glycogen in peripheral blood mononuclear cells with periodic acid schiff staining. *J. Visualized Experiments: JoVE.* **94**, 52199 (2014).
56. Saleh, M. et al. Therapeutic intervention with dietary Chitosan nanoparticles alleviates fish pathological and molecular systemic inflammatory responses against infections. *Mar. Drugs.* **20** (7), 425 (2022).
57. Dytham, C. *Choosing and using statistics: a biologist's guide* (Wiley, 2011).
58. Thummabancha, K., Onparn, N. & Srisapoom, P. Analysis of hematologic alterations, immune responses and Metallothionein gene expression in Nile tilapia (*Oreochromis niloticus*) exposed to silver nanoparticles. *J. Immunotoxicol.* **13** (6), 909–917 (2016).
59. Kavitha, C. et al. Toxicity of Moringa Oleifera seed extract on some hematological and biochemical profiles in a freshwater fish, *Cyprinus Carpio*. *%J Experimental Toxicologic Pathol.* **64** (7–8), 681–687 (2012).
60. Joshp, P., Bose, M. & Harish, D. Changes in certain haematological parameters in a silurid Cat fish *Clarias Batrachus* (Linn) exposed to cadmium chloride. *%J Pollution Res.* **21** (2), 129–131 (2002).
61. Khalil, R. M., Reda & Awad, A. Efficacy of spirulina platensis diet supplements on disease resistance and immune-related gene expression in *Cyprinus Carpio L.* exposed to herbicide atrazine. *%J Fish. Shellfish Immunol.* **67**, 119–128 (2017).
62. Härdig, J. & Höglund, L. B. On accuracy in estimating fish blood variables. *Comp. Biochem. Physiol. Part. A: Physiol.* **75** (1), 35–40 (1983).
63. Osman, A., Hamed, M. & Sayed, A. Protective role of spirulina platensis against UVA-induced haemato-biochemical and cellular alterations in *Clarias Gariepinus*. *J. Photochem. Photobiol. B.* **191**, 59–64 (2019).
64. Alkaladi, A. et al. Hematological and biochemical investigations on the effect of vitamin E and C on *Oreochromis niloticus* exposed to zinc oxide nanoparticles. *Saudi J. Biol. Sci.* **22** (5), 556–563 (2015).
65. Rochman et al. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. *Sci. Rep.* **3**, 3263 (2013).
66. Fathy, M. et al. Hemotoxic effects of some herbicides on juvenile of Nile tilapia *Oreochromis niloticus*. *Environ. Sci. Pollut. Res. Int.* **26** (30), 30857–30865 (2019).
67. Owolabi, O. D., J.S.J.I.J.o, T. & Omotosho *Atrazine-mediated Oxidative Stress Responses Lipid Peroxidation Tissues Clarias Gariepinus* **11**(2): 29–38. (2017).
68. Atli, G. et al. Alterations in the serum biomarkers belonging to different metabolic systems of fish (*Oreochromis niloticus*) after Cd and Pb exposures. *Environ. Toxicol. Pharmacol.* **40** (2), 508–515 (2015).
69. Öner, M., Atli, G. & Canli, M. Changes in serum biochemical parameters of freshwater fish *Oreochromis niloticus* following prolonged metal (Ag, Cd, Cr, Cu, Zn) exposures. Alterations in the serum biomarkers belonging to different metabolic systems of fish (*Oreochromis niloticus*) after Cd and Pb exposures. *Environ. Toxicol. Chem.* **27** (2), 360–366 (2008).
70. Canli, E. G. & Canli, M. Low water conductivity increases the effects of copper on the serum parameters in fish (*Oreochromis niloticus*). *Environ. Toxicol. Pharmacol.* **39** (2), 606–613 (2015).
71. Authman, M. M. et al. Heavy Metals Pollution and their Effects on Gills and Liver of the Nile Catfish Inhabiting El-Rahawy Drain, Egypt. *J Glob Vet.* **10** (2), 103–115 (2013).
72. Lu et al. Uptake and accumulation of polystyrene microplastics in zebrafish (*Danio rerio*) and toxic effects in liver. *%J Environ. Sci. Technol.* **50** (7), 4054–4060 (2016).
73. Ahmad M. I. et al. Pendimethalin-induced oxidative stress, DNA damage and activation of anti-inflammatory and apoptotic markers in male rats. *Sci. Rep.* **8** (1), 17139 (2018).
74. Ferreira, P. et al. Effects of multi-stressors on juveniles of the marine fish *Pomatoschistus microps*: gold nanoparticles, microplastics and temperature. *Aquat. Toxicol.* **170**, 89–103 (2016).
75. Wang, S. et al. Interactions effects of nano-microplastics and heavy metals in hybrid Snakehead (*Channa maculata*♀ × *Channa argus*♂). **124**: pp. 74–81. (2022).
76. Jiang, C. et al. Molecular characterization and expression profiles of two Interleukin genes IL-8 and IL-10 in Pacific Cod (*Gadus macrocephalus*). *Aquaculture Rep.* **21**, 100788 (2021).
77. Sawhney, A. K. & Johal, M. S. Erythrocyte alterations induced by malathion in *Channa punctatus* (Bloch). *Bull. Environ. Contam. Toxicol.* **64** (3), 398–405 (2000).
78. Chukhlovina, A. B. Apoptosis and red blood cell echinocytosis: common features. *Scanning Microsc.* **10** (3), 795–803 (1996). discussion 803–4.
79. Ateeq, B. et al. *Induction of micronuclei and erythrocyte alterations in the catfish Clarias Batrachus by 2, 4-dichlorophenoxyacetic acid and Butachlor.* **518**(2): pp. 135–144. (2002).
80. Brown, L. *Aquaculture for veterinarians. Fish husbandry and medicine* (1993).
81. Buckley, J. A., Whitmore, C. M. & Matsuda, R. I. Changes in blood chemistry and blood cell morphology in Coho salmon (*Oncorhynchus kisutch*) following exposure to sublethal levels of total residual Chlorine in municipal wastewater. *J. Fish. Res. Board Can.* **33** (4), 776–782 (1976).
82. Araújo, C., de Andrade Vieira, J. E. & Malafaia, G. Toxicity and trophic transfer of polyethylene microplastics from *Poecilia reticulata* to *Danio rerio*. *%J Sci. Total Environ.* **742**, 140217 (2020).
83. Kaposi, K. L. et al. *Ingestion of Microplastic Has Limited Impact on a Marine Larva* 48p. 1638–1645 (Environmental Science & Technology, 2014). 3.
84. Hamed, M., Osman, A. G. M., Badrey, A. E. A., Soliman, H. A. M., and Sayed, A. E. D. H. Microplastics-Induced Eryptosis and Poikilocytosis in Early-Juvenile Nile Tilapia (*Oreochromis niloticus*), *Front. Physiol.* (2021) 12.
85. Brancatelli, G. et al. Hepatic sinusoidal dilatation. *Abdom. Radiol. (NY).* **43** (8), 2011–2022 (2018).
86. von Moos, N., Burkhardt-Holm, P. & Kohler, A. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis L.* after an experimental exposure. *Environ. Sci. Technol.* **46** (20), 11327–11335 (2012).
87. Nemcsók, J. et al. *Effects of copper, zinc and Paraquat on acetylcholinesterase activity in carp (Cyprinus Carpio L.).* **5**(1): pp. 23–31. (1984).
88. Handy, R. D., Runnalls, T. & Russell, P. M. Histopathologic biomarkers in three spined sticklebacks, *Gasterosteus aculeatus*, from several rivers in Southern England that Meet the freshwater fisheries directive. *Ecotoxicology* **11** (6), 467–479 (2002).
89. Salim, F. Histopathological effect of heavy metal on different organs of fresh water fish tissues from Garmat Ali river adjacent to Al-Najebya power station. *Kufa J. Vet. Med. Sci.* **6** (1), 141–153 (2015).
90. Hamed et al. Antioxidants and molecular damage in Nile tilapia (*Oreochromis niloticus*) after exposure to microplastics. *Environ. Sci. Pollut. Res. Int.* **27** (13), 14581–14588 (2020).

91. Li, B. et al. Microplastics in fishes and their living environments surrounding a plastic production area. *Sci. Total Environ.* **727**, 138662 (2020).
92. Van Wettere, A. J. et al. Anchoring hepatic gene expression with development of fibrosis and neoplasia in a toxicant-induced fish model of liver injury. *Toxicol. Pathol.* **41** (5), 744–760 (2013).
93. Zuo, L. Z. et al. Sorption and desorption of phenanthrene on biodegradable poly (butylene adipate co-terephthalate) microplastics. 215: pp. 25–32. (2019).
94. Karbalaeei, S. et al. Toxicity of polystyrene microplastics on juvenile *Oncorhynchus Mykiss* (rainbow trout) after individual and combined exposure with chlorpyrifos. *J. Hazard. Mater.* **403**, 123980 (2021).
95. Hanachi, P. et al. Combined polystyrene microplastics and Chlorpyrifos decrease levels of nutritional parameters in muscle of rainbow trout (*Oncorhynchus mykiss*). *Environ. Sci. Pollut. Res.* **28**, 64908–64920 (2021).
96. Garrido, S. et al. *Effect of microplastics on the toxicity of chlorpyrifos to the microalgae Isochrysis galbana, clone t-ISO*. 173: pp. 103–109. (2019).
97. Nematdoost Haghi, B. & Banaee, M. Effects of micro-plastic particles on Paraquat toxicity to common carp (*Cyprinus carpio*): biochemical changes. *J. Int. J. Environ. Sci. Technol.* **14** (3), 521–530 (2017).
98. Lee, H., Shim, W. J. & J.-H. J.S.o.t.t.e. Kwon. *Sorption Capacity Plast. Debris Hydrophobic Org. Chemicals.* **470**, 1545–1552 (2014).
99. Comanche-Webb, X. M. *Surface interactions of polyethylene microplastics and sodium laureth ether sulfate*, (2024).

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Author contributions

Alyaa Elsayed: Conceptualization, Methodology, Formal analysis, Writing – original draft. Hamdy A. M. Soliman: Conceptualization, Methodology, Formal analysis, Writing – original draft, Supervision, Fatma Ahmed: Conceptualization, Methodology, Formal analysis, Writing – original draft, Supervising, Hanem S. Abdel-Tawab: Methodology, Practical, Writing – original draft, Alaa El-Din H. Sayed: Conceptualization, Methodology, Practical, Writing – original draft. All authors are aware of the submission and agree to it.

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Declarations

Competing interests

The authors declare no competing interests.

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