Microplastics induced endocrine disruption, alteration in testicular tissue in tilapia (*Oreochromis niloticus*) pre-fed on *Amphora coffeaeformis*

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Abstract

This study examines the potential defending effects of the diatom, *Amphora coffeaeformis, as a feed additive* against the deleterious effects (mainly on gonads) caused by microplastics (MPs) in the Nile tilapia, *Oreochromes niloticus*. Male tilapia groups were pre-fed diets with four different supplementation levels of *A. coffeaeformis* (0%, 2.5%, 5% and 7.5%) for 70 days, then were exposed to 10 mg/L MPs for 15 days. The results showed significantly ($p < 0.05$) higher numbers of erythrocytes, but significant decreases ($p < 0.05$) in the number of RBCs, Hb, Ht, platelets, and eosinophil percentages. Testicular histological degenerative changes and testis-ova were found in the MPs-exposed fish. Thus, *A. coffeaeformis* supplementation displayed ameliorative properties that detoxified the negative effects of MPs. This study provides a better understanding of the reproductive injuries caused by MPs and provides evidence for the use of *A. coffeaeformis* as a natural remedy in freshwater tilapia.

1. Introduction

In recent years, concerns have been raised about the incorporation of microplastics (MPs) in food webs. MPs are synthetic polymers with a diameter of less than 5 mm (Thompson et al., 2009). Increased industrial production of plastic has led to excessive quantities of plastic along coasts, and in rivers, seas, and oceans around the world, where they accumulate and fragment into smaller particles of microplastics and nanoplastics (Barnes et al., 2009). MPs have a negative effect on marine organisms, and can combine with other pollutants, such as plastic additives or organic pollutants, to create more damage (Browne et al., 2013; Hammer et al., 2012). At the present time, MPs characterize the greatest category of pollutants in the world (Derraik, 2002; Galgani et al., 2013).

Studies have reported that MPs may induce oxidative stress and exert negative effects on the antioxidant defenses of some invertebrates, such as monogonont rotifers (*Brachionus koreanus*), copepods (*Paracyclopina nana)*, and Chinese mitten crabs (*Eriocheir sinensis*) (Jeong et al., 2016; Yu et al., 2018). Fish exposed to MPs can modify antioxidant biomarkers and boost lipid peroxidation in some species, such as sheepshead minnows (*Cyprinodon variegatus*) (Choi et al., 2018), red tilapia (*Oreochromis sp.*) (Ding et al., 2018) zebrafish (*Danio rerio*) (Qiao et al., 2019; Wan et al., 2019), and Nile tilapia (*Oreochromis niloticus*) (Hamed et al., 2020). In another example, Barboza et al. (2018) found that MPs amplified lipid oxidation in European sea bass (*Dicentrarchus labrax*) brains, triggering oxidative stress and lipid damage. It was also found that MPs stimulated DNA damage in Nile tilapia (*O. niloticus*) (Hamed et al., 2020). Exposure to MPs has also been found to cause other injurious effects, such as neural dysfunction in gobies (*Pomatoschistus microps*) (Oliveira et al., 2013), hepatic stress in medakas (*Oryzias latipes*) (Rochman et al., 2014), increased mortality in European sea bass (*D. labrax*) (Mazurais et al., 2015), and immune system damage in fathead minnows (*Pimephales promelas*) (Greven et al., 2016).

MPs are also a possible cause of fish reproductive stress, which causes reproductive disruption, but the stress response in fish gonads caused by MPs exposure is still poorly understood. It has been reported

that MPs cause endocrine impairment and disrupt the hypothalamus-pituitary-gonadal (HPG) axis and steroidogenesis pathway (Karami et al., 2016; Rochman et al., 2014; Wan et al., 2019). MPs have also been found to delay female gonadal maturation, decrease fecundity (Lönnstedt and Eklöv, 2016; Wan et al., 2019), increase the amount of reactive oxygen species (ROS) in the gonads of both male and female zebrafish, cause reproductive stress by increasing apoptosis levels and histological alterations in testes (Qiang and Cheng, 2021), and decrease the plasma 17b-estradiol (E2) and testosterone (T) levels in female marine medaka (*Oryzias melastigma*) (Wan et al., 2019).

Recent research has focused on finding effective natural antioxidants to replace synthetic antioxidants currently in use (Glodde et al., 2018; Kumosani et al., 2017; Taghvaei and Jafari, 2015). Benthic diatoms are microalgae that are recognized as natural antioxidants (El-Sayed et al., 2018; Lee et al., 2008). *Amphora coffeaeformis* is a single-celled microalgae present in most aquatic environments that can tolerate various salinities (Sala et al., 1998). Its cell wall is made of opaline silica (Buhmann et al., 2014) and it is an important food source for many invertebrates, fish species, and zooplankton (Kaparapu, 2018). Diatoms such as *A. coffeaeformis*, have a high capability for metal absorption and a high proliferation rate (Anantharaj et al., 2011), which enables them to resist the toxic effects of environmental contaminants (Gaur and Rai, 2001). These features support the use of diatoms as antioxidant mediators, and detoxifying agents in stress-induced fishes (Sheikhzadeh et al., 2012).

A. coffeaeformis analysis reveals that they are composed of substantial amounts of lipids, proteins, minerals, sugars, carotenoids, chlorophylls, fatty acids, and polyphenols (Chtourou et al., 2015). *A. coffeaeformis* has also displayed anti-inflammatory factors (Lauritano et al., 2016), as wellas antibacterial (Boukhris et al., 2017), antiviral (Abdel-Wahab, 2018), and antioxidant activity, which limits the harmful effects of free radicals (El-Sayed et al., 2018; Mekkawy et al., 2020). *A. coffeaeformis* shows promise as a feed additive, given its bioactive antioxidant constituents, including carotenoids, astaxanthin, canthaxanthin, b-glucans, polyunsaturated fatty acids, sulfated polysaccharides, and vitamins C and E (El-Sayed et al., 2018). Thus, *A. coffeaeformis* as a feed additive in Nile tilapia diets could replace antibiotics (Ayoub et al., 2019b), and detoxify and protect against arsenic-induced oxidation, as has occurred in African catfish (*Clarias gariepinus*) liver tissues (Ayoub et al., 2019a). *A. coffeaeformis* supplementation has been shown to improve fish immunity by reducing pathogenic bacteria and increasing the innate immune system response of fish (Saleh et al., 2020). It can also enhance male gonadal maturity, increase female fish fecundity (Saleh et al., 2020), and reduce DNA lesions (Karawita et al., 2007).

This study investigated the effect of *A. coffeaeformis* supplementation as pre-feeding on the *Oreochromis niloticus* intoxicated with MPs. This objective accomplished via the assessment of hematological and biochemical parameters, antioxidant enzymes, hormonal levels, and the reproductive status of male fish. This study will help to elucidate the effects of MPs exposure on fish health, with special concern to gonadal health, and attempts to ameliorate the negative impacts of MPs by supplementing *A. coffeaeformis* in fish diets.

2. Materials And Methods

2.1. Experimental design

Nile tilapia fingerlings (360) were obtained from the Baltim experimental farm (NIOF), Egypt and were transferred to fish nutrition laboratory (NIOF) and fed on a commercial diet (25% crude protein, 4.5 L, Aller Aqua, Egypt) for one week to adjust to the experimental conditions. The fish (average body weight ca. 4 g) were distributed in 12 tanks with 200 L capacity on triplicate basis. Water temperature was 27.4 ± 1.2°C. Oxygen was supplied and kept above 7.0 mg/L. The natural photoperiod was 11-h light: 13-h dark throughout the experiment period. The experimental diets contained ascending levels of *A. coffeaeformis* (0%, 2.5%, 5 % and 7.5%) to their diets, as described by Saleh et al. (Saleh et al., 2020) (Table 1). The test diets were given three times a day to satiation, and the fish were fed the experimental diets for 70 days. After this period 10 male fish were selected from each tank and placed into separate aquaria for extra 15 days under MPs treatment on triplicate basis. Microplastic was added to the each aquarium at a concentration of 10 mg/ L according to Hamed et al. (2019).

Table 1
Composition and proximate analyses (% DM) of amphora-supplemented diets

Ingredient	Diets (g/Kg)				
	A0	A0	A1	A2	A3
Fish meal[1]	160	160	160	160	160
Soybean meal[2]	350	350	350	350	350
Yellow corn	200	200	200	200	200
Wheat flour	220	220	195	170	145
Sunflower oil	50	50	50	50	50
Vitamins& minerals Premix[3]	20	20	20	20	20
Amphora meal (g/kg)	0	0	25	50	75
Proximate composition (%DM)					
Crude protein	28.89	28.89	29.50	29.90	29.34
Lipid	7.97	7.97	8.30	8.22	7.99
Ash	7.2	7.2	7.00	6.50	7.10
Fiber	3.66	3.66	3.70	3.90	3.85
NFE [4]	52.26	52.26	50.50	51.48	51.72
GE (MJ/Kg)[5]	18.34	18.34	19.16	19.20	18.98
Dry matter	90.48	90.48	91.00	89.90	90.89
[1]lab made (68% protein), [2] (42% protein)					
[3] Vitamins and minerals premix (mg kg^{-1}): p-amino benzoic acid (9.48); D-biotin (0.38); inositol (379.20); niacin (37.92); Ca pantothenate (56.88); pyridoxine HCl (11.38); riboflavin (7.58); thiamine HCl (3.79); L-ascorbyl-2-phosphate Mg (APM) (296.00); folic acid (0.76); cyanocobalamine (0.08); menadione (3.80), vitamin A palmitate (17.85); a-tocopherol (18.96); calciferol (1.14). K2PO4 (2.011); Ca3 (PO4)2 (2.736); Mg SO47H2O (3.058); NaH2PO4 2H2O (0.795).					
[4]Nitrogen-free extracts (NFE) = 100– [% ash + % lipid + % protein + % fiber].					
[5]Gross energy was quantified based on 23.6, 39.5 and 17.2 kJ/g for protein, lipid, and carbohydrates, respectively (NRC, 2011).					

The experimental fish groups included the control group (A0MP0) where fish were fed a control diet and reared in normal conditions, a group (A0MP) where the fish were fed the control diet, but exposed to MP-

polluted water, and an additional three test groups (A1MP, A2MP and A3MP), where fish were reared in MP-polluted water while receiving increasing concentrations of *A. coffeaeformis* (2.5, 5 & 7.5%, respectively) in their diets.

At the end of the day 15 of MPs exposure, six fish from each group were randomly chosen and sampled. Blood was collected from the caudal vein to measure antioxidant enzymes, hormone levels, and lipid peroxidation. After fish were anesthetized and weighed separately, they were directly killed by decapitation and then dissected. Gonads were excised from each fish, weighed and GSI calculations were completed using the following formula: GSI = gonads weight/gutted weight × 100. Afterwards, the gonads were fixed in neutral buffered formalin (10%).

Fish maintenance and experimental procedures were approved by the Research Committee of the NIOF, Egypt, and were in accordance with the Guide for Use and Care of Laboratory Animals (European Communities Council Directive 2010/63/EU).

2.2. Diatom preparation

A. coffeaeformis powder was obtained from the Microalgae Culture Center at the National Research Center, NRC, Egypt. The production procedure was carried out according to El-Sayed et al. (2018). Analysis of the *A. coffeaeformis* powder samples was conducted to determine their biochemical composition (AOAC, 1995), which included 26.6% crude protein, 9.9% crude lipid, 45.4% ash, 3.3% fiber and 14.8% total carbohydrate content. The diets were isonitrogenous (ca. 30% CP), isolipidic (8% L) and isoenergetic (19 MJ/kg).

2.3. Microplastics

The MPs consisted of raw powder with irregular-shaped particles. More than 90% of the MPs were > 100 nm in size. The MP powder was purchased from Toxemerge Pty Ltd. (Melbourne, Australia). A stock solution was prepared from the powder using purified water (Milli-Q) according to the manufacturer's instructions and stored at 4 ºC in the dark. The stock solution (2.5 g MP/L) was sonicated before each use. Additional dilutions were prepared from this stock immediately every time the rearing water was changed. The characterization of the MP particles was performed using light and transmission electron microscopy at TEMU, Assiut University (JEOL JEM-1200 EX II) (Hamed et al., 2019).

2.4. Hematological parameters

The blood samples were collected from the caudal vein of the fish using heparinized syringes, and immediately smeared onto clean glass slides. Blood smears (six slides from each fish) were dried, then fixed in absolute methanol for 1 min and stained with hematoxylin and eosin stain (H&E) per Mekkawy et al. (2011). In each slide group, 10,000 cells (minimum of 1,000 cells per slide) were examined under a 40× objective lens with a 10× eyepiece using an OMAX microscope with a 14 MP USB digital camera (CS M837ZFLRC140U) to identify any micronucleated, morphological, or nuclear abnormalities in the red blood cells (RBCs) per Al-Sabti and Metcalfe (1995). The RBCs and white blood cell (WBCs) counts, hematocrit (HCT), and hemoglobin (HB) were estimated using an automated hematology analyzer (BCC-

3000B; Changchun Dirui Industrial Co., Ltd). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated using the formulae mentioned by Dacie and Lewis (1991).

2.5. Biochemical parameters

The remaining blood samples were taken with non-heparinized syringes then left to clot at 4°C. The coagulated blood samples were centrifuged at 4,000 *g* for 10 min for serum separation. The analyzed biomarkers included: aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities, glucose (GL), cholesterol, total protein (TP), albumin (AL), globulin (GLO), creatinine (CR), and uric acid (UA). These were determined using test kits from HUMAN GmbH Company, Wiesbaden, Germany.

2.6. Antioxidant enzyme activities

Superoxide dismutase (SOD) was evaluated per Nishikimi et al. (1972). Catalase (CAT) was determined per Aebi (1984). Total antioxidant capacity (TAC) was measured according to Koracevic et al. (2001).

2.7. Hormone measurements

The follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (T) were measured using ELISA kits (Diagnostics Biochem Canada Inc.; Ontario, Canada). FSH was measured according to Knobil (1980) using a test kit (Cat. No. CANFSH-4060). LH was measured per Cumming et al. (1985) using a test kit (Cat. No. CAN-LH-4040). T was also measured using a test kit (CAN-TE-250) per Check et al. (Check et al.). All hormones were measured at 450 nm using an automatic immunodiagnostic analyzer (Sorin Biomedica, Model: 0-2730; S/N = 0654, Chemila SP.A., Italy).

2.8. Histological analysis

The fixed testicular samples were dehydrated in ascending grades of ethanol, then were cleared in methyl benzoate, and embedded in paraffin wax. Paraffin sections of 5 µm thickness were cut and stained with H&E stain, then examined microscopically.

2.9. Statistical analysis

Data are expressed as mean ± standard deviation. Significant differences between the treated and control groups were tested using one-way analysis of variance (ANOVA) followed by the Tukey-HSD test for multiple comparisons. Probability of a significant difference was set at $p < 0.05$. The analysis was carried out using the SPSS® version 23.0 package (SPSS, 1998).

3. Results

3.1. Hematological parameters

The blood smears from all treated groups had altered erythrocyte shapes, including acanthocytes, crenated cells, teardrop-like cells, and sickle cells. Altered nucleus shapes were also found, including blebbed nuclei, notched nuclei, vacuolated nuclei, and bilobed nuclei (Fig. 1b–f).

The fish group that was only exposed to MPs (A0MP) had a significantly high number of altered erythrocytes ($p < 0.05$) compared to the other groups (Fig. 2). The group fed the highest level of *A. coffeaeformis* (A3MP) had a significantly ($p < 0.05$) smaller number of altered erythrocytes compared to the other two *A. coffeaeformis* fed groups (A1MP and A2MP), but still displayed a significantly higher ($p < 0.05$) amount than the control group (Fig. 2).

The fish blood analysis results displayed a significant decrease ($p < 0.05$) in RBCs, HB, Ht and platelets in the control group (A0MP0), A1MP and A2MP fish groups. In contrast, these blood biomarkers were not significantly different in the A3MP group compared to the control ($p < 0.05$; Table 2). WBCs were significantly ($p < 0.05$) increased in A0MP and A1MP compared to A2MP; however, MCV, MCH, and MCHC percentages were not significantly different between the different treatment groups. WBCs differentiation demonstrated a remarkable decrease in the percentage of eosinophils in A0MP and A1MP, which coincided with a significant increase in neutrophils ($p < 0.05$). No change was found in the percentage of monocytes and lymphocytes.

Table 2
Hematological parameters of Nile tilapia (*Oreochromis niloticus*) fed different *A. coffeaeformis* concentrations, then exposed to 10 mg/L microplastics.

Fish group					
	A0MP0	**A0MP**	**A1MP**	**A2MP**	**A3MP**
RBC's (million/mm3)	2.036 ± 0.056^{a}	1.813 ± 0.047^{b}	1.833 ± 0.047^{b}	1.920 ± 0.060^{ab}	2.023 ± 0.066^{a}
Hb(g/dl)	8.46 ± 0.11^{a}	8.00 ± 0.10^{b}	8.13 ± 0.25^{ab}	7.96 ± 0.12^{b}	8.33 ± 0.05^{ab}
Ht (PCV) (g/dl)	25.80 ± 0.34^{a}	23.00 ± 1.00^{b}	23.33 ± 0.57^{b}	24.53 ± 0.23^{ab}	25.86 ± 0.46^{a}
MCV (µm³)	126.73 ± 3.52	126.91 ± 6.92	127.31 ± 3.74	133.87 ± 3.30	127.96 ± 5.74
MCH (Pg)	41.595 ± 1.39	44.147 ± 1.70	44.376 ± 1.47	43.434 ± 1.16	41.209 ± 1.05
MCHC %	32.824 ± 0.89	34.819 ± 1.33	34.885 ± 1.82	32.444 ± 0.19	32.224 ± 0.70
Platelets (Thousands/ mm3)	315.00 ± 1.00^{a}	311.33 ± 0.57^{b}	309.66 ± 0.57^{b}	310.66 ± 0.57^{b}	314.00^{a}
WBC's (Thousands/ mm3)	850.66 ± 5.13^{ab}	861.66 ± 5.77^{a}	861.66 ± 5.13^{a}	846.33 ± 1.52^{b}	851.33 ± 6.35^{ab}
Lymphocytes %	88.33 ± 0.57	88.66 ± 0.57	88.33 ± 0.57	88.33 ± 0.57	88.00
Monocytes %	3.66 ± 0.57	2.66 ± 0.57	3.00	3.33 ± 0.57	2.66 ± 0.57
Neutrophils %	6.00^{b}	7.66 ± 0.57^{a}	7.66 ± 0.57^{a}	6.00^{b}	7.33 ± 0.57^{a}
Eosinophils %	2.00^{a}	1.00^{b}	1.00^{b}	2.33 ± 0.57^{a}	2.00^{a}

Data are presented as mean ± SD, n = 6; means with different superscript letter within the same row for each parameter are significantly different ($p < 0.05$).

3.2. Biochemical parameters

No significant differences ($p < 0.05$) were found in liver enzyme levels (AST and ALT) among the treatment groups (Table 3). ALP levels were the highest in A0MP but remained at control levels in fish fed *A. coffeaeformis*. The serum glucose levels significantly increased ($p < 0.05$) in A0MP, A1MP, and A2MP compared to the control, but A3MP had serum glucose at control levels. The cholesterol and serum total protein levels were unchanged between the different treatment groups ($p < 0.05$). An obvious elevation in

serum uric acid values were recorded in A0MP, A1MP, and A2MP, but A3MP had uric acid levels at control levels. The uric acid levels displayed a negative correlation with increasing amounts of *A. coffeaeformis* (Table 3). Serum albumin levels and albumin-to-globulin ratios (A/G) were significantly elevated ($p < 0.05$) in A0MP and A1MP. A2MP and A3MP had the same serum albumin and A/G values as the control.

Table 3
Biochemical parameters of Nile tilapia (*Oreochromis niloticus*) fed different *A. coffeaeformis* concentrations, then exposed to 10 mg/L microplastics.

Group	A0MP0	A0MP	A1MP	A2MP	A3MP
AST (u/l)	55.00 ± 1.04	53.03 ± 2.02	52.33 ± 1.67	53.26 ± 0.90	54.43 ± 1.00
ALT (u/l)	27.86 ± 0.32	28.03 ± 0.64	27.63 ± 1.59	27.46 ± 0.55	28.36 ± 0.49
ALP (u/l)	24.53 ± 0.05^{b}	27.23 ± 0.28^{a}	26.36 ± 0.55^{ab}	25.03 ± 1.33^{b}	25.00 ± 0.52^{b}
Glucose (mg/dl)	97.86 ± 1.27^{c}	111.73 ± 1.15^{a}	114.10 ± 1.53^{a}	105.20 ± 3.34^{b}	98.86 ± 0.05^{c}
Cholesterol (mg/dl)	191.33 ± 5.85	197.33 ± 2.08	191.00 ± 11.26	190.66 ± 3.78	188.33 ± 1.15
Total protein (g/dl)	4.93 ± 0.41	5.26 ± 0.05	5.40 ± 0.20	4.86 ± 0.05	4.86 ± 0.20
uric acid (mg/dl)	12.20 ± 0.17 c	13.43 ± 0.05 a	13.16 ± 0.25 a	12.73 ± 0.05 b	12.40 ± 0.17 bc
Creatinine (mg/dl)	0.53 ± 0.09	0.64 ± 0.02	0.61 ± 0.05	0.55 ± 0.01	0.59 ± 0.07
Albumin (g/dl)	1.03 ± 0.01^{c}	1.27 ± 0.08^{ab}	1.41 ± 0.16^{a}	1.09 ± 0.04^{bc}	1.12 ± 0.02^{bc}
Globulin (g/dl)	2.13 ± 0.01	2.18 ± 0.04	2.17 ± 0.09	2.14 ± 0.05	2.11 ± 0.05
A/G	0.483 ± 0.002^{c}	0.582 ± 0.028^{ab}	0.647 ± 0.071^{a}	0.511 ± 0.009^{bc}	0.532 ± 0.008^{bc}

Data are presented as mean ± SD, n = 6, means with different superscript letter within the same row for each parameter are significantly different ($p < 0.05$). Abbreviations: A/G, albumin-globulin ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase

3.3. Antioxidant enzyme activities

TAC levels were unchanged between (A1MP, A2MP, A3MP) and control, while TAC level was only significant higher in A0MP than A1MP group (Fig. 3a). A significant elevation ($p < 0.05$) in the serum levels of SOD and CAT enzymes in A0MP were observed compared to the control, while the other three fish groups fed *A. coffeaeformis* had levels that were comparable to the control group (Fig. 3b, c).

3.4. Hormonal activity and GSI

The serum LH and T profiles demonstrated pronounced suppression in A0MP. The LH and T serum levels in A3MP were close to those of the control group. The FSH serum levels showed insignificant ($p < 0.05$) changes between the different groups (Fig. 4a, b and c). Moreover, the GSI displayed significant increases ($p < 0.05$) in A3MP (Fig. 4d).

3.5. Testicular histology

Light microscopic examination showed that transverse sectioned testes from the control group consisted of lobules with different spermatogenic stages that were separated from each other by interstitial tissue. An accumulation of mature spermatozoa was observed in the lumen of the testicular lobules (Fig. 5a).

The A0MP group had a small number of spermatozoa or no spermatozoa in the lumen of the testicular lobule. Vacuolated seminiferous epithelium and a slight proliferation of the interstitial connective tissue were also detected in the testes, but the testes still displayed considerable amounts of spermatogonia and spermatocytes in the tubule cysts. Other sections of the testes had perinucleolar oocytes inside the testicular lobules (testis-ova) (Fig. 5b, c & d).

A1MP had proliferated interstitial connective tissue, a marked decrease of the spermatozoa in the lumen of the testicular lobules, and vacuolization of the seminiferous epithelium. The development of a considerable amount of early perinucleolar oocytes were found inside the testicular lobules (testis-ova), and degenerative changes with necrotic areas were also observed (Fig. 5e &f).

A2MP and A3MP had no testicular alterations. Sections showed a large number of spermatozoa occupying most of the testicular lobules (Fig. 5g&h).

4. Discussion

Hematological parameters are the primary indicators of fish health (Thummabancha et al., 2016). In this study, MPs induced erythrocyte alterations in all tested groups compared to the control (A0MP0). Fish groups fed *A. coffeaeformis*-supplemented diets expressed less significant erythrocyte alterations than the A0MP0 group. A remarkable reduction in RBCs, Hb, Ht, platelets, and eosinophil percentages after exposure to MPs were detected. Furthermore, the neutrophil percentage significantly increased after MPs exposure. In general, the hematological results indicated that the highest amphora supplementation level (7.5%) ameliorates the effects of MPs, since they maintained the hematological parameters at control levels. These results concur with those of Hamed et al. (2019), who found that MPs exposure induced hematological deterioration in juvenile Nile tilapia. Moreover, immune system efficiency is affected by MPs uptake, which disturbs the organism's defense and health (Espinosa et al., 2017).

Serum biochemical parameters are good indicators for the general health status of animals and can provide early signs of serious changes in stressed organisms (Folmar, 1993; Garima and Himanshu, 2015; Jacobson-Kram and Keller, 2001). It has been reported that MPs exposure can induce changes in the biochemical parameters of various fish species, including the common goby (*Pomatoschistus*

microps) (Oliveira et al., 2013), the common carp (*Cyprinus carpio*) (Haghi and Banaee, 2016), and Nile tilapia (Hamed et al., 2019). In general, the different serum biomarkers act as indicators for organ dysfunction and the impairment of biological processes. In this study, some serum biomarkers (ALP, glucose, uric acid, albumin, and A/G ratio) were significantly increased due to MPs exposure. These biomarkers were not significantly changed and maintained as control levels when *A. coffeaeformis* supplementation used with 5% and 7.5% diets.

The results of this study agree with those of Mekkawy et al. (2020), who recorded that *A. coffeaeformis* dietary supplementation improved arsenic-induced hematological parameters in African catfish (*Clarias gariepinus*), and those of El-Sayed et al. (2018), who proved that dietary supplementation of *A. coffeaeformis* removed elements causing liver tissue damage induced by paracetamol induction in rats. The ameliorative effect of *A. coffeaeformis* may be attributed to its bioactive components, which have many health benefits (Mekkawy et al., 2020; Saleh et al., 2020) that protect organisms against stressors. Ayoub et al. (Ayoub et al., 2019a) found that *A. coffeaeformis* supplementation in fish diets can increase disease resistant and improve hematological and biochemical parameters in Nile tilapia.

Antioxidant biomarkers are used to identify the effects of multiple environmental stressors in aquatic organisms (Hook et al., 2014). SOD and CAT levels in this study were significantly elevated attributable to MPs exposure, while TAC had close to control levels in all tested groups, but were significantly different between A2MP and A0MP. The disruption of antioxidant enzyme activities triggered by MPs exposure has been recorded in some studied aquatic organisms (Prinz and Korez, 2020) and fish, including the common goby (Luís et al., 2015), zebrafish (Lu et al., 2016), sheepshead minnows (Choi et al., 2018), and red tilapia (Ding et al., 2018). Opposing the present results, Karami et al. (2016) found that MPs had no effects on the antioxidant enzyme activities in zebrafish. Changes in CAT and SOD enzymes were limited by *A. coffeaeformis* supplementation. These results agree with El-Sayed et al. (2018) and Moneeb et al. (2020), who suggested that *A. coffeaeformis* extract can restore antioxidant enzymes to control levels. *A. coffeaeformis* has antioxidative properties and is considered a natural antioxidant (Lee et al., 2008; Lee et al., 2009) that is able to stimulate a specific set of biochemical and physiological actions to repel the toxic effects of environmental pollutants (Gaur and Rai, 2001).

Fish reproductive success has a long-term effect on population constancy and the integrity of entire aquatic ecosystems. The current results demonstrated that exposure to MPs pollution can negatively impact male tilapia fertility, and induce oxidative stress. The potential negative impacts of MPs were reduced in fish fed higher *A. coffeaeformis* levels.

It is suggested that MPs indirectly act on the hypothalamus-pituitary axis to alter the synthesis and secretion of gonadotropin, which leads to the interruption of sex steroid production and testicular degeneration. The alterations seen in this study have also been recorded in male marine medaka (*Oryzias melastigma*) and may be explained by the up-regulation of gene expression for genes involved in the HPG axis and the steroidogenesis pathway (Wan et al., 2019). MPs also down-regulated the transcription of gonadotropin-releasing hormone (GnRH) in the hypothalamus of African catfish (*Clarias gariepinus*)

(Karami et al., 2016), and vitellogenin and choriogenin in the liver of male Japanese medaka (*Oryzias latipes*) (Rochman et al., 2014). In accordance with the histological alterations described in this study, Rochman et al. (2014) defined abnormal male germ cell growth and suggested that this abnormal growth may be due to small clusters of oogonia that undergo atrophy when male Japanese medaka were exposed to environmentally relevant MPs concentrations. Wang et al. (2019) explained different histological alterations in testicular tissues after MPs exposure, including an increase in the interstitial tissue, a disorder of the arrangement of seminiferous lobules, the dissolution of the basal membrane, and a loose arrangement of spermatocytes. Moreover, a reduction in the testis basement membrane thickness was also recorded (Qiang and Cheng, 2021). In contrast to the present results, MPs exposure significantly increased the level of T in male marine medaka (Wan et al., 2019).

This study indicated that the testis histological alterations found in fish exposed to MPs were the most improved by the higher two doses of *A. coffeaeformis* supplementation in A2MP (5%) and A3MP (7.5%), which maintained the LH and T levels as in control fish and improved GSI values.

Altogether, the current results suggest that MP exposure reduces reproductive performance in Nile tilapia indirectly through oxidative stress, which agrees with Wang et al. (2019). Qiang and Cheng (2021) proposed that overall stress in detoxification and the induction of antioxidant enzyme activities are contributing factors in reproductive impairments. Gonads are most predisposed to oxidative stress, since gonads display a higher frequency of cell division, mitochondrial oxygen intake, and unsaturated fatty acids compared to other tissues (Asadi et al., 2017; Ciani et al., 2015). It has been previously shown that stressors, such as chemical exposure, can induce testicular oxidative stress, cause apoptosis in germ cells, and negatively affect spermatogenesis (Asadi et al., 2017).

In conclusion, 10 mg/L MPs exposure in fish for 15 days induced hematological and biochemical changes in *O. niloticus*. Furthermore, MPs prompted oxidative stress, hormonal disruption and testicular damage, and induced testis-ova in male tilapia. The highest tested supplementation level of *A. coffeaeformis* (A3MP; 7.5%) prevented the oxidative stress, testicular damage, and negative hematological alterations. The present results suggest that MPs indirectly affect the HPG axis and subsequently the gonads through oxidative stress. Additional research work is needed to determine the mechanisms responsible for the endocrine disruption, histomorphological alterations, and possible reproductive impairments induced by MP exposure.

Declarations

Authors' contributions

Experimental design: RFI, NES, AHS. Experiment and analysis: RFI, NES, AHS. Data interpretation: RFI, NES, AHS. Writing and revision: RFI, NES, AHS. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analyzed during this study are included in the research article.

Acknowledgment

It is not applicable.

Ethical approval

Experimental conditions and fish handling were approved by the Research Ethical Committee of the Faculty of Science, Assuit University, Assuit, Egypt according to the guidelines approved by the institutional animal care and use committee (IACUC).

Consent to participate

Not applicable

Consent for publication

Not applicable

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Figures

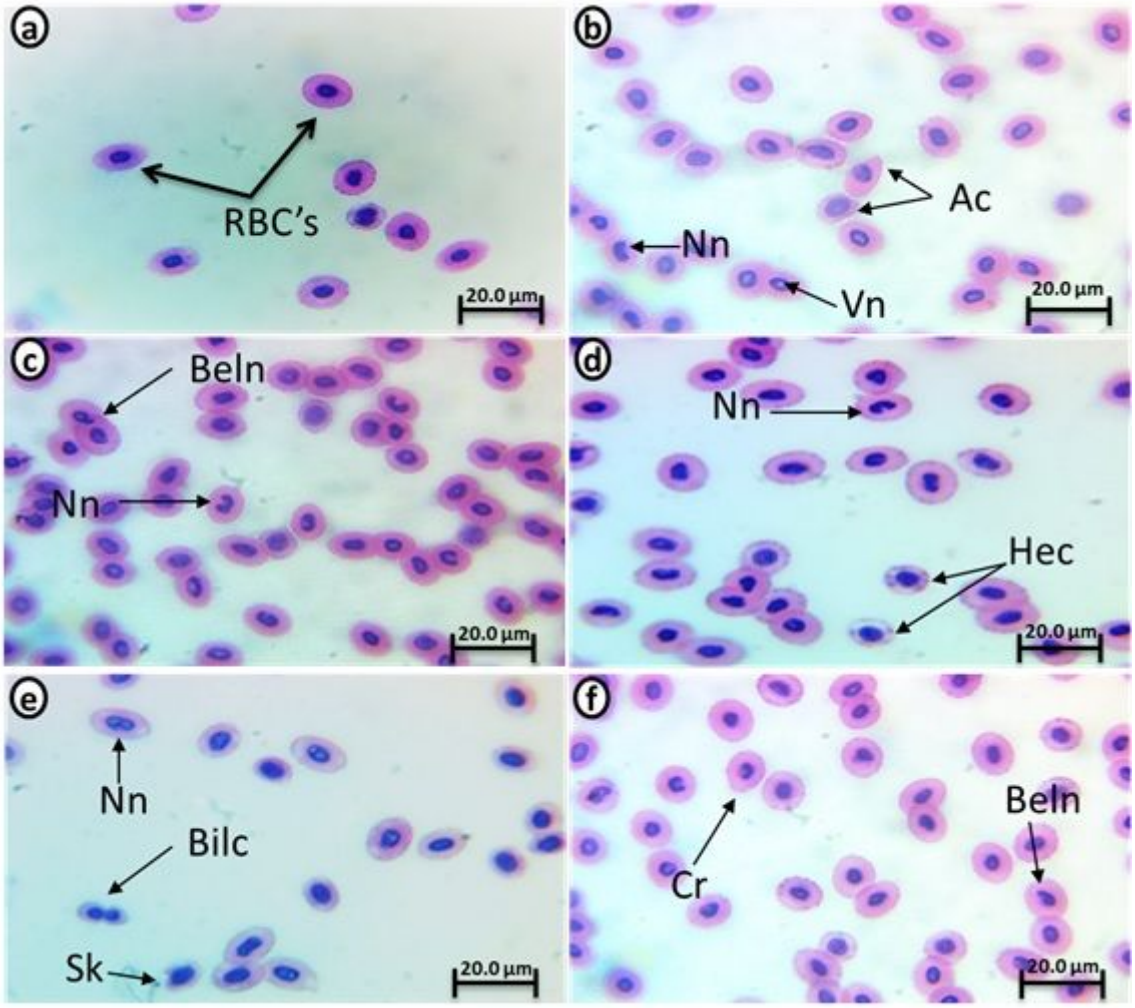

Figure 1

Representative blood smears from Nile tilapia (Oreochromis niloticus). (a) Control (A0MP0); (b, c) (A0MP group) exposed to MPs; (d) A1MP group exposed to MPs + 2.5 % amphora; (e) A2MP group exposed to MPs + 5% amphora; and (f) A3MP exposed to MPs + 7.5% amphora. (RBCs) Red blood cells (Ac) acanthocytes; (Cr) crenated cells; (Beln) belebbed nucleus; (Vn) vacuolated nucleus; (Hec) hemolyzed cells; (Bilc) bilobed cells; (Nn) notched nucleus; and (Sk) sickle cells. H&E stain was used; scale bar 20 µm.

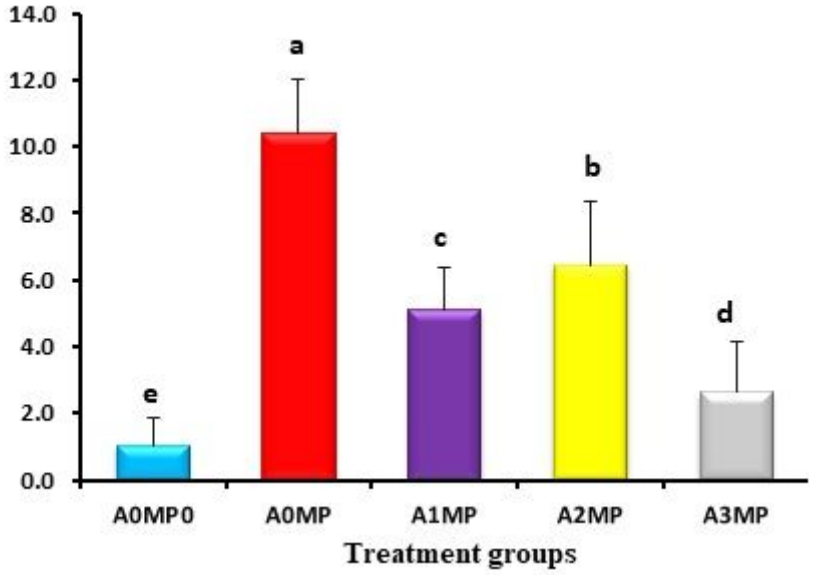

Figure 2

Erythrocyte alterations (%) of O. niloticus fed different levels of A. coffeaeformis-supplemented diets, then exposed to 10 mg/L microplastic. Data are represented as mean ± S D.

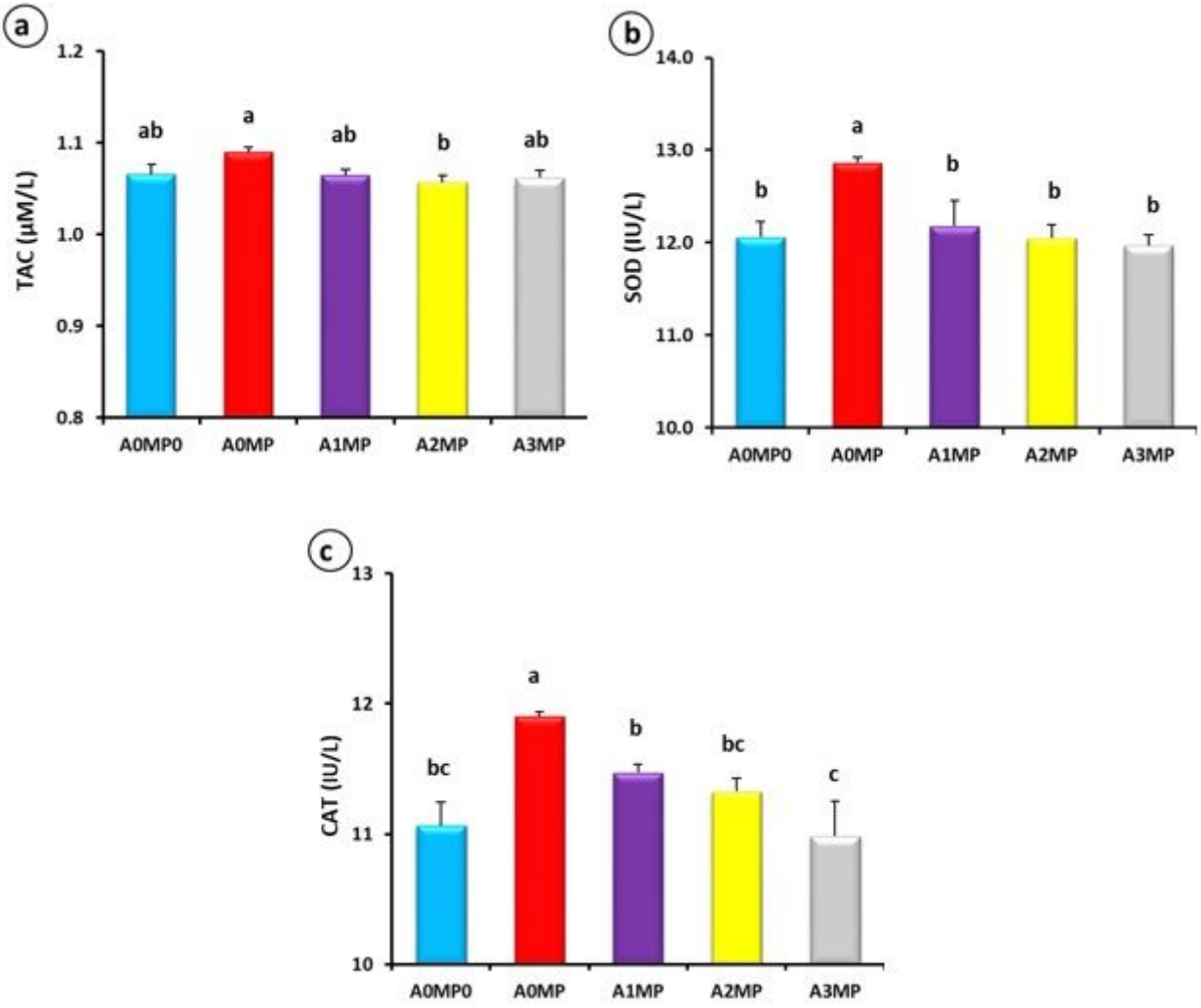

Figure 3

Antioxidant enzyme activity (TAC, SOD and CAT) in Nile tilapia (Oreochromis niloticus) fed with different A. coffeaeformis concentrations, then exposed to 10 mg/L microplastics. Data are presented as mean ± SD. n = 6. Means with different superscript letters are significantly different ($p < 0.05$).

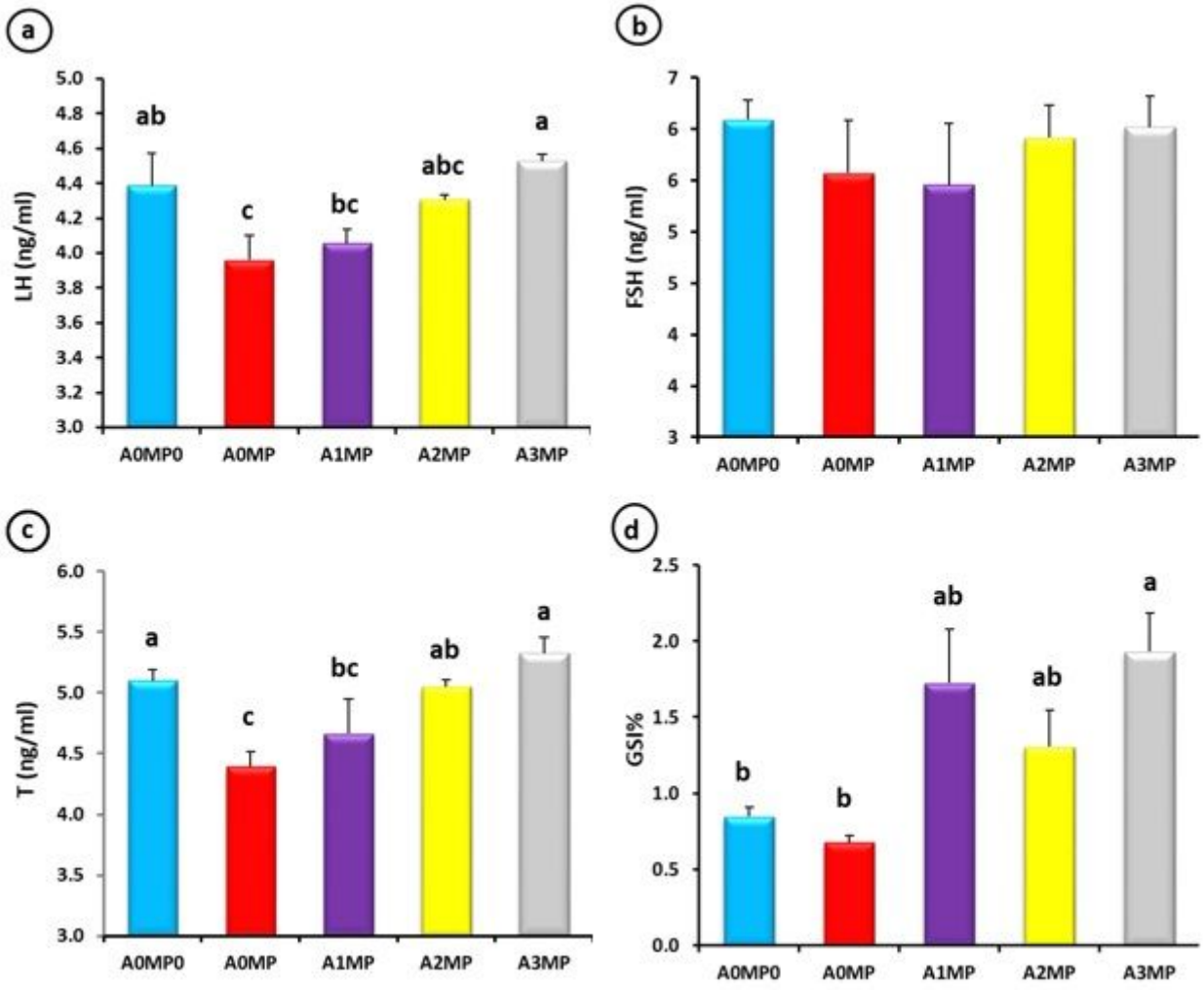

Figure 4

Hormonal activity (LH, FSH and T) and GSI% value in Nile tilapia (Oreochromis niloticus) fed different A. coffeaeformis concentrations, and then exposed to 10 mg/L microplastics. Data are presented as mean ± SD. n = 6. Means with different superscript letters are significantly different ($p < 0.05$).

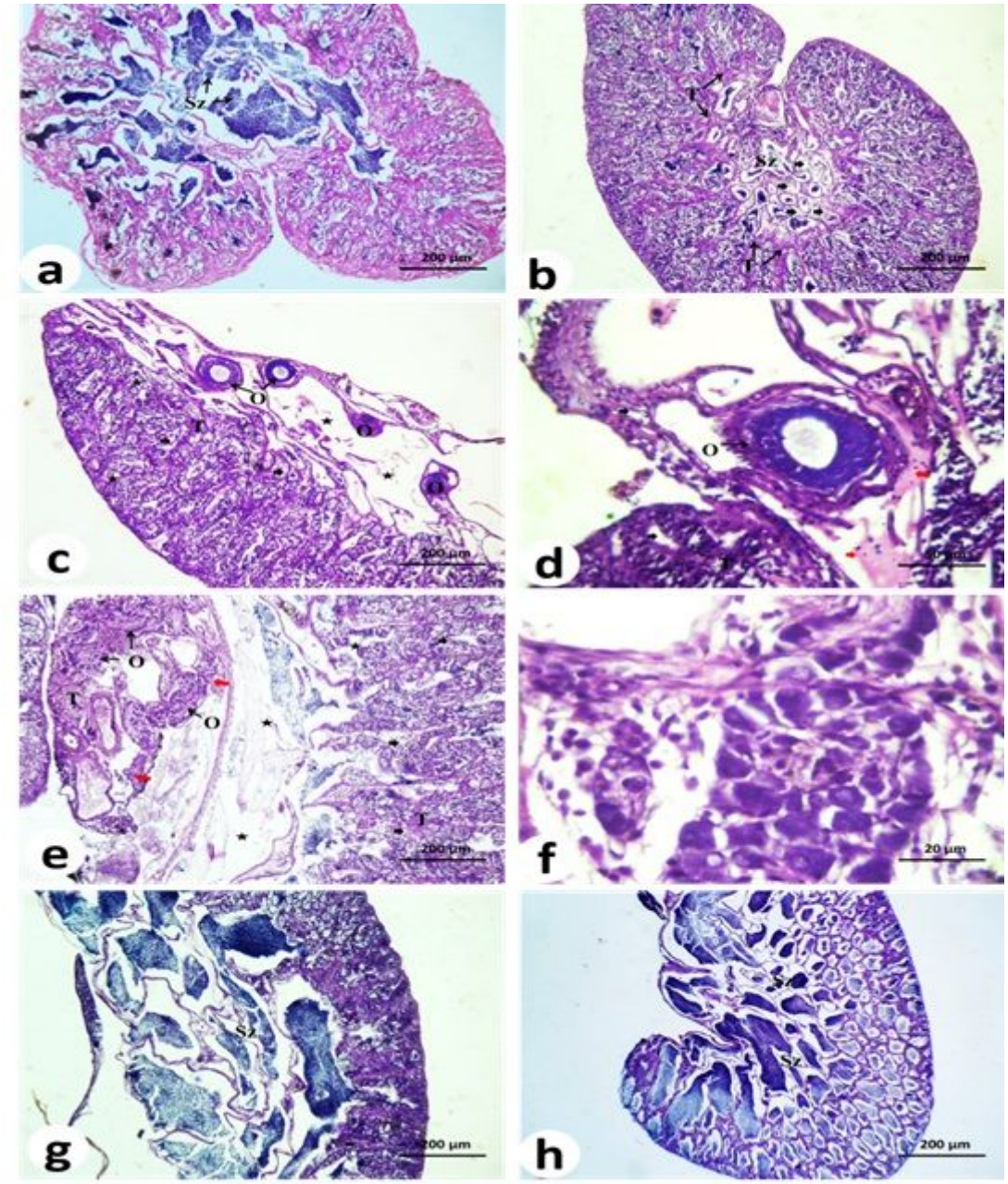

Figure 5

Microscopic pictures of O. niloticus testes after 15 days of microplastic exposure: (a) shows the cystic arrangement in control testis with different spermatogenic cells and spermatozoa (SZ) in the lumen of the cysts. (b-d) show sections of testis from A0MP with different alterations, proliferation of interstitial tissue (T), vacuolization of seminiferous epithelium (arrowheads), and a small number of spermatozoa (SZ) or a lack of spermatozoa in the lumen of the testicular lobules (asterisks). Different oocyte developmental stages were seen inside the testicular lobule (O) and necrosis (red arrow) were also present. (e) Testis sections from A1MP with proliferation of the interstitial tissue (T), vacuolization of the seminiferous epithelium (arrowheads), a lack of spermatozoa from the lumen of the testicular lobules (asterisks), and necrosis (red arrow). A nest of early perinucleus oocytes (O) were also present to form the testes-ova. (f) Magnification of the early perinucleus oocytes. (g) Section from A2MP showed normal

cystic arrangement with a fair number of mature spermatozoa (SZ). (h) Section from A3MP with cysts occupied by mature spermatozoa (SZ). H&E staining was used.

Supplementary Files

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