Diet Supplemented With Purslane, *Portulaca oleracea* Linnaeus, 1753 Resolves Bisphenol A Impact on North African Catfish, *Clarias gariepinus* (Burchell, 1822)

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Abstract

Bisphenol A (BPA) is one of the most widely used synthetic compounds in the world. Since BPA is a suspected xenoestrogen and oxidative stressful agent, its potential hazardous impacts were evaluated on the health and the reproductive status of the male North African catfish, *Clarias gariepinus* (Burchell, 1822). Ninety mature male North African catfish were divided into six groups (in three replicates); Group 1 & Group 4 fed basal diet (control), Group 2 & Group 5 diets supplemented with 3 % purslane, *Portulaca oleracea* Linnaeus, 1753 powder, and Group 3 & Group 6 diets supplemented with 5 % purslane powder. Group 4, Group 5, and Group 6 were exposed to 50 $\mu g.L^{-1}$ BPA for 14 days. Group 4 showed a significant increase in the luteinising hormone, 17β estradiol, and malondialdehyde levels, with a decrease in the testosterone, superoxide dismutase, and catalase concentrations. Simultaneously, there were elevated liver and kidney markers with severe degenerative changes in the testes, liver, and kidney. Dietary supplementation of purslane powder returned the measured parameters to their normal values with the correction of the tissue architectures and details. Interestingly, purslane in the non-BPA treated groups raised the testosterone and 17β estradiol over the control values. It is suggested that purslane could be used in aquaculture and cultivated near polluted water areas.

Keywords: reproductive hormones disruption; antioxidants; GPER; oxidative stress; quercetin

Introduction

The North African catfish, *Clarias gariepinus* (Burchell, 1822), is freshwater species. Under favourable conditions, their gonads show a continuous cycle with mature spermatozoa throughout the year, their pituitaries store large amounts of gonadotropin (Van Oordt and Goos, 1987), and their luteinising hormone (LH) is able to activate both the catfish luteinising-hormone receptor and the follicle-stimulating hormone receptor while follicle-stimulating hormone (FSH) is absent (Vischer et al., 2003). So, the North African catfish is an interesting model to study the xenoestrogenic effect of bisphenol A (BPA) on reproductive hormone functions.

Pollution of aquatic ecosystems with a wide range of pollutants is one of the most important environmental problems that can threaten the life of aquatic organisms (Navabian et al., 2020; Budovich, 2021; Wirnkor et al., 2021; Al-Tameemi et al., 2022). BPA is an essential component in the epoxy resins and polyvinyl chloride, polyesters, and polyacrylates, which have wide industrial uses, including the manufacture of plastic wares and food, can linings (Mandich et al., 2007). In a meta-analysis study, Wu and Seebacher (2020) stated that the BPA levels were higher in freshwater systems than in marine and estuarine systems. Moreover, a study by Zahran et al. (2020) in Egypt found that the BPA concentrations were 6.5 $mg.L^{-1}$ in water samples and 25.9 and 48.07 $\mu g.kg^{-1}$ in the liver and muscle, respectively, of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758).

BPA exposure has an overall negative effect on aquatic organisms, interrupting a broad range of biological functions. The biological effects of BPA are most likely mediated by its endocrine-disrupting action, particu-

larly of estrogen receptors, with a significant decrease in reproductive output that alters population dynamics and persistence so that BPA has the potential to have serious consequences for biodiversity (Wu and Seebacher, 2020). In male fish, Wang et al. (2019) reviewed that BPA exposure can harm the development of male gonads, including decreased serum androgen levels, decreased sperm counts and motility in the epididymis, apoptosis of sperm cells, and sperm DNA damage. Furthermore, Faheem and Lone (2017) found raised catalase (CAT) and glutathione-S-transferase activity with increased lipid peroxidation concentration while reducing glutathione content damaging the vital organs in freshwater cyprinid, *Ctenopharyngodon idella* (Valenciennes, 1844).

Purslane plant herbal supplements have been efficiently used in fish (Ghafarifarsani et al., 2021a, b, c; Raissy et al., 2022) and other vertebrates to improve growth, immunity and reproduction and also resistance to pollutants (Fattepur et al., 2018; Ridzuan et al., 2019; Fattepur et al., 2020; Halim et al., 2020; Roslane and Shariff, 2021). In addition, herbs-supplemented diet could mitigate the negative effects of pollutants exposure in common carp, *Cyprinus carpio* (Linnaeus, 1758) (Taheri Mirghaed et al., 2019; Rajabiesterabadi et al., 2020a, b; Hoseini et al., 2018) and in Nile tilapia (Naiel et al., 2019; Abdelhamid et al., 2020). Purslane, *Portulaca oleracea* Linnaeus, 1753 belongs to the Portulacaceae family. Many countries used purslane as a folk medicine to cure burns, headaches, and illnesses associated with the intestine, liver, stomach, and arthritis. Purslane possesses a wide spectrum of pharmacological properties such as antioxidant, anti-inflammatory, hepato-protective, and anticancer activities (Farag and Shakour, 2019). Interestingly, Imai et al. (2007) demonstrated that purslane has the ability to remove BPA efficiently from water within 24 h when the ratio of the whole plant was at 40 $g.L^{-1}$ water.

The present study was conducted to a) assess the effects of BPA on reproductive hormones, antioxidants, and liver and kidney biomarkers, as well as on testes, liver, and kidney of male catfish b) assess the protective effect of dietary supplementation of purslane powder.

Materials and Methods

Ethical approval

The animal study was approved by the committee of Animal Welfare and Research Ethics, Faculty of Veterinary Medicine, Zagazig University (ZU-IACUC /2/F/213/2021).

Chemicals

BPA (CAS 80-50-7, 97 % pure) was purchased from Aldrich Company. Diagnostic kits for assaying serum E2 (17β estradiol) and LH were from Roche Company USA. Serum antioxidants enzymes and lipid peroxides were from BioMED, Egypt and serum liver and kidney biomarkers were from Spinreact, Spain.

Plant

Wild mature purslane plant was purchased from Harraz market, Sharkia governorate, Egypt. It was identified and authenticated in Botany Department, Faculty of Science, Zagazig University.

Fish

Ninety apparently healthy mature male North African catfish with a length of 30 ± 5 cm were obtained from a semi-intensive aquaculture facility of the fish research station (World Fish Center, Abbassa, Egypt). The fish were acclimated for 2 weeks in glass aquaria measuring 100 × 40 × 40 cm filled with de-chlorinated tap water (100 effective litres each). Continuous aeration was maintained in each aquarium using an electric air pump and heaters at 26 °C with natural photoperiod to maintain the temperature. The water parameters, including dissolved oxygen, pH, ammonia, nitrate, and nitrite concentrations, were measured according to APHA (1998) and maintained at acceptable limits, 6.5 ± 0.5, 7.1 ± 0.8, 0.003, 0.31, and 0.42 $mg.L^{-1}$, respectively .

Fish were fed a basal diet (30 % protein) daily at 3 % of their body weight divided into 3 times throughout the adaptation period.

Diet

Three diets were prepared: diet 1 (basal diet 30 % protein), diet 2 (basal diet supplemented with 3 % purslane), and diet 3 (basal diet supplemented with 5 % purslane). The diets (Table 1) were prepared in the Fish Research Center, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Experimental design

Fish were divided into six groups with three replicates with five fish in each aquarium. Group 1, Group 2, and Group 3 were reared in de-chlorinated tap water and were fed diet 1, diet 2, and diet 3, respectively with a daily exchange of 30 % water with de-chlorinated tap water. Group 4, Group 5, and Group 6 were reared in de-chlorinated tap water with 50 $\mu g.L^{-1}$ of BPA and fed diet 1, diet 2, and diet 3, respectively and the daily exchanged water had the same concentration of BPA for 14 days. The BPA dose and duration of 14 days were selected according to Mandich et al. (2007) and purslane doses used after Abdel-Razek et al. (2019).

Collection of serum

Fish were fasted overnight and anaesthetised for sample collection after 7, 14 days from the beginning of the experiment. Five blood samples were collected

Table 1. Ingredients used to prepare the experimental diets incorporated purslane, *Portulaca oleracea* at 0 % (Diet 1), 3 % (Diet 2) and 5 % (Diet 3) and the proximate analysis of the diets.

Ingredients	Diet 1	Diet 2	Diet 3
Ground corn	20.3	20.3	20.3
Soybean meal (44 % CP)	43.0	43.0	43.0
Fish meal (65 % CP)	10.4	10.4	10.4
Wheat bran	15.5	12.5	10.5
Starch	4.0	4.0	4.0
Corn oil	1.5	1.5	1.5
Vitamins premix[1]	1.0	1.0	1.0
Minerals premix[2]	2.0	2.0	2.0
Cod fish oil	2.3	2.3	2.3
Purslane powder	0.0	3.0	5.0
Total	100	100	100
Proximate analysis (%)			
Dry matter	91.8	91.1	91.0
Crude protein	30.0	30.0	30.0
Total ash	6.3	7.0	7.6
Crude fibre	5.8	6.1	6.3
Gross energy (KJ %)	19.6	19.5	19.4

[1]Vitamin premix (per kg of premix): thiamine: 2.5 g; riboflavin: 2.5 g; pyridoxine: 2.0 g; inositol: 100.0 g; biotin: 0.3 g; pantothenic acid: 100.0 g; folic acid: 0.75 g; para-aminobenzoic acid: 2.5 g; choline: 200.0 g; nicotinic acid: 10.0 g; cyanocobalamine: 0.005 g; α-tocopherol acetate: 20.1 g; menadione: 2.0 g; retinol palmitate: 100,000 IU; cholecalciferol: 500,000 IU.
[2]Mineral premix (per kg of premix): $CaHPO_4.2H_2O$: 727.2 g; $MgCO_3.7H_2O$: 127.5 g; KCl: 50.0 g; NaCl: 60.0 g; $FeC_6H_5O_7.3H_2O$: 25.0 g; $ZnCO_3$: 5.5 g; $MnCl_2.4H_2O$: 2.5 g; $CuCl_2$: 0.785 g; $CoCl_3.6H_2O$: 0.477 g; $CaIO_3.6H_2O$: 0.295 g; $CrCl_3.6H_2O$: 0.128 g; $AlCl_3.6H_2O$: 0.54 g; Na_2SeO_3: 0.3 g.
Gross energy was calculated according to National Research Council (1993).

from each group from the caudal vein into centrifuge tubes without anticoagulant and then centrifuged at 3000 rpm for 15 min to obtain serum for the biochemical and hormonal analysis.

Tissue samples

The fish of all groups were dissected, and liver, kidney, and testes were fixed in 10 % formalin for histopathological examination.

Biochemical assay

Estimation of the liver and kidney functions: The serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, and creatinine were determined using 7150 Automatic Analyzer (Hitachi, Japan).

Reproductive hormonal assays

Levels of LH, E2, and testosterone (T) hormones were determined according to Johnson et al. (1993), Tietz (1995), and Carlstrom et al. (1988), respectively, using electrochemiluminescence immune assay (ECLIA) kits by Roche Elecsys 2010.

Serum antioxidant enzymes and lipid peroxidation assays

The superoxide dismutase (SOD) activity was estimated as described by Spitz and Oberley (1989). The CAT activity was determined according to the method of Aebi (1984). The lipid peroxides concentration was determined as malondialdehyde (MDA) according to Jentzsch et al. (1996).

Histopathological examination

The fixed liver, kidney, and testes specimens were dehydrated, cleared and embedded in paraffin wax, sectioned at 4–5 µm thickness and stained with haematoxylin and eosin stain (H&E), then examined microscopically.

Statistical analysis

Levene's test was used to test the homogeneity of the variances. The data with non-equal variances were transformed using Box-Cox transformation. The data were subjected to two-way ANOVA test (SPSS software version 23) with the purslane and BPA as variables. This was followed by post hoc Duncan's test to make multiple comparisons between averages, where the averages followed by the same letter are not significantly different from each other at 0.05 probability level.

Results

Table 2 shows the effects of BPA and purslane on reproductive hormones. The interaction effect of dietary purslane, especially at 3 % and water containing BPA on LH, E2, and T levels were significant at $P \leq 0.05$. BPA in the absence of dietary purslane (Group 4) elevated LH and E2 over the control values (Group 1), and lowered T, while the dietary purslane in the BPA-free water (Group 2 and Group 3) significantly

Table 2. Effect on reproductive hormones in North African catfish, *Clarias gariepinus* exposed to bisphenol A (BPA) and purslane, *Portulaca oleracea*, for 2 weeks.

		Luteinizing hormone (mIU.mL^{-1})		17β estradiol (pg.mL^{-1})		Testosterone (ng.mL^{-1})	
		Week 1	Week 2	Week 1	Week 2	Week 1	Week 2
BPA effect							
0		1.15 ± 0.02^{b}	1.19 ± 0.01^{b}	51.09 ± 2.35^{b}	49.83 ± 2.00^{b}	1.65 ± 0.05^{a}	1.67 ± 0.05^{a}
50		1.56 ± 0.15^{a}	1.70 ± 0.16^{a}	73.04 ± 4.23^{a}	75.07 ± 2.70^{a}	0.99 ± 0.13^{b}	1.06 ± 0.14^{b}
Purslane effect							
0		1.73 ± 0.22^{a}	1.88 ± 0.22^{a}	66.93 ± 9.31^{a}	64.20 ± 8.04^{a}	0.91 ± 0.20^{c}	0.99 ± 0.21^{c}
3 %		1.14 ± 0.02^{c}	1.20 ± 0.01^{c}	58.81 ± 0.55^{b}	61.20 ± 1.67^{a}	1.60 ± 0.09^{a}	1.65 ± 0.08^{a}
5 %		1.19 ± 0.01^{b}	1.26 ± 0.03^{b}	60.45 ± 1.92^{b}	61.90 ± 3.37^{a}	1.45 ± 0.07^{b}	1.45 ± 0.07^{b}
Interaction effect BPA + Purslane							
0	0 %	1.08 ± 0.04^{c}	1.23 ± 0.01^{c}	39.26 ± 1.68^{d}	40.40 ± 1.62^{e}	1.50 ± 0.07^{bc}	1.60 ± 0.12^{ab}
0	3 %	1.18 ± 0.01^{b}	1.18 ± 0.01^{d}	58.00 ± 0.71^{c}	56.80 ± 0.45^{d}	1.80 ± 0.08^{a}	1.80 ± 0.07^{a}
0	5 %	1.18 ± 0.01^{b}	1.18 ± 0.00^{d}	56.00 ± 1.22^{c}	52.28 ± 1.80^{d}	1.64 ± 0.06^{ab}	1.60 ± 0.07^{ab}
50	0 %	2.37 ± 0.03^{a}	2.54 ± 0.01^{a}	94.60 ± 2.07^{a}	88.00 ± 2.21^{a}	0.31 ± 0.03^{e}	0.37 ± 0.02^{d}
50	3 %	1.10 ± 0.03^{c}	1.23 ± 0.01^{c}	59.62 ± 0.72^{c}	65.60 ± 1.65^{c}	1.40 ± 0.08^{cd}	1.50 ± 0.10^{bc}
50	5 %	1.20 ± 0.02^{b}	1.35 ± 0.02^{b}	64.90 ± 2.28^{b}	71.60 ± 1.15^{b}	1.26 ± 0.05^{d}	1.30 ± 0.05^{c}
Homogeneity test (*P* value)		0.10	0.16	0.12	0.28	0.89	0.21

Data are mean ± SE. Different letters in the same column are significantly different ($P \leq 0.05$).

elevated all tested hormones. The coexistence of BPA and dietary purslane (Group 5 and Group 6) significantly returned LH and, to some extent T to the control values, while the E2 became higher than control but lower than group 4.

Table 3 shows the oxidant and antioxidant biomarkers. Dietary purslane and water contained BPA had a significant ($P \leq 0.05$) interaction effect on SOD, CAT, and MDA levels. Dietary purslane in the absence of BPA (Group 2 and Group 3) elevated SOD and CAT over the control values (Group 1), and lowered MDA, while the BPA in the nonexistence of purslane (Group 4) reversed the situation. In the end of the 1st week, the simultaneous presence of BPA and dietary purslane (Group 5 and Group 6) significantly raised MDA and decreased SOD and CAT, but by the end of the 2nd week, dietary purslane at 3 % (Group 5) returned SOD, MDA, and to some extent CAT to the control values.

Data presented in Table 4 explored an alteration in liver and kidney functions. Dietary purslane and water containing BPA had a significant ($P \leq 0.05$) interaction effect on ALT, AST, urea, and creatinine levels. BPA in

Table 3. Values of some antioxidant enzymes and malondialdehyde in North African catfish, *Clarias gariepinus*, exposed to bisphenol A (BPA) and purslane, *Portulaca oleracea*, for 2 weeks.

		Superoxide dismutase (I.U.mL^{-1})		Catalase (I.U.mL^{-1})		Malondialdehyde (nmol.mL^{-1})	
		Week 1	Week 2	Week 1	Week 2	Week 1	Week 2
BPA effect							
0		24.64 ± 0.62^{a}	23.73 ± 0.52^{a}	15.99 ± 0.70^{a}	15.65 ± 0.34^{a}	1.59 ± 0.02^{b}	1.53 ± 0.06^{b}
50		17.75 ± 0.53^{b}	19.75 ± 0.65^{b}	9.81 ± 0.45^{b}	12.07 ± 0.46^{b}	2.31 ± 0.23^{a}	2.74 ± 0.39^{a}
Purslane effect							
0 %		19.12 ± 1.05^{c}	19.85 ± 0.89^{c}	10.20 ± 0.76^{c}	12.60 ± 0.76^{c}	2.54 ± 0.32^{a}	3.25 ± 0.52^{a}
3 %		23.34 ± 1.24^{a}	23.64 ± 0.77^{a}	14.75 ± 1.19^{a}	15.285 ± 0.68^{a}	1.57 ± 0.03^{c}	1.60 ± 0.04^{b}
5 %		21.12 ± 1.37^{b}	21.73 ± 0.87^{b}	13.75 ± 1.23^{b}	13.70 ± 0.62^{b}	1.75 ± 0.02^{b}	1.57 ± 0.10^{b}
Interaction effect BPA + Purslane							
0	0 %	22.02 ± 0.40^{c}	21.80 ± 0.50^{bc}	12.41 ± 0.31^{b}	14.70 ± 0.49^{bc}	1.58 ± 0.01^{e}	1.70 ± 0.05^{bc}
0	3 %	26.90 ± 0.36^{a}	25.60 ± 0.52^{a}	18.17 ± 0.24^{a}	17.05 ± 0.28^{a}	1.50 ± 0.02^{f}	1.60 ± 0.05^{c}
0	5 %	24.99 ± 0.84^{b}	23.80 ± 0.73^{ab}	17.40 ± 0.37^{a}	15.20 ± 0.33^{b}	1.70 ± 0.01^{c}	1.30 ± 0.08^{d}
50	0 %	16.23 ± 0.76^{e}	17.89 ± 1.18^{d}	8.00 ± 0.32^{d}	10.50 ± 0.42^{e}	3.50 ± 0.01^{a}	4.80 ± 0.08^{a}
50	3 %	19.78 ± 0.68^{d}	21.69 ± 0.69^{bc}	11.34 ± 0.71^{b}	13.52 ± 0.66^{cd}	1.64 ± 0.02^{d}	1.60 ± 0.07^{c}
50	5 %	17.24 ± 0.51^{e}	19.66 ± 0.85^{cd}	10.10 ± 0.24^{c}	12.20 ± 0.70^{d}	1.79 ± 0.01^{b}	1.83 ± 0.04^{b}
Homogeneity test (*P* value)		0.58	0.75	0.40	0.60	0.73	0.88

Data are mean ± SE. Different letters in the same column are significantly different ($P \leq 0.05$).

Table 4. Mean values of some liver enzymes, urea and creatinine in North African catfish, *Clarias gariepinus*, exposed to bisphenol A (BPA) and purslane, *Portulaca oleracea*, for 2 weeks.

	ALT (U.L^{-1})		AST (U.L^{-1})		Urea (mg.L^{-1})		Creatinine (mg.L^{-1})	
	Week1	Week2	Week1	Week2	Week1	Week2	Week1	Week2
BPA effect								
0	20.05 ± 0.90^{b}	19.81 ± 0.91^{b}	122.46 ± 0.64^{b}	125.10 ± 0.73^{b}	8.45 ± 0.10^{b}	7.66 ± 0.12^{b}	0.28 ± 0.01^{b}	0.27 ± 0.01^{b}
50	35.97 ± 1.83^{a}	49.43 ± 5.72^{a}	152.79 ± 4.30^{a}	166.23 ± 10.55^{a}	13.34 ± 1.13^{a}	10.83 ± 0.80^{a}	0.87 ± 0.14^{a}	0.80 ± 0.18^{a}
Purslane effect								
0 %	33.40 ± 3.00^{a}	51.72 ± 9.03^{a}	144.19 ± 8.13^{a}	170.65 ± 16.09^{a}	13.56 ± 1.81^{a}	11.14 ± 1.19^{a}	0.93 ± 0.22^{a}	1.00 ± 0.25^{a}
3 %	22.37 ± 1.56^{c}	22.85 ± 1.88^{c}	126.35 ± 1.45^{c}	124.45 ± 0.55^{c}	8.575 ± 0.11^{c}	7.33 ± 0.15^{c}	0.32 ± 0.02c	0.27 ± 0.01^{c}
5 %	28.27 ± 3.56^{b}	29.30 ± 3.90^{b}	142.33 ± 5.59^{b}	141.90 ± 4.65^{b}	10.56 ± 0.52^{b}	9.27 ± 0.38^{b}	0.47 ± 0.06^{b}	0.34 ± 0.02^{b}
Interaction effect BPA + Purslane								
0 0 %	24.62 ± 0.74^{d}	24.62 ± 0.04^{d}	119.80 ± 0.04^{f}	122.40 ± 0.64^{e}	8.12 ± 0.00^{d}	7.61 ± 0.05^{cd}	0.27 ± 0.01^{d}	0.26 ± 0.02^{c}
0 3 %	17.90 ± 0.35^{e}	17.22 ± 0.01^{f}	122.00 ± 0.20^{e}	124.90 ± 0.87^{d}	8.24 ± 0.01d	7.20 ± 0.16^{d}	0.27 ± 0.01^{d}	0.26 ± 0.01^{c}
0 5 %	17.62 ± 0.19^{e}	17.60 ± 0.00^{e}	125.57 ± 0.07^{d}	128.00 ± 0.71^{c}	9.00 ± 0.02^{c}	8.17 ± 0.12^{c}	0.30 ± 0.01^{d}	0.29 ± 0.01^{c}
50 0 %	42.17 ± 1.21^{a}	78.82 ± 0.04^{a}	168.58 ± 0.31^{a}	218.90 ± 0.64^{a}	19.00 ± 0.06^{a}	14.66 ± 0.35^{a}	1.59 ± 0.02^{a}	1.74 ± 0.02^{a}
50 3 %	26.83 ± 0.96^{c}	28.48 ± 0.07^{c}	130.70 ± 0.07^{c}	124.00 ± 0.71^{de}	8.91 ± 0.01^{c}	7.46 ± 0.27^{d}	0.37 ± 0.01^{c}	0.28 ± 0.01^{c}
50 5 %	38.92 ± 0.52^{b}	41.00 ± 0.06^{b}	159.10 ± 0.31^{b}	155.80 ± 0.49^{b}	12.12 ± 0.03^{b}	10.36 ± 0.18^{b}	0.64 ± 0.01^{b}	0.38 ± 0.01^{b}
Homogeneity test (*P* value)	0.08	0.12	0.07	0.77	0.07	0.26	0.44	0.43

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.
Data are mean ± SE. Different letters in the same column are significantly different ($P \leq 0.05$).

the purslane-free diet (Group 4) significantly raised all these parameters. Reversely, dietary purslane in the BPA-free water (Group 2 and Group 3) elevated AST over the control values (Group 1), and lowered ALT, but urea and creatinine showed non-significant alterations. At the end of the 1st week, the parallel presence of BPA and dietary purslane (Group 5 and Group 6) could not retain the control parameters, but by the end of the 2nd week, dietary purslane at 3 % (Group 5) returned urea and creatinine, and to some extent AST to the control values, while ALT showed significant elevations but lower than Group 4.

The histopathological findings confirmed the preceding data, where the testes of catfish exposed to BPA (Group 4) for 7 days exhibited focal tubular cystic changes with degeneration of cells lining the seminiferous tubules, spermatogonia, and spermatocytes in addition to hyposermatogenesis (Fig. 1A). The liver revealed marked hydropic degeneration and some hepatocytes revealed early microsteatosis (Fig.1B), while the kidney suffered interstitial lymphocytic infiltration associated with tubular pressure atrophy and degenerative changes of its epithelial lining (Fig. 1C).

The lesions become more progressive after extending the period to 14 days, where the testes showed moderate cystic changes in a large number of the seminiferous tubules, hyposeprmatogenesis, and separation of the germinal epithelia from the basement membrane. In addition, degenerative and necrotic changes in spermatogonia and spermatocytes with thickening of the interstitial septa and empty seminiferous tubules were predominant (Fig. 2A). Some seminiferous tubules suffered destruction in the walls and separation of the germinal epithelia from the basement membrane (Fig. 2B). The liver experienced thrombosed portal blood vessel and hydropic degeneration of hepatocytes (Fig. 2C), whereas the kidney suffered a massive interstitial lymphocytic infiltration and many glomeruli showed atrophic changes with degenerative and early necrotic changes in most of the tubular epithelium besides, focal mildly activated melano-macrophage centres (Fig. 2D). Intriguing, the addition of 3 % purslane with BPA for 7 days could improve the histopathological changes of the vital organs, where testes showed focal cystic dilatation of seminiferous tubules with degenerative changes in spermatocytes and mild sloughing of the germinal layer (Fig. 2E).

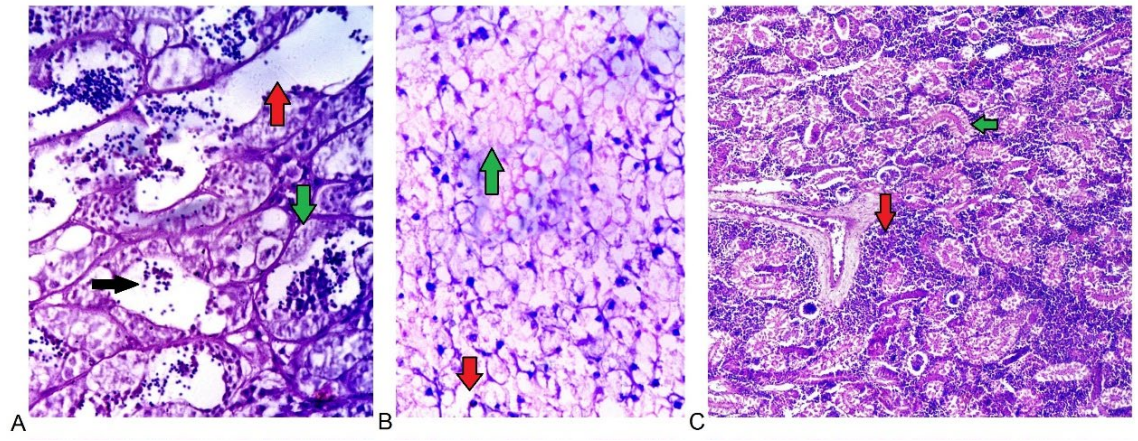

Fig. 1. Male North African catfish *Clarias gariepinus*, exposed to 50 ug.L^{-1} of bisphenol A (BPA) for 7 days. A) Testes showing focal tubular cystic change (red arrow), degeneration of cells lining seminiferous tubules spermatogonia and spermatocytes (green arrow), hypospermatogenesis (black arrow) (H&E ×200); B) Liver showing hepatocytes with early microsteatosis (red arrow), marked hydropic degeneration (green arrow) (H&E ×200); C) Kidney showing interstitial lymphocytic infiltration (red arrow), degenerative change of epithelial lining (green arrow) (H&E ×200).

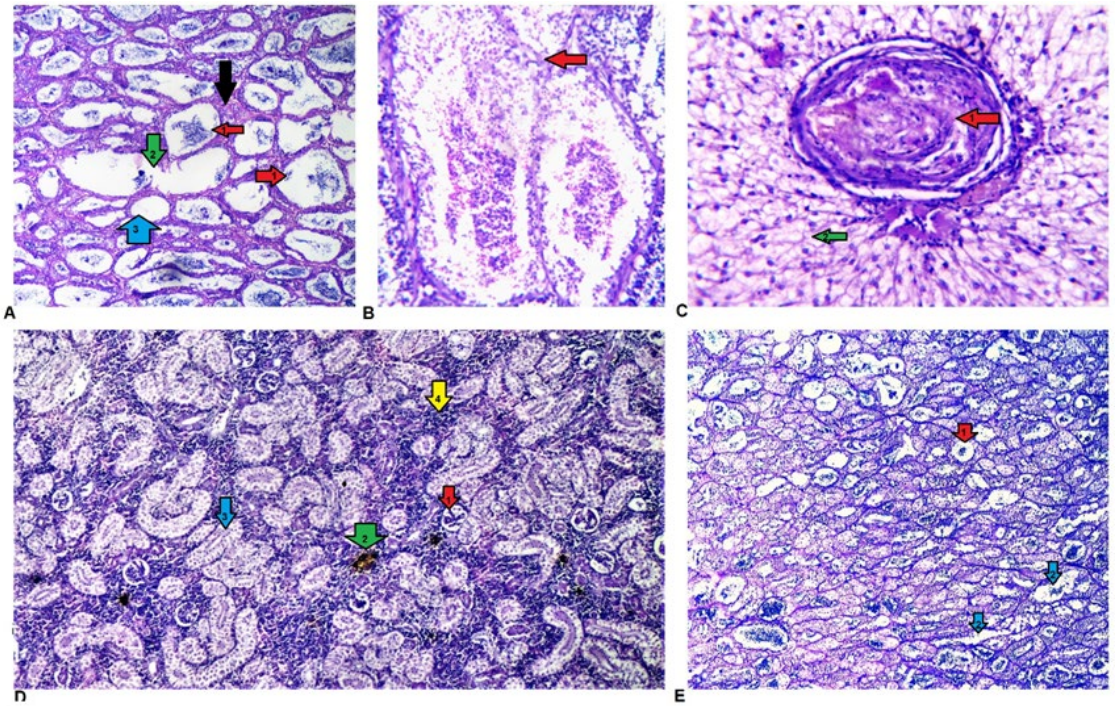

Fig. 2. Male North African catfish *Clarias gariepinus*, exposed to 50 ug.L^{-1} of bisphenol A (BPA) for 14 days. A) Testes showing separation of germinal epithelia from the basement membrane beside degenerative and necrotic changes in spermatogonia and spermatocytes (red arrow), moderate cystic changes in large number of seminiferous tubules with hyposeprmatogenesis, (green arrow), thickening of the interstitial septa (black arrow), empty seminiferous tubules (blue arrow) (H&E ×200); B) Testes showing destruction in walls of the seminiferous tubules with separation of germinal epithelia from the basement membrane (arrow) (H&E ×400); C) Liver showing thrombosed portal blood vessel (red arrow), hydropic degeneration of hepatocytes (green arrow) (H&E ×400); D) Kidney showing atrophic changes of glomeruli (red arrow), focal mildly activated melano-macrophage centres (green arrow), degenerative and early necrotic changes in tubular epithelium (blue arrow), massive interstitial lymphocytic infiltration (yellow arrow) (H&E ×100); E) Testes after 7 days with 3 % purslane showing mild sloughing of germinal layer (red arrow), focal cystic dilatation of seminiferous tubules with degenerative changes in spermatocytes (blue arrow) (H&E ×100).

The liver had apparently normal hepatic parenchyma with mild hydropic degeneration of some hepatocytes (Fig. 3A), while the kidney showed a focal hydropic degeneration of the epithelial cells lining the renal tubules with a moderate interstitial lymphocytic infiltration (Fig. 3B).

After 14 days of bisphenol exposure with 3 % purslane (Group 5), catfish organs showed pronounced improvement, where the seminiferous tubules of the testes appeared normal and filled with spermatid while few others revealed mild degenerative changes with sloughing of the germinal layer (Fig. 3C). The liver and

kidney showed the same lesion as in the BPA treated group with 3 % purslane after 7 days (Figs. 3A, B).

There was insignificantimprovement in the pathology of catfish exposed to BPA with 5 % purslane for 7 or 14 days of the experiment than the previous group (BPA and 3 % purslane. The alteration in testes had moderate degenerative and early necrotic changes in the spermatocytes, focal cystic changes, and moderate hyposermatogenesis (Fig. 4A). The liver showed mild portal vein dilatation and moderate hydropic degeneration (Fig. 4B). The kidney suffered a thickening of the renal arteriolar wall due to hyalinisation, moderate hydropic degeneration, and early necrotic changes in some tubular epithelium of the renal tubules with few interstitial lymphocytic infiltrations and some glomeruli suffered atrophic changes (Fig. 4C).

The histopathological findings of the catfish in groups treated with 3 % or 5 % purslane for 1 and 2 weeks showed complete normal architectures and tissue details, while the testicular tissue appeared with a regular arrangement of the seminiferous tubules on the intact basement membrane and the lumens were filled with spermatid and spermatozoa with a well-developed interstitium (Fig. 5A). The liver showed apparently normal hepatic parenchyma and vascular structures with a mild focal hydropic degeneration (Fig. 5B), and the kidney revealed normal glomeruli with a mild hydropic degeneration of the epithelial lining the renal tubules (Fig. 5C).

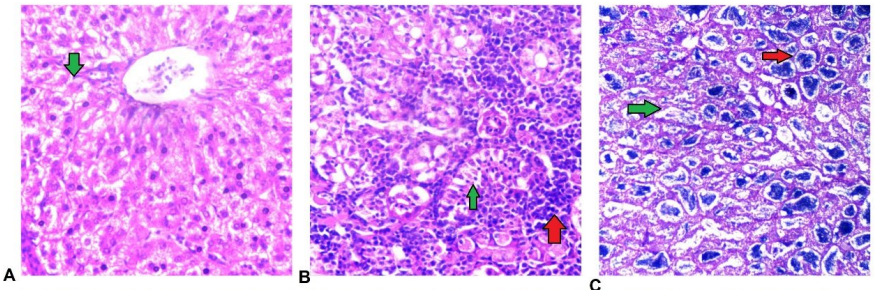

Fig. 3. Male North African catfish *Clarias gariepinus*, exposed to 50 ug.L^{-1} of bisphenol A (BPA) and 3 % purslane, *Portulaca oleracea*, for 14 days. A) Liver showing apparently normal hepatic parenchyma with mild hydropic degeneration (arrow) (H&E ×400); B) Kidney showing moderate interstitial lymphocytic infiltration (red arrow), focal hydropic degeneration of epithelial cells lining of the renal tubules (green arrow) (H&E ×200); C) Testes showing normal seminiferous tubules filled with spermatid (red arrow), mild degenerative changes with sloughing of the germinal layer (green arrow) (H&E ×100).

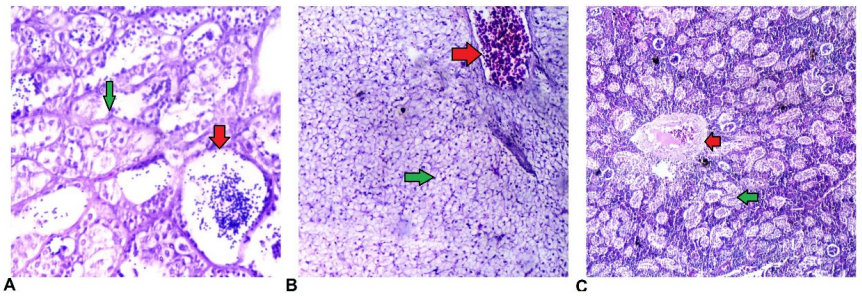

Fig. 4. Male North African catfish *Clarias gariepinus*, exposed to 50 ug.L^{-1} of bisphenol A (BPA) and 5 % purslane, *Portulaca oleracea*, for 14 days. A) Testes showing focal cystic changes and moderate hyposermatogenesis (red arrow), moderate degenerative and early necrotic changes in the spermatocytes (green arrow) (H&E ×200); B) Liver showing mild portal vein dilatation (red arrow), moderate hydropic degeneration (green arrow) (H&E ×100); C) Kidney showing renal arteriolar wall thickening (red arrow), moderate hydropic degeneration with early necrotic changes in some tubular epithelium of the renal tubules (green arrow) (H&E ×100).

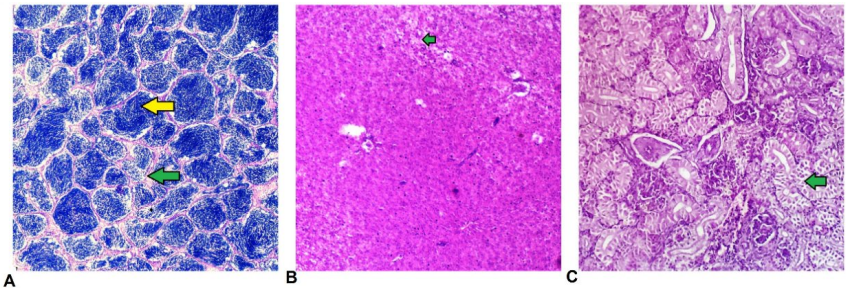

Fig. 5. Male North African catfish *Clarias gariepinus*, exposed to 3 % purslane, *Portulaca oleracea*, for 14 days. A) Testes showing well developed interstitium (green arrow), regular arrangement of seminiferous tubules on the intact basement membrane with lumen filled with spermatid and spermatozoa (yellow arrow). B) Liver showing normal hepatic parenchyma and vascular structures with mild focal hydropic degeneration (arrow). C) Kidney showing normal glomeruli with mild hydropic degeneration of epithelial lining renal tubules (arrow). (H&E ×100).

Discussion

BPA is an emerging contaminant associated with oxidative stress Abdel-Tawwab and Hamed (2018) and endocrine disruption Wu and Seebacher (2020). The present study revealed elevated levels of LH and E2 with a lowered level of T after 2 weeks of 50 µg.L^{-1} BPA exposure. Inconsistent with these results, Mandich et al. (2007) and Faheem et al. (2017) recorded a significant reduction in T levels with increased E2 levels after exposure to 100 µg.L^{-1} BPA for 14 days in *C. carpio* and Pakistani major carp, *Catla catla* (Hamilton, 1822), respectively. This reduction in the T levels may be attributed to two reasons. The first could be, suppression of T production because of (a) Injury of the Leydig cells (LCs) due to the oxidative stress that BPA disrupts testis maturation through apoptosis of germ cells and Leydig cells, thus decreased in 11-ketotestosterone levels that disrupt spermatogenesis (Wang et al., 2019); (b) BPA can bind to both estrogen receptors (ER) and estrogen-related receptors (ERR) which block LCs gene expression. Moreover, BPA binds to the androgen receptor as an antagonist to block the activation of LC genes. BPA can also bind to G-coupled protein estrogen receptor (GPER), which inhibits the differentiation of LCs (Li et al., 2020). The second reason for the reduction or T levels could be due to the over-expression of cyp19a1a (aromatase) in response to BPA exposure caused an increase in the irreversible conversion of T into E2 (Liu et al., 2014). The increased irreversible conversion of T into E2 with high LH could explain the elevated E2 in our results.

It has been documented that BPA exposure up-regulated the gonadotropin-releasing hormones (GnRH) mRNA protein in zebrafish, *Danio rerio* (Hamilton, 1822) and minnow *Gobiocypris rarus* (Vosges et al., 2010). Moreover, Harding et al. (2013) observed signalling changes in GnRH system and some reproductive-related genes via an ER-activated pathway. These xenoestrogenic effects of BPA with the sharp decline in T besides the increased E2 caused the LH to exceed the double value of its counterpart in Group1 through the positive feedback mechanism (Dickey and Swanson, 1998; Cavaco et al., 2001). Conversely, Zahran et al. (2020) reported a reduction in LH and an elevation in T level in male Nile tilapia in BPA (6.5 mg.L^{-1}) polluted sites. While Wang et al. (2019) found no obvious change in the expression of FSHβ and LHβ in the brain tissue of male goldfish exposed to 50 µg.L^{-1} BPA. Also, Mandich et al. (2007) found no significant change in E2 levels in adult male *C. carpio*. The discrepancy in these results may be related to different experimental protocols, e.g., BPA dose, age, and length of exposure, or species variations.

The histopathological picture of the testes reflected the previous results as the walls of some seminiferous tubules were destroyed while others were empty with the complete disappearance of primary, secondary spermatogonial cells and spermatid. In addition, thickening of the interstitial tissue was prevalent. These findings were similar to that of *C. carpio* and zebrafish, *D. rerio*, subjected to 100 µg.L^{-1} BPA (Al-Sakran et al., 2016; Lora et al., 2016).

Antioxidant biomarkers were used to detect various environmental stressors effects in some aquatic organisms (Hook et al., 2014). The obtained data showed lowered antioxidant enzyme activities including SOD and CAT with increased MDA concentration in BPA subjected North African catfish. The same results were obtained by Abdel-Tawwab and Hamed (2018) in Nile tilapia and Akram et al. (2021) in freshwater bighead carp, *Hypophthalmichthys nobilis* (Richardson, 1845).

The histopathological results (Fig. 2D) showed activation of melano-macrophage centres of the kidney, which could be a consequence of oxidative stress. The overall decrease in T would compromise the immunity that the T and 11-ketotestosterone are involved in regulating the response of professional phagocytes in gilthead seabream (Aguila et al., 2012). Costantini and Møller (2008) also reported that oxidative stress could be a key mediating factor in the activation of the immune system,) i.e., immune system produces highly reactive species that destroy pathogens but can also cause oxidative damage to host tissues, and these adverse effects may therefore impair further investment in immune responses. To offset these toxic effects, animals rely on a complex system of antioxidants (Marri and Richner, 2015). Moreover, Biller-Takahashi et al. (2015) found that oxidative stress limited the production of immune components in pacu, *Piaractus mesopotamicus* (Holmberg, 1887), i.e., when the equilibrium between the oxidant and antioxidant production is lost, the immune system responses produce lower amounts of protective proteins and cells. This imbalance indicates that the antioxidant status in the fish probably limited the immunity in an attempt to avoid over-production of reactive oxygen species.

Besides the elevation of ALT, AST, urea and creatinine levels in the BPA-subjected groups as seen in the present study, Abdel-Tawwab and Hamed (2018) found an alteration in liver and kidney functions in the Nile tilapia exposed to BPA; indicating that the BPA toxicity induced several pathological processes in different fish organs. The histopathological alterations in the liver and kidney of BPA-exposed catfish in the present study agreed with the results in *C. catla* exposed to 1 ppm BPA (Faheem et al., 2016).

Diverse compounds have been isolated from purslane, such as flavonoids (kaempferol, luteolin, quercetin, isorhamnetin, and rutin), phenolic acids (caffeic acid, p-coumaric acid, and ferulic acid), fatty acids, vitamins (A, E, C, and B complex), and minerals (Nemzer et al., 2020).

In the present study, the dietary purslane powder restored the normal levels of the measured hormones

in the BPA-exposed catfish. Similarly, Al-Bishri et al. (2017) recorded that adding the purslane enhanced the fertility measured hormones and decreased antifertility in epileptic rats. These results were attributed to the antioxidant activity of purslane besides its docosahexaenoic acid (DHA) contents (Farag and Shakour, 2019), where the supplementation of 0.2 % DHA alone was able to restore male mice fertility, spermiogenesis, sperm morphology, and sperm count (Roqueta-Rivera et al., 2010).

In the present study feeding, purslane-containing diets exhibited an improvement in the antioxidant activity in the catfish subjected to BPA. This finding is consistent with Abdel-Razek et al. (2019), who reported that dietary purslane supplementation enhanced the SOD and CAT activities accompanied by a decline in MDA concentration in Aeromonas-infected Nile tilapia. Moreover, the purslane directed the disrupted ALT, AST, CAT, SOD, and MDA toward the normalisation in rats intoxicated by CCl_4, Cd, and Al (Eidi et al., 2015; Seif et al., 2019; Samir et al., 2022). Meanwhile, Mahdavinia et al. (2019) reported that quercetin (the main flavonoid in purslane) ameliorated the BPA-elevated ALT, AST, and CAT and decreased the MDA rate. Noteworthy flavonoids should not be regarded as a pure antioxidant that may exert a pro-oxidant action when their concentration exceeds certain limits. However, the pro-oxidant activity of flavonoids seems to be more helpful to the cell than to be cytotoxic (Procházková et al., 2011), which could explain why the 3 % purslane-supplemented diet showed a better tendency toward normalisation than 5 % purslane-supplemented diet in the present results. The flavonoids could scavenge ROS and RNS using its hydroxyl groups following the proton–electron sequential transfer mechanism; where flavonoids firstly donate a proton to free radicals associated with BPA exposure resulting in a flavonoid anion, which is more stable and better electron donor forming a stable flavonoid (Al-Mamary and Moussa, 2021).

The 3 % purslane and to some extent 5 % purslane supplementations in this work could ameliorate the liver and kidney elevated enzymes, pointing out the various anti-inflammatory contents of this plant. Noteworthy, quercetin is one of the most effective inducers of phase II detoxification enzymes (Procházková et al., 2011). In addition, Ganeshpurkar and Saluja (2017) reviewed that the rutin afforded a protective effect on the damage to human sperm induced by lipid peroxidation and possible protection to the testicular tissue with a decrement in the levels of serum ALT, AST, urea, and creatinine. Moreover, purslane exhibited potent radical scavenging activities more than the natural antioxidants, i.e., Vitamin C and E (Farag and Shakour, 2019).

The histopathological changes observed in the testes, liver, and kidney of BPA-treated catfish improved after being fed 3 % purslane-containing diets for 14 days. These findings agreed with the results obtained by Seif et al. (2019) who reported that the administration of purslane extract helped liver and kidney tissues to ameliorate through its anti-inflammatory components. The improvement of the testes, liver, and kidney integrity may be mediated by the purslane's anti-inflammatory properties and antioxidant.

The interesting result is the ability of purslane to increase T, and E2 levels in the non-BPA treated groups which may be due to the presence of quercetin in purslane, which has been found to act as an agonist of the GPER (Prossnitz and Barton, 2014). Kotula-Balak et al. (2018) showed that GPER is involved in a proper LCs function and the maintenance of its architecture and supervising its steroidogenic function by estrogen during the male life. Moreover, recent studies indicated that GPER is involved in modulating GnRH release and gonadotropins secretion (Chimento et al., 2014). Concurrently, GPER was shown to regulate the proliferative and apoptotic pathways involved in spermatogenesis throughout rat reproductive development (Lucas et al., 2014). All these reports could explain the high spermatogenesis levels with the elevated T and E2 in the purslane-only treated groups in the present study.

Conclusion

The present study concluded that bisphenol A (BPA) induced reproductive disorders in male North African catfish, *Clarias gariepinus* via endocrine disrupting and oxidative damage as evidence of the hormonal, biochemical, and histopathological alterations in the BPA treated catfish. The purslane, *Portulaca oleracea* (at the ratio of 3 %) could be considered as a protective herbal against the BPA adverse effects. Future research is needed to enable a reliable risk assessment of the BPA exposure for mature male fish to support this work.

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Author contributions: All the authors contributed to the study conception, design, material preparation, data collection, analysis and read and approved the final manuscript.

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