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Nephrotoxicity of Single or Combined Exposure to Microplastics (MPs) and Sodium Lauryl Sulfate (SLS) in African Catfish (*Clarias gariepinus*)

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Abstract: It is unclear how fish in freshwater ecosystems are affected by the combined presence of microplastics (MPs) and anionic surfactant detergents. So, our investigations focus on the nephrotoxic effects of both Polyethylene microplastics (PE-MPs) and Sodium lauryl sulfate (SLS) in single or combined exposure on African Catfish (*Clarias gariepinus*). In the current study, rearing water of catfish (*Clarias gariepinus*) was supplied with 0, 10 mg/L PE-MPs, 4 mg/L SLS, or their combination for 15 days. Blood analysis for the kidney functions and histological appraisals for the renal tissue integrity, collagen density (types I & III), and polysaccharide deposits were investigated. Exposure to the irregular-shaped PE-MPs (10 mg/L) or SLS (4 mg/L) revealed nephrotoxicity affecting the kidney of the exposed fish. Significant increases in the serum creatinine and uric acid levels and severe pathological lesions were recorded in the renal tissue of PE-MPs (10 mg/L) and SLS (4 mg/L)-exposed groups. In addition, significantly higher fibrosis as well as hypoglycemia were observed in all the exposed groups compared to the control group. Nevertheless, the combined exposure to these substances revealed non-significant differences in the serum uric acid level and mild pathological lesions as well as lower fibrosis and hypoglycemia. In conclusion, PE-MPs and SLS are nephrotoxic for fish either singularly or in combination. Unexpectedly, an antagonistic effect in the form of lower nephrotoxicity was observed upon the combined exposure of 10 mg/L PE-MPs + 4 mg/L SLS might be due to some interaction between both substances that altered their toxicity.
Keywords: Collagen (I & III), Fish, Renal Fibrosis, Renal Hypoglycemia, Toxicity.

1. Introduction

Plastics are used widely these days in a variety of daily uses everywhere in the world. As a result, the need for plastic has grown in the modern world to raise standards of living. In most industrialized nations, excessive use of plastic is regarded as a serious warning [1]. Regretfully, it is anticipated that in the coming decades, the number of plastics entering the water ecosystem will rise to the point where the mass of plastics in the marine and freshwater ecosystems will surpass that of fish [1, 2]. All plastic particles of a diameter less than 5 mm are classified as "microplastics" (MPs) by the European Food Safety Authority (EFSA) [3].

MPs are tiny artificial solid particles of plastic that are primarily made of functional additives and polymer blends with varying densities, sizes, shapes, colors, and chemical compositions [4]. Regarding their origin, they are divided into primary and secondary categories. Any plastic particles released into the environment directly are considered primary MPs that consist of plastic pellets, microbeads, fragments, and microfibers [4]. On the other hand, secondary MPs come from the breakdown of bigger plastic items that have an uneven or regular shape after being exposed to the marine as well as freshwater environment [1, 5]. They are produced by abrasion, photooxidation, mechanical wear acting as a wave, and biological deterioration of tiny plastic fragments [6, 7]. Numerous fish species' gastrointestinal tracts, gills, and excrement have been found to contain MPs that may have caused a range of harm to fish, including oxidative stress, toxicity (physical and chemical), genetic modifications, severe hematological, biochemical, or histological appraisals and high mortality [8]. Furthermore, some scientists contend that MPs may serve as a vector in this regard, transferring pathogens from the environment to living organisms. As a result, the inherent toxicity of plastics as well as their capacity to absorb, concentrate, and release environmental pollutants into living things may combine to cause negative effects of MPs [4]. Noteworthy, deposits of MPs in freshwater sediments can reach the same huge amount as in the highest contaminated marine sediments, and the MPs mass drifting through one river can exceed the mass of its living organisms from zooplankton and fish larvae [9]. Moreover, MPs of the freshwater rivers might persist in the drinking water and elicit direct harm to human and terrestrial animals' health status [10].

Sodium Lauryl Sulphate (SLS) is an anionic surfactant detergent that is made from alcohol sulfates [11]. In several reports, SLS has another synonym namely, Sodium Dodecyl Sulphate or SDS. SLS is frequently found in cosmetics, toothpaste, shampoos, shaving foams, and bubble baths, among other household goods. Additionally, it is used as a leather softening agent and a wool cleaning agent [11]. However, detergents have toxic effects on consumers and even slightly surpass the minimal threshold for entry into the freshwater

ecosystem, they harm the aquatic life. These detergents harm the respiratory system (gills), the skin, and the outer layer of protective mucus coatings. At least, when present at low concentrations, it will destroy the developing embryos and eggs [11]. Because SLS is easily biodegradable and has a low bioaccumulation rate, that is, it does not linger in the environment for very long, it was formally categorized as "environmentally friendly" [12]. However, high concentrations of anionic detergents can change the structural arrangement of the plasma membrane and jeopardize its functionality because they have a strong tendency to bind to the lipid component of the membrane [13]. Therefore, it has been reported that SLS is toxic and has a hazardous impact on the survival of aquatic animals like fish as well as microbes like yeasts and bacteria and to a lesser extent, toxic to mammals [11]. Fish, Echinodermata, Mollusca, Crustacea, Bacteria, and Microalgae have all been shown to be affected by SLS [14, 16]. Interestingly, SLS is frequently used as a reference toxicant in toxicity tests due to its quick-acting, nonselective, and constant toxicity [15].

Histopathological alterations are frequently employed as biomarkers to assess the health of fish exposed to pollutants as fish exposed to chemical pollutants are likely to develop lesions in various body organs, including the liver, kidneys, and gills. SLS and MPs were reported to affect the normal histology of fish gills, liver, kidneys, and intestines [13, 17].

The kidney is partially responsible for the metabolism of xenobiotics and is essential to the maintenance of a stable internal environment [11]. Here, the current study focuses on the nephrotoxic effects on catfish (*Clarias gariepinus*) caused by single as well as combined exposure to polyethylene MPs (PE-MPs) and SLS. For our investigations, blood biochemical indices, kidney functions, and histological and pathological lesions were estimated. Since the histology of the kidney has not received as much attention as other organs, evaluations of the toxic effects of MPs and SLS on fish renal histology and biochemistry have been assessed in the current study.

2. Materials and methods

2.1. Chemicals

PE-MPs were purchased as a raw powder from Toxemerge Pty Ltd. (Melbourne, Australia). SLS (>99% purity) was purchased from Sigma–Aldrich Company (St. Louis, MO, USA). Kits for the analysis of biochemical indices of the kidney function parameters, creatinine, and uric acid, including RANDOX Laboratories Ltd., PD410, United Kingdom, and Stanbio LDH (UV-Rate) USA, were bought from Bio-Diagnostic Co., Cairo, Egypt.

2.2. Physical Characteristics of the PE-MPs Particles

Following the manufacturer's instructions, purified water (Milli-Q) was used to obtain a stock suspension containing 1g PE-MPs/L. The particles were examined for their surface morphology under an Olympus light microscope at 40x magnification. Photomicrographs were taken using the digital camera (BX50F4, Olympus Optical Co., LTP, Japan) attached to the microscope.

2.3. Experimental Design

2.3.1. Fish Rearing and Healthy Status Assurance

Experimental ethics for fish care has been approved by the Research Ethics Committee of the Faculty of Science, Assiut University. Healthy *C. gariepinus* of 250-300g average body weight and 20-25cm average length were purchased from a “category 1” private farm and transported to the wet Laboratory of Fish Biology and Pollution, Faculty of Science, Assiut University. The pathogen-free and healthy status were checked, and the fish were acclimated to the laboratory conditions for four weeks before initiating the experiment. Fish were raised in 100-L fiberglass tanks filled up with dechlorinated tap water, which was maintained at 20.5°C, pH=7.4, and dissolved O_2=6.9 mg/L. The fish were acclimated to a 12:12h light/dark photoperiod and were fed twice daily on a commercial basal diet at 3% body weight.

2.3.2. Fish Exposure

A total of 64 *C. gariepinus* were assigned for four groups in duplicate (8 fish/tank; 16 fish/group) for a 15-day toxicants exposure trial. The first group contained the control fish, while the other three groups contained the toxicants-exposed fish. The second group was exposed to PE-MPs (10 mg/L) and named (PE-MPs-exposed), the third group was exposed to SLS (4 mg/L) and named (SLS-exposed) and the fourth group was exposed to their combination (PE-MPs; 10 mg/L + SLS; 4 mg/L) and named (PE-MPs+SLS-exposed). The exposure doses were selected with the toxic values observed in previous literature [16, 19].

For our experiments, stock suspensions containing 1 g/L of PE-MPs or SLS in purified water (Milli-Q) were designated and kept at 4°C in the dark according to the manufacturer’s instructions. Before each rearing water exchange, the stock solutions were stirred and instantly diluted in the fresh rearing water to adjust the detected exposure doses [17]. The exposure regimen was carried out by changing half of the water in tanks and redosing the PE-MPs and SLS every day after 2 days of the previous exposure. During the 15 days of the exposure, all groups were kept in the same acclimation setup and fed by the same regimen (3% body weight).

2.3.3. Tissue Sampling

After 15 days of the exposure treatment, six random fish were caught individually from each group (i.e., 3 fish from each tank) to be subjected to the kidney functions and histological analyses. The individually caught fish were sedated immediately by placing them in ice to avoid stress during further processing as previously described by Hamed et al. [18]. Blood samples were withdrawn in heparinized syringes from the caudal vasculature of the freshly sampled fish to be processed for the biochemical indices of kidney functions. Then after, the ice-sedated fish were dissected individually and tissue pieces of kidneys were elicited, rinsed, and fixed in 10% neutral buffered formalin to be further processed for the pathological and histochemical examinations.

2.4. Bioassays

2.4.1. Blood Biochemical Analysis

A fraction of the drawn blood was allowed to clot at 4°C before being centrifuged for 20 minutes at 5000rpm at 4°C. The serum was then separated and analyzed for the biochemical kidney function parameters, creatinine, and uric acid, following the kit's instructions [21].

2.4.2. Histology and Histochemistry Processing

Kidney samples were fixed for at least 48 hours in 10% neutral buffered formalin. Then after, the fixed samples were routinely processed for dehydration in graded ethanol concentrations (70, 90, 100%), clearing in xylene and paraffin wax impregnation. Ultrathin sections at 5µm were ultra-sectioned by rotary microtome, dewaxed in xylene, and stained according to the standard protocols [22]. To detect tissue degeneration, random samples were counterstained with Harris's Hematoxylin and Eosin (H & E). For the estimation of the polysaccharides content (mainly glycogen), random samples were subjected to the Periodic Acid Schiff (PAS) reaction. For fibrosis estimation, random samples were stained with Sirius red. All the stained sections were visualized and photographed under an Olympus microscope connected to a digital camera (BX50F4, Olympus Optical Co., LTP, Japan).

2.5. Statistical Analysis

Standard error (SE) and means were computed using basic descriptive statistics. A one-way analysis of variance (ANOVA) and Duncan's multiple range test (MRT) were applied to examine the significance of the comparison between means and standard errors. A Statistical Package for Social Sciences (SPSS) software (version 17.0) was used for all statistical analyses, with a significance level of 0.05. P-values of <0.01 (**) are considered highly significant and <0.001 (***) are considered very highly significant [23].

Renal fibrosis was evaluated statistically via the semi-quantitative measurements of renal collagen fibers, types I and III, in Sirius red-stained sections using ImageJ software (Public Domain, BSD-2, https://imagej.net/Ops, version 1.41o) according to Saleh et al. [24] with minor modification in the number of replicate photomicrographs and data interpretation to match our findings. Triplicate randomly selected digital images (acquired at 40×) of the Sirius red-stained sections from each dissected fish (i.e., 18 images from each group) (n=18) were subjected to the software procedures. The semi-quantification was estimated in two parameters for each group: (i) the expanding pattern of these fibers in the overall area (%) and (ii) the density of fibers per 0.5mm^2 area of each selected image. Obtained data were interpreted as means ± SE and displayed in histograms. In the same way, renal polysaccharide depositions (the positively stained area) were semi-quantified in the overall area (%) of randomly selected digital images of PAS-stained sections from each group (acquired at 40×, n=18).

3. Results

3.1. Surface Characteristics of PE-MPs

The morphology of the PE-MPs particles under a light microscope at 40x magnification is found to be irregular and vary in size as shown in Fig. 1.

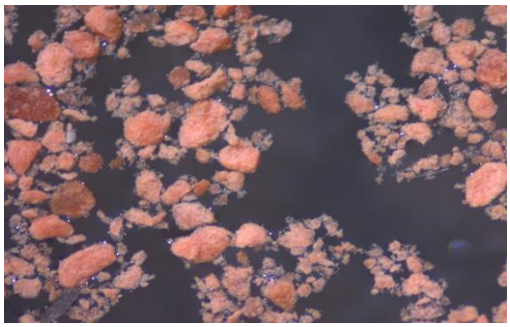

Figure 1. Photomicrograph displaying the irregular-shaped particles of the PE-MPs in a water medium under a light microscope at 40x magnification.

3.2. Kidney Functions Indicators in Fish Serum

An elevation in the kidney functions of *C. gariepinus* was observed after exposure to PE-MPs or SLS or their combination for 15 days. A significant increase (P <0.05) of creatinine was recorded in all the exposed groups compared to the control group. In addition, the level of uric acid increased significantly (P <0.05) following exposure to 10 mg/L PE-MPs or 4 mg/L SLS for 15 days compared to the control group. However, no statistically significant change between groups was recorded in the uric acid level by the exposure to the PE-MPs/SLS combination (Table 1).

Table 1. Kidney functions analysis in *C. gariepinus* serum after 15 days of exposure to PE-MPs, SLS, or their combination.

Parameter	Control	PE-MPs	SLS	PE-MPs + SLS
Creatinine (mg/dL)	0.34 ± 0.0^a	0.44 ± 0.01^b	0.44 ± 0.1^b	0.43 ± 0.01^b
Uric acid (mmol/L)	21.5 ± 0.2^a	22.4 ± 0.3^b	22.4 ± 0.3^b	21.9 ± 0.2^{ab}

Data are represented as means ± SE. Values with different superscript letters in the same row are significantly different (P <0.05).

3.3. Renal Tissue Integrity

3.3.1. Renal Pathology

H&E counter-stained sections of the kidney displayed the morphology of renal tubules in all the experimental groups. As shown in Fig. 2 A, the kidney sections from the control group have normal renal tubules with good morphology and intact glomeruli with regular Bowman's space. Cells of the whole tubules contain homogenous acidophilic cytoplasm with vesicular nuclei and a few with pyknotic ones. Still, all tubules lining cells connect with the basement membrane and separation between the cells of tubules leaves narrow unstained space or edema. In addition, hemopoietic tissues were observed between the renal tubules.

Kidney sections from the PE-MPs-exposed group (Fig. 2 B) showed excessive degeneration in renal morphology in both tubules and Bowman's capsule. Whole tubules dilated, edematous, lost their basal membrane with disorganization of their epithelial lining, and complete degeneration was noticed in their core with many pyknotic Nuclei. In addition, the renal tubules are surrounded by dissociated acidophilic cytoplasm.

Other tubules containing remnants of their basement membrane were observed and others with brush borders. Different stages of hemopoietic tissues between tubules were observed. Large melano-macrophage centers were observed and deformation of Bowman's capsule with irregular glomeruli with regular Bowman's space.

Kidney sections from the SLS-exposed group (Fig. 2 C) showed marked degeneration in renal morphology. Whole tubules contain lining cuboidal epithelial cells with homogenous acidophilic cytoplasm having both vesicular and pyknotic nuclei. The lining epithelium lost contact with each other leaving unstained spaces or edema, but still, the basement membrane was observed. Some other tubules were observed to lose their connection with the basement membrane due to edema and their lining epithelium moved towards their core, which contains faint acidophilic cytoplasm. Increased hemopoietic tissue cells between tubules, especially red blood corpuscles were observed.

Kidney sections from PE-MPs + SLS-exposed fish (Fig. 2 D) showed moderate degeneration in the renal structure. Dilation of both convoluted tubules and corpuscles was noticed. The main bulk of tubules degenerated with much edema and the tubules lost their epithelial lining. Other tubules contain luminal casts and pyknotic nuclei in the core of tubules. Still, the basal membrane was observed in a few tubules. Bowman's capsules showed severe deformation in their surface, which appeared with many infoldings or projections and dilated blood capillaries of glomeruli. In addition, hemorrhages containing hemopoietic cells were observed between the convoluted tubules.

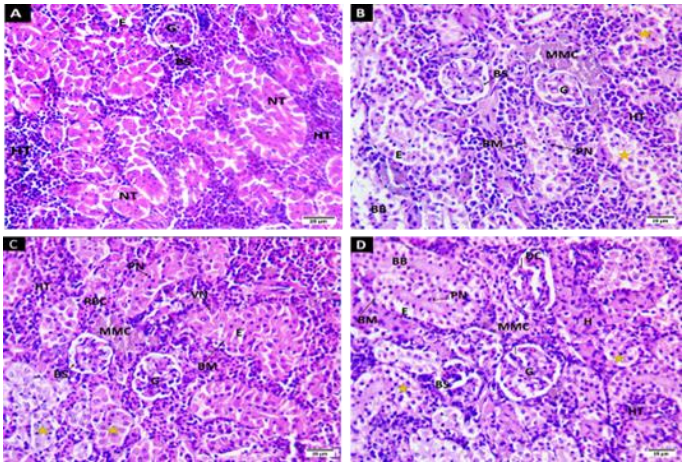

Figure 2. Photomicrographs of Hematoxylin and Eosin (H&E)-counterstained sections from the kidney of catfish *C. gariepinus* (×400; Scale bar =25µm). **A)** A section from a control fish showing normal renal integrity, including renal tubules (NT) with some edema (E), glomeruli (G), Bowman's space (BS), and hemopoietic tissue (HT). **B)** A section from fish exposed to PE-MPs (10 mg/L) shows complete degeneration in both tubules and Bowman's capsule with many pyknotic Nuclei (PN), edema (E), accumulations of melano-macrophage centers (MMC), brush border (BB), basement membrane (BM), and Bowman's space (BS) expansion. **C)** Kidney section from a fish exposed to SLS (4 mg/L) showing marked degeneration in renal morphology with unstained spaces of edema (E) and red blood cells (RBC) and vesicular nucleus (VN). **D)** Kidney section from a fish exposed to PE-MPs+SLS shows mild histological appraisals of renal tissue. Few residual hydropic degenerations (D), dilated blood capillaries (DC), and Hemorrhage (H) were noticed. In addition, the bulk of tubules degenerated and lost their epithelial lining from their basal membrane (yellow star).

Localization and Quantification of Renal Fibrosis Visual examination of Sirius red-stained kidney sections was employed for the localization of collagen I & III fibers as an optical sign of renal fibrosis (Fig. 3). Sections from the control group showed sparse collagen fibers in the connective tissue around glomeruli and renal tubules (Fig. 3 A). In fish exposed to PE-MPs (10 mg/L) or SLS (4 mg/L) (Figs. 3 B, and C), large accumulations of collagen fibers were noticed in the connective tissue surrounding the renal tubules and their associated blood vessels that show substantial thickening of their walls. Compared to the other exposed groups, decreased accumulations of collagen fibers were noticed in the connective tissue of the combined PE-MPs + SLS-exposed group (Fig. 3 D).

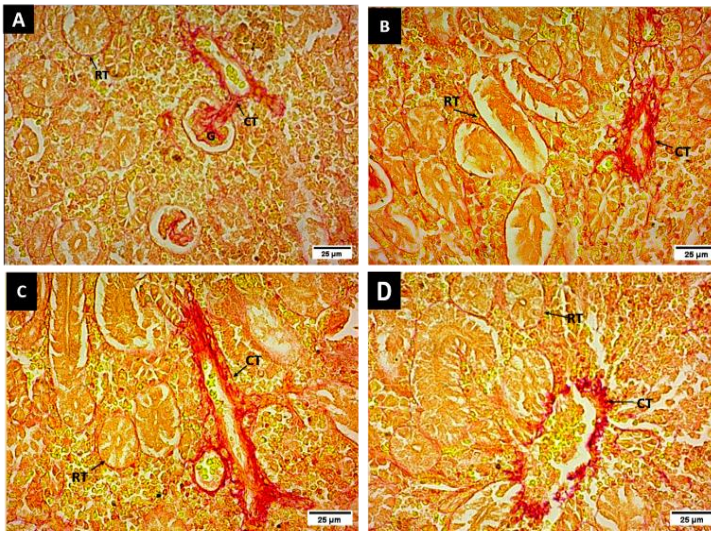

Figure 3. Photomicrographs of Sirius red-stained sections from control and exposed fish kidney (×400; scale bars =25µm). **A)** A control section shows sparse fibers of connective tissue (CT) surrounding the renal tubules (RT), renal corpuscles (RC), and glomerulus (G). **B)** A section from a PE-MPs-exposed fish shows connective tissue accumulations (CT) around the renal tubules (RT) and their associated blood vessels. **C)** A section from an SLS-exposed fish shows fiber accumulations of connective tissue (CT) around renal tubules (RT) and blood vessels with substantial thickness in the blood vessel walls. **D)** A section from a fish exposed to PE-MPs + SLS shows less accumulations of collagen I & III fibers in the connective tissue (CT).

Software semi-quantification of the renal collagen I & III fibers configured the visual observations of these fibers' locations and density. Fig. 4 displays the statistical variation in the expanding pattern of renal collagen fibers and their density calculations. Compared to the control group, a very high significant ($P < 0.001$) increase was recorded in the level of renal fibrosis of all the exposed groups; evidenced in their total area expansion and the density per 0.5 mm^2 area calculations. Notably, a significant decrease ($P < 0.05$) was recorded in the fibrosis of the combined PE-MPs + SLS-exposed group compared to the other two exposed groups.

3.3.2. Localization and Quantification of Renal Polysaccharides Depositions

Sections of fish kidneys stained by periodic acid Schiff's reagent for polysaccharides were employed for the localization

of the renal polysaccharides as displayed in Fig. 5. In the control group, extensive reactivity of polysaccharides was observed in the basement membrane and brush border of renal glomeruli and tubules. On the contrary, the PE-MPs or SLS-exposed groups showed depletion in polysaccharide contents of the same locations. At the same time, an augmentation in these contents was noticed in the combined PE-MPs+SLS-exposed group compared to the other two exposed groups.

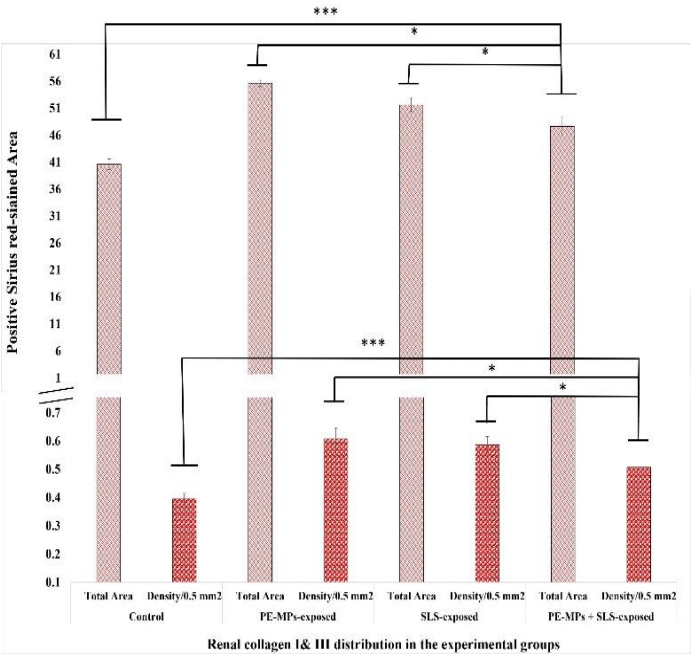

Figure 4. Bar histogram displays the expanding pattern (% area) and the density per 0.5mm^2 area of the renal collagen fibers (types I & III) in Sirius red-stained sections of kidney from control, PE-MPs, SLS or PE-MPs+SLS-exposed *C. gariepinus*. Data were obtained by ImageJ software and represented as Means ± SE of 18 sections per group (n=18). Superscript symbols on bars indicate the statistically significant different values between groups (P <0.05).

The statistical variation in the semi-quantification of the polysaccharide depositions between the experimental groups is displayed in the bar histogram (Fig. 6). Compared to the control group, the level of renal glycogen depositions showed a very high significant decrease (P <0.001) in both the SLS and PE-MPs-exposed groups, which indicates marked hypoglycemia. The lower level was recorded by the SLS-exposed group followed by the PE-MPs-exposed group. On the other hand, a depletion of renal polysaccharides level with no statistically significant difference (P > 0.05) was recorded by the combined PE-MPs + SLS group compared to the control group. Comparing between groups, high significant (P < 0.01) augmentation in the renal polysaccharides was recorded in the combined PE-MPs+SLS group compared to the other SLS and PE-MPs-exposed groups.

4. Discussion

Several hazardous effects are behind the exposure to environmental toxicants such as MPs and SLS. MPs sizes and forms (fiber-shaped, fragmented, etc) play a crucial role in their absorption as they can enter a fish's body from the water or sediment through gill respiration or swallowing behavior and accumulate in the gastrointestinal tract, muscles, organs, and circulatory system [25, 29]. Importantly, the consumption of MPs has been linked to several major side effects in many studies, which might be caused by inflammation and oxidative stress [30, 31]. In addition, anorexia and lethargy were reported as clinical signs associated with the ingestion of large-sized MPs (120–220 µm) [7]. Moreover, MPs are absorbed mechanically through the intestinal mucosa leaving serious damage to the digestive tract and its associated organs [32]. It's interesting to note that MPs serve as carriers for fish pathogens; therefore, MPs-exposed fish are more susceptible to infections [33].

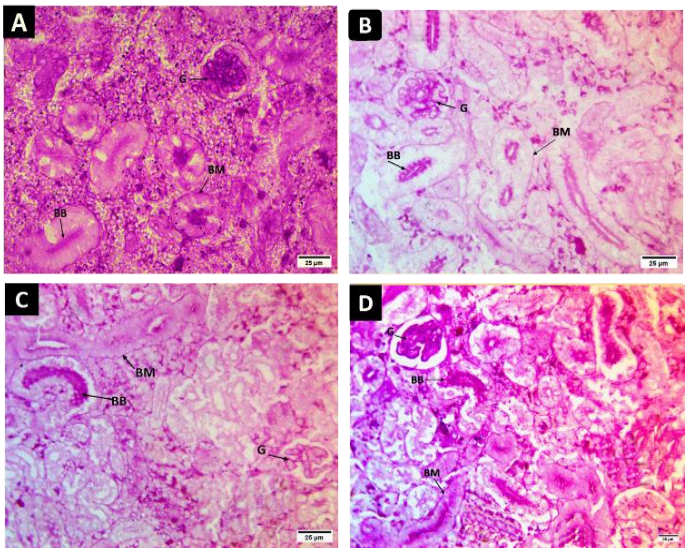

Figure 5. Photomicrographs of Periodic acid Schiff-stained sections of kidney from control and exposed fish (PAS; ×400; Scale bar =25µm). **A)** A control section shows extensive PAS reactivity and glycogen depositions in the basement membrane (BM) and brush border (BB) of the renal tubules. **B)** and **c)** sections from PE-MPs- and SLS-exposed fish show glycogen depletion in the basement membranes (BM) and brush borders (BB) of glomeruli (G) and renal tubules. **D)** A section from a PE-MPs+SLS-exposed fish shows a reversal in the glycogen depletion observed in the basement membrane (BM) and brush border (BB).

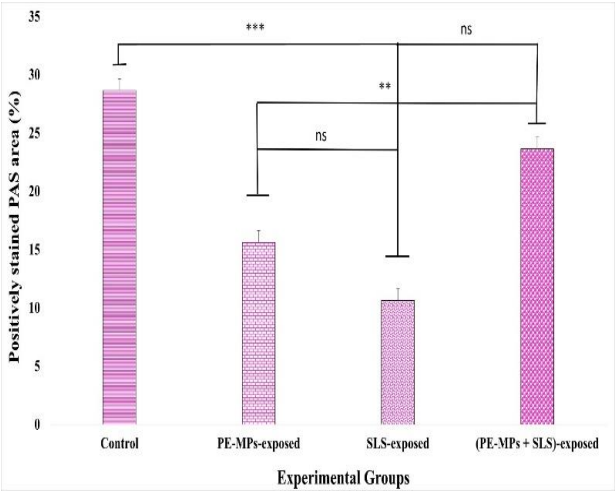

Figure 6. Bar histogram displays the expanding pattern (% area) of the renal polysaccharides' depositions in PAS-stained sections of the kidney. Data were obtained by ImageJ software and represented as Means ± SE of 18 sections per group (n=18). Superscript symbols on bars indicate the statistically significant different values between groups (P <0.05).

The surfactant SLS is also reported to elicit several harms upon consumption. Recent research has indicated that the harmful effects of SLS may be associated with the disturbance of the osmotic balance and the development of oxidative stress [14, 33]. Notably, the different anionic surfactants affect differently the enzyme activities of fish owing to their different physicochemical characteristics [35]. The current study was conducted to evaluate the fish nephrotoxicity initiated by MPs and SLS exposure either alone or in combination. We are interested in investigating the *C. gariepinus* kidney functions and renal tissue integrity after 15 days of exposure trial. The biochemical analysis of fish serum revealed significantly elevated levels of creatinine after 15 days of exposure to PE-MPs (10 mg/L), SLS (4 mg/L), or their combination compared to the control unexposed fish. Notably, the level of uric acid was elevated by the single exposure to PE-MPs or SLS but was not significantly changed between the experimental groups upon their combined exposure, indicating an antagonistic effect. Our data indicated the determined effect on fish upon single exposure to MPs or SLS, which agrees with the findings reported in several previous studies. Sayed and Authman [16] reported a significant increase in serum creatinine level and a nonsignificant change of uric acid in *C. gariepinus* exposed to a low dose of SLS (0.1 mg/L) for two weeks. Hamed et al. [18] reported a dose-dependent significant increase of creatinine and uric acid levels in the blood of Nile Tilapia (*Oreochromis niloticus)* early juveniles exposed to 1, 10, and 100 mg/L of MPs for 15 days. Similarly, *C. lazera* catfish experimentally exposed to varying doses of SLS displayed elevated urea and creatinine levels in serum [36].

On the other hand, the nonsignificant change of uric acid levels during the combined exposure to PE-MPs and SLS implies that their combined interaction can alter their toxicity and/or bioavailability. Reversely, MPs in fish diets were reported to damage the cells, suppress the innate defense system, and facilitate reaching additives as well as pathogens to the bloodstream from the gut lumen [37]. More recently, high concentrations of MPs in rainbow trout (Oncorhynchus mykiss) diet provided higher delivery, bioavailability, and bioaccumulation rate for the Enrofloxacin antibiotic; whereas amplified its toxicity and reduced its efficacy [38]. It is worth mentioning that deteriorated effects of MPs exposure were reported on the creatinine level of fish blood when combined with other stress-motivated factors. Creatinine levels increased significantly in the plasma of rainbow trout co-exposed to MPs and *Yersinia ruckeri* virulent bacteria [33].

On the histological levels, the smaller-sized MPs are likely to accumulate inside the fish body organs since they can pass through the digestive tract into the circulatory system, whereas larger-sized MPs typically amass in the digestive tract and are expelled more quickly [39]. Pathological investigation in the current study showed that 15 days of exposure to PE-MPs (10 mg/L) and/or SLS (4 mg/L) revealed glomerulopathy and nephrogenesis in catfish *C. gariepinus*. Exposure to MPs was reported to elicit pathological damage in different tissues of fish bodies. Wild fish exposed to high MPs pollution in their living environment showed pathological damage in the liver tissue [26]. In-vitro exposure to 10 and 100 mg/L of MPs for 14 days showed severe damage to almost all the internal organs of Nile tilapia early juveniles [40].

In a similar line to our study, it was reported that renal tissue disruption occurred in *C. gariepinus* after 15 days of feeding on a diet supplemented with MPs (500 mg/kg). This disruption was manifested in the form of tissue dissociation, shrinkage or regional glomerular hypertrophy, accumulation of melano-macrophages, and expansion of Bowman's space [17]. In an earlier report, diets amended with 500, 1000, or 2000 µg/g 10 µm fluorescent spherical polystyrene MPs for 10 weeks caused glomerulopathy and nephrogenesis of outbred and translucent Japanese medaka (*Oryzias latipes*) during their maturation in a dose-dependent manner without leaving mortalities, growth discrepancies or behavioral changes [37]. In disagreement with our findings, no pathological lesions were reported associated with pristine MPs ingestion by fish [7].

As for SLS exposure, several earlier studies reported the hazardous histological traits of several organs such as gills, liver, kidneys, and spleen tissues of catfish *C. lazera* exposed to low concentrations of SLS (0.11, 0.22, and 0.44 mg/L) displayed several histopathological alterations [36]. In agreement with our findings, severe blood vessel dilation was frequently observed in renal conjunction with an excess of extravasated erythrocytes [36]. In a recent study, SLS was less likely to be used for kidney allotransplantation as it couldn't preserve the extracellular matrix architecture of kidney tissue when used as a solvent in an automatic decellularization and perfuse device [41].

In-deep histological investigations of our study revealed significant augmentation in fibrosis while depletion in the polysaccharide's deposition was recorded in both the PE-MPs and SLS exposed groups compared to the control unexposed group. However, the combination of PE-MPs and SLS exposure revealed an antagonistic effect that lowers renal tissue degradation regarding both parameters. Increasing the collagen I & III in the connective tissue of the kidney indicates fibrosis and decreasing the polysaccharides than the control indicates tissue degradation [42]. Regarding energy reserves, living organisms can control the use of glycogen or lipids according to the stress level imposed upon them [19]. Lower levels of polysaccharides in the kidney tissue in exposed fish than in the control group indicate a reduction in glycogen metabolism, which is referred to as "hypoglycemia". In agreement with our findings, Sayed et al. [17] reported high connective tissue fiber accumulation and hypoglycemia in the kidney as well as the intestine of *C. gariepinus* fed with diets mixed with 500 mg/kg MPs for 15 days. In the same study [17], Masson's trichrome staining demonstrated the promoted collagen deposition in fish exposed to MPs and illustrated that the coadministration of natural antioxidants (lycopene, chlorella, or citric acid) during the administration of MPs decreased the accumulation of connective tissue fibers in different tissues [17]. Similarly, the toxicity of Voliam flexi® insecticide revealed increased intensity of hepatic collagen deposition in *C. gariepinus* juveniles [43]. Common carp continuously exposed to 10 mg/L PE-MPs for two weeks showed severe depletion in the hepatic polysaccharide contents [42]. Recently, single exposure to MPs or combined with polycyclic aromatic hydrocarbons was reported to reduce fish

muscle glycogens owing to osmoregulatory disturbances [45].

Overall, our findings indicate that there is some interaction between the PE-MPs and SLS eliciting unexpected antagonistic effects upon their combined exposure to fish. Our results disagree with Ammar et al. [44] who reported the synergistic harm effects of PE-MPs and the plastic additive 4-nonylphenol on juvenile common carp upon combined exposure for two weeks. Similarly, Rainieri et al. [46] reported the synergistic hazardous effects of dietary MPs combined with some organic contaminants mixture on zebrafish fed for 3 weeks.

4. Conclusion

Relevant information about the possible biological risk of the ensuing release of two chemical toxicants PE-MPs and/or SLS in aquatic ecosystems is provided by the current study. The kidney functions and tissue integrity of catfish *C. gariepinus* were assessed *in vitro* following exposure to both environmental toxicants, either individually or in combination. PE-MPs and SLS are found to be nephrotoxic for fish, while their synergism was observed to cause some interaction altering their toxicity, which elicited lower nephrotoxicity. This leads us to believe that ecological assessments are still missing from the toxicity evaluation, though, because a wide range of natural environmental factors can synergize the effect and counteract the toxicity as well as structure of pollutants, which alters their toxic effects in nature. Considering our findings, we recommend carrying out further upcoming studies on the significance of water contamination with MPs and SLS on fish disease susceptibility and propagation.

CRediT authorship contribution statement:

Conceptualization, H.S. and A.H.S.; methodology, H.S., A.E., F. A. and A.H.S.; software, H.S., A.E., F. A. and A.H.S; validation, H.S., A.E., F. A. and A.H.S.; formal analysis, H.S., A.E., F. A. and A.H.S.; investigation, H.S., A.E., F. A. and A.H.S; resources, H.S., A.E., F. A. and A.H.S.; data curation, H.S., A.E., F. A. and A.H.S; writing-original draft preparation, A.E., and F. A..; writing-review and editing, H.S., F. A. and A.H.S visualization, H.S., F. A. and A.H.S; supervision, H.S., F. A. and A.H.S. All authors have read and agreed to the published version of the manuscript."

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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