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Ameliorative role of chitosan nanoparticles against bisphenol-A induced behavioral, biochemical changes and nephrotoxicity in the African catfish, *Clarias gariepinus*

Naema M. Hussein[1], Rokaya M. A. Saeed[1], Adel A. Shaheen[2], Heba S. Hamed[1*]

1-Department of Zoology, Faculty of women for Arts, Science & Education, Ain Sham University, Cairo 11757, Egypt.

2- Department of Fish Diseases and Management، Faculty of Veterinary Medicine, Banha University, Banha, Egypt.

Corresponding Author:hebasalah84@women.asu.edu.eg

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ABSTRACT

The Present study aimed to investigate the protective effect of Chitosan nanoparticles (CSNPs) on the behavioral, biochemical alterations and oxidative stress biomarkers of African catfish, *Clarias gariepinus* exposed to (1.43µg/L) Bisphenol-A (BPA) for 30 days. Fish were allocated into five groups as follows: group I was control , group II was treated with Chitosan nanoparticles (0.66ml/L) , group III was treated with BPA (1.43µg/L), group IV was treated with BPA (1.43µg/L) plus CSNPs (0.33ml/L), group V was treated with BPA (1.43µg/L) plus CSNPs (0.66ml/L). At the end of experiment, biochemical and oxidative stress biomarkers were analyzed in the blood and kidney tissues. Serum alanine aminotransferase (ALT), total proteins, Albumin, globulin, Alkaline Phosphatase (ALP), Acetylcholinesterase (AChE) were significantly decreased in (group III) exposed to BPA compared to (group I). Meanwhile, Cortisol, Glucose, Urea, Creatinine, Uric acid, Aspartate aminotransferase (AST) were increased in catfish exposed to (1.43µg/L) BPA compared to the control fish. Marked increments in renal Superoxide dismutase (SOD) and malondialdehyde (MDA) levels and significant reductions in the activity of renal Glutathione peroxidase (GSH-px) , total antioxidant capacity (TAC), and Catalase (CAT) were detected in BPA-exposed fish (group III) compared to the control group (group I) and CNPs (0.66ml/L.) (group II). However, administration of fish with the two tested doses (0.33 and0.66ml/L) of CSNPs attenuated the BPA- induced biochemical changes oxidative stress in catfish. Such improvement was more obvious in catfish received the higher dose (0.66ml/L) of CSNPs, where the tested parameters were restored to the control group in comparison to catfish exposed to BPA alone (group III) after 30 days of exposure. The obtained results induced that CSNPs administration ameliorated the toxic effects of BPA on African catfish.

INTRODUCTION

Recently, BPA is expansively used for aquatic toxicity testing owing to its adversarial effects on wild life, mainly fishes. Fishes are widely used to normalize the health of aquatic systems because their biological responses serve as biological indicators of environmental contamination. Bisphenol-A, an organic compound having two phenol

functional groups is used to make polycarbonate polymers and epoxy resins, along with other materials working to make plastics **(Vasu *et al.*, 2019)**. The aquatic ecosystems are exposed to BPA through the urban sanitation, petrochemical industry discharge, and landfill leachates causing a severe problems to the aquatic organism's health (Flint *et al*., 2012; Rubin, 2011). Thus, its detrimental effects on aquatic animals have also received great concern **(Buxton and Kolpin, 2005; Huang *et al*., 2012; Abdel-Tawwab and Hamed, 2018)**.

The main chemicals produced internationally is Bisphenol-A and its demand is increasing due to the ever-increasing demand and creation of plastic products. Bisphenol-A is used in almost all recent industries. It was manufactured in 1891 and nowadays the current manufacture is estimated at about 4 billion kilograms per year globally **(Environment Canada Report, 2009; Vasu *et al*., 2019)**. Therefore, it is inevitable that aquatic organisms including, humans, are vulnerable to toxic and endocrine-disruptive effects by BPA. Indeed, a large body of research has reported adverse effects of BPA on development, growth performance, biochemical profile, and oxidative stress biomarkers of tilapia fish **(Hamed and Abdel-Tawwab, 2017; Abdel-Tawwab and Hamed, 2018)**.

Chitosan (CS) is a natural established polymer, non-toxic immunostimulant resulting from the de-acetylation procedure of chitin, a major shell element of shellfish such as crab, shrimp, and crayfish **(Harikrishnana *et al.*, 2012)**. Chitosan nanoparticles (CSNPs) exhibit more superior activities than CS. Where, nanoparticles have larger surface area compared to large particles producing more dissolution pressure with a corresponding increase in saturation solubility **(Müller and Böhm, 1998)**.

African catfish, *Clarias gariepinus* has been used in toxicological studies as an excellent animal model since it has a well-documented biology **(Mahmoud *et al.*, 2009; Mekkawy *et al*., 2011; Sayed and Hamed, 2017)**. *C. gariepinus* was chosen in this experiment because it has high growth rate, high stocking density capacities, and can resist low water quality **(Akinwole and faturoti, 2007; Adewola *et al*., 2008; Karami *et al*., 2010; Marzouk *et al*., 2017)**.

The purpose of the current study was (a) to evaluate whether BPA induces difference in the biochemical parameters and the oxidant defense mechanism in the kidneys tissues of the exposed African catfish, and (b) to investigate the probable ameliorative roles of chitosan nanoparticles in alleviating the toxic effects of BPA in the exposed fish.

MATERIALS AND METHODS

Chemicals

Bisphenol-A (98% purity) was purchased from Sigma Aldrich Trade Co., Egypt**.** Chitosan was purchased from Oxford labs Co., Cairo, Egypt. Stock solutions of chitosan nanoparticles at the concentrations (0.33ml/L and 0.66ml/L) were prepared according to **Tang *et al*. (2007)**.

AST, ALT, ALP, Urea, Creatinine, Uric acid, Glucose, Cortisol, AChE , Total Proteins, Albumin and antioxidants kits (MDA, SOD, CAT, GSH-Px ,and TAC) were purchased from Biodiagnostic Co., Dokki, Egypt.

Fish rearing

African catfish, *Clarias.gariepinus* (250±50 g and body length 33.5±2.0 cm), were brought from Abbassa fish farm, Egypt. fish were distributed in glass aquaria containing 85L. of dechlorinated tap, and kept under laboratory circumstances for 15 days for acclimatization. Fish were fed a basal diet containing 32% protein.

Half lethal concentration of Bisphenol-A (BPA)

The half lethal concentration of BPA that caused 50% mortality (LC_{50}) in African catfish after 96 h. of exposure was determined (14.30µg/l) and it was carried out according to **Litchfield and Wilcoxon (1949)**.

Experimental design

Fish were divided into five groups, and each group consisted of 3 replicates of 15 catfish per aquarium. Each group was exposed for 30 days the following treatments:

Group I: fish served as control group and fed on free basal diet .

Group II: fish served as control group with addition of Chitosan nanoparticles (CNPs) (0.66 ml/L).

Group III: fish exposed to (1.43µg/L) of BPA for 30 days.

Group IV:: fish exposed to (1.43µg/L) of BPA + CSNPs (0.33ml/ L water) for 30 days.

Group V: fish exposed to (1.43µg/L) of BPA + (CNPs) (0.66ml/ L water) for 30 days.

During this experimental period, any fish mortalities or abnormalities were documented daily, and water was continuously changed every 48 h..

Clinical investigation

Behavioral abnormalities, mortality of fish and post-mortem lesions were observed according to **Amlacher (1970)**.

Biochemical analysis

After the experiment, 8 fish from each group were anaesthetized with 0.02% benzocaine. Blood samples were collected in clean centrifuge tubes from the caudal veins, allowed to clot, and then centrifuged at 3000×g at 4 °C for 15 min. Serum glucose and cortisol were determined according to **Trinder (1959)** and **Yalow and Berson (1971)**, respectively. AST and ALT were estimated according to the method described by **Reitman and Frankel (1957)**. Serum ALP. was determined according to the method described by **Tietz *et al.* (1983)**. Urea and creatinine were measured according to **Henry *et al.* (1974)** and **Fabiny and Eringhasuen (1971)**, respectively. Serum uric acid was recorded according to **Barham and Trinder (1972)**. Serum acetylcholinesterase (AChE), total proteins, albumin, and globulin were recorded according to **Kendel and Bottger (1967), Gornal *et al.* (1949), Dumas and Biggs (1972),** and **Coles (1974)**, respectively.

Renal malondialdehyde (MDA) and oxidative stress biomarkers

Samples of kidneys tissues were homogenized in cold phosphate buffered saline (0.1M,pH7.4)using a Potter-Elvejhem glass/Teflon homogenizer,then centrifuged. Supernatants were stored at -20 °C until analysis. MDA levels were detected according to Mihara and Uchiyama (1978). SOD, CAT , GSH-PX, and TAC activities were estimated according to **Nishikimi *et al*. (1972), Aebi (1984), Beutler *etal*. (1963),** and **Koracevic *et al.* (2001)**, respectively.

Data analysis

The data were presented as means ± SE. Data were subjected to two-way ANOVA to evaluate effects of BPA toxicity and chitosan nanoparticles administration. followed by Tukey's-B post hoc test to compare between groups, $P<0.05$ was considered statistically significant. All the data analyses were done using SPSS program version 20.

RESULTS

1. Clinical investigations

Catfish in group (I) showed normal behavior. Whereas, fish exposed to bisphenol-A (BPA) (1.43µg/L) for 30 days, swam near the surface of water. Irregular swimming activities were also noted. The most clinical signs and PM lesions associated with the exposure of *C. gariepinus* to BPA were displayed by moving in the way of the air pump (Fig.1). Trials to jump out of aquarium, calmness swimming and continuing immobile at a definite site mostly at mid water level for a long time were also noticed, Although the postmortem examination shown different grades of congestion, hemorrhage in the ovaries as well as, distention of gall bladder that were documented as prominent lesions (Fig. 2).

2. Biochemical parameters

Catfish exposed to BPA showed increments in the values of serum AST, Urea, Creatinine, Uric acid , Glucose, and Cortisol compared to the control group (Tables1&2). Significant ($P<0.05$) decrease in the levels of ALT, ALP, AChE were recorded in the sera of BPA-intoxicated catfish compared to the control ones (Tables 1&2). Also, marked ($P < 0.05$) reduction in the levels of Total protein, albumin, and globulin in the blood of BPA-exposed fish (Table 2) compared to the control group. Administration of CSNPs with the two tested doses (0.33and 0.66ml/L) caused marked ($P < 0.05$) decreases in biochemical parameters compared to the BPA-exposed fish group (group III). Combined treatment with BPA at low CSNPs dose (0.33ml/L) and high CSNPs dose (0.66ml/L) restored all the tested parameters to nearly the normal levels particularly, at group (V) which received the high dose of CSNPs .

Fig.1: *Clarias gariepinus* exposed to bisphenol- A (0.143 µg/ L.) moved towards the way of the air pump.

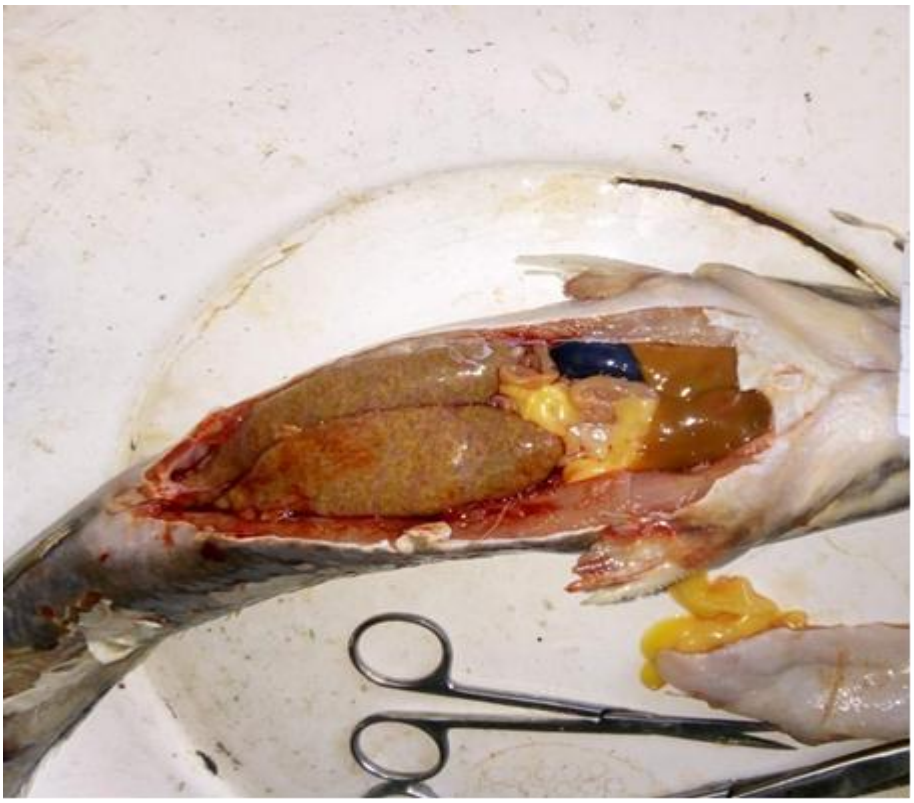

Fig. 2: Haemorrhage in the ovaries, and distension of the gall bladder of *C. gariepinus* exposed to bisphenol- A (0.143 µg/ L.) for 30 days.

Table 1: Changes in the blood biochemical parameters (means ± SD.) in African catfish, *C. gariepinus*, exposed to BPA (1.43 µg/L) and treated with CNPs (0.33and 0.66 ml/L), respectively for 30 days.

Groups / Parameters	Group I	Group II	Group III	Group IV	Group V
AST µ/l	47.35±0.49^{c}	47.31±0.79^{c}	60.44±0.61^{a}	52.63±0.53^{b}	49.05±0.33^{c}
ALT µ/l	18.23 ±0.17^{a}	17.58±0.28^{b}	11.90±0.34^{c}	17.43±0.09b	17.66±0.37^{b}
ALP µ/l	34.54±0.51^{a}	34.61±0.40^{a}	24.29±0.18^{c}	29.85±0.36^{b}	34.84±0.63^{a}
Urea mg/dl	13.63±0.18^{c}	13.41±0.43^{c}	21.52±0.43^{a}	16.53±0.07^{b}	13.48±0.22^{c}
Creatinine mg/dl	0.28±0.01^{d}	0.28±0.04^{d}	0.98±0.01^{a}	0.50±0.02^{b}	0.31±0.01^{c}
Uric Acid mg/dl	10.34±0.24^{b}	10.68±0.43^{b}	15.93±0.08^{a}	11.84±0.13^{c}	10.47±0.33^{b}

Means with different superscript letters in the same row for each parameter are significantly different (P<0.05).

Table 2: Changes in the stress indicators and total proteins levels (means ± SD.) in African catfish, *C. gariepinus*, exposed to BPA (1.43 µg/L) and treated with CNPs (0.33and 0.66 ml/L), respectively for 30 days.

Groups / Parameters	Group I	Group II	Group III	Group IV	Group V
Glucose mg/dl	85.90±0.44^{c}	85.92±0.51^{c}	119.36±1.20^{a}	100.07±2.05^{b}	89.17±0.90^{c}
Cortisol µg/dl	10.98±0.18^{c}	10.98±0.09^{c}	20.11±0.19^{a}	14.21±0.35^{b}	10.58±0.26^{c}
AchE µ/l	546.53±1.8^{a}	544.17±2.28^{a}	387.38±3.17^{d}	500.14±3.07^{c}	539.15±1.08^{b}
Total proteins g/dl	6.90±0.32^{a}	6.65±0.35^{a}	4.22±0.19^{d}	5.89±0.07^{c}	6.05±0.21^{b}
Albumin g/dl	2.97±0.04^{a}	2.94±0.02^{a}	2.04±0.01^{d}	2.87±0.05^{c}	2.90±0.02^{ab}
Globulin g/dl	3.93±0.17^{a}	3.71±0.22^{b}	2.18±0.11^{d}	3.02±0.14^{c}	3.15±0.12^{bc}
A/G ratio	0.76±0.0	0.79±0.02^{a}	0.94±0.04^{b}	0.95±0.01^{b}	0.92±0.01^{b}

Means with different superscript letters in the same row for each parameter are significantly different (P<0.05).

3. Renal malondialdehyde level (MDA) and antioxidant enzymes

The presented data in (Table 3) showed marked (P<0.05) increase in renal MDA and SOD levels of group (III) exposed to BPA for 30 days. Also, significant depletion (P <0.05) in CAT, GSH-Px, and TAC enzymes of the same tissue was recorded compared to the control fish. Co-administration with CSNPs at both the low and high doses improved the oxidative stress of kidney tissues of BPA- exposed catfish especially in group (V) which treated with the high CSNPs dose (0.66ml/L) for 30 days.

Table 3: Changes in renal Malondialdehyde (MDA) and oxidative stress biomarkers (mean ± SD.) in African catfish, *C. gariepinus*, exposed to BPA (1.43 µg/L) and treated with CNPs (0.33and 0.66 ml/L), respectively for 30 days.

Groups / Parameters	Group I	Group II	Group III	Group IV	Group V
MDA nmol/g protein	41.15±0.61^{d}	41.37±0.66^{d}	94.54±1.43^{a}	64.22±0.62^{b}	48.43±0.39^{c}
SOD µg/mg protein	42.38±0.33^{d}	43.03±0.67^{d}	85.24±0.80^{a}	64.51±1.14^{b}	52.26±1.17^{c}
CAT µg/mg protein	134.25±0.62^{a}	131.33±0.51^{ab}	101.90±0.28^{d}	121.17±0.09^{c}	129.79±0.25^{b}
GSH-Px mg/g protein	56.91±0.23^{a}	57.46±0.25^{a}	36.06±1.43^{c}	48.68±0.71^{b}	56.25±0.37^{a}
TAC µmol/mgprotein	39.27±0.16^{ab}	40.54±0.18^{a}	20.83±0.51^{d}	31.07±0.42^{c}	38.41±0.80^{b}

Means with different superscript letters in the same row for each parameter are significantly different ($P<0.05$).

DISCUSSION

Previous studies cleared that Phenols containing bisphenols are broadly extent in the surrounding environment including food, and drinking water and indoor microenvironments **(Igbinosa *et al.*, 2013)**. They are toxic to aquatic organisms including fish. .Meanwhile, chitosan is a natural polymer derived from chitin-de-acetylation process of crustaceans which has biomedical applications such as water treatment. In our results, CSNPs can improve the biochemical parameters results by reducing BPA toxicity.

Bisphenol -A (BPA) is classified as a slightly to moderately toxic substance based on its acute toxicity to adult fish, however, it can significantly impact the growth and morphology, as well as, the behavioral and histological structure of fish embryos/larvae/fry during their early life ages **(Pastva *et al.*, 2001; Lam *et al.*, 2011; Kinch *et al.*, 2015)**. However, chitosan nanoparticles (CSNPs) administration for 30 days reduced the abnormal behaviors of catfish, being in a better condition.

BPA- exposed fish showed loss of balance and irregular swimming performance. **Prasanth *et al.* (2005)** explained the abnormal behavior in catfish exposed to BPA due to the accumulation of acetylcholine at synaptic junctions causing lack in muscular coordination of catfish.

Assessment of AST, ALT levels in serum are good indicators for diagnosis of liver damage in animals exposed to xenobiotics **(Samipillai and Jagadeesan, 2005)**. The present study showed that BPA toxicity increased AST and decreased ALT and ALP levels in the blood of the African catfish for 30 days. Our investigation agreed with

Sayed and Hamed (2017) who found a reduction in ALP level after exposure to Nonylphenol for 14 days in African catfish. The changes in liver enzymes may be due to the damage in hepatic cells caused by BPA toxication **(Hamed and Osman, 2017)**. Furthermore, serum urea, creatinine and uric acid levels were increased significantly in group (III) exposed to BPA for 30 days. The increase in renal products could be due to reduced glomerular filtration rates **(Hamed and Osman, 2017)**.

The high levels of AST, urea, creatinine, and uric acid were also supported by **AbdelKhalek *et al.* (2015), Hamed (2015, 2016), Abdel-Tawwab and Hamed (2020), and Hamed *et al.* (2021)**. Administration of CSNPs at low and high doses with BPA-exposed fish decreased significantly serum AST, urea, creatinine, and uric acid levels and increased serum ALT, ALP. The results were more obvious at the high dose of CSNPs (0.66ml/L).

The present work showed a marked elevation in serum glucose of *C. gariepinus* exposed to sublethal concentration of bisphenol A (1.43µg/L) for 30 days. The results are supported by **Crestani *et al.* (2006), Nieves-Puigdoller *et al.* (2007), Velisek *et al.* (2009), Bakhshwan *et al.* (2009), Nascimento *et al.* (2012), Hamed and Abdel-Tawwab (2017), Abdel-Tawwab and Hamed (2018), Meng *et al.* (2018)**. The raise in glucose levels could be attributed to the raise in glucogenolysis process in stressed fish to satisfy its energy needs **(Winkalar *et al.*, 2007; Hamed and Osman, 2017; Hamed and Abdel- Tawwab, 2017; Abdel-Tawwab and Hamed, 2018)**. Interestingly, Supplementation of CSNPs restored the level of glucose to the control level, particularly, in group (V), which treated with CSNPs (0.66 ml /L).

Blood cortisol is the major corticosteroid in fish and may have a significant effect on its dynamics **(Wendelar Bonga, 1997; Mommsen *et al.*, 1999)**. Regarding serum cortisol, the results revealed that there was a significant increase in cortisol level of bisphenol A exposed fish during the exposure period in comparison to the control group. Our investigation agreed with **Waring and moore (2004), Gad and Ibrahim (2005), Nieves-Puigdoller *et al.* (2007), Soso *et al.* (2007), Cericato *et al.* (2008 and 2009), Dogan and Can (2011), Kadry *et al.* (2012), Abdel-Tawwab and Hamed (2020)**. The results may be explained by the activation of hypothalamo-pituitary-inter renal axis with their release of steroid cortisol in blood stream due to stress **(Ibrahim, 1992; Reddy and Leatherland, 1998; Bakhshwan *et al.*, 2009; Kadry *et al.*, 2012; Abdel-Tawwab and Hamed, 2020)**.

Similarly, increased cortisol may be essential for organizing energy for responding to and restoring destruction produced by contaminants, but then may have negative concerns for disease conflict and growth **(Wendelaar Bonga, 1997; Kadry *et al.*, 2012)**. Catfish exposed to BPA and co-administrated with CNPs (groups IV and V) showed marked decline in serum cortisol values, which was more pronounced in group (V) exposed to BPA (1.43µg/L) and treated with high dose of CNPs (0.66ml/L) for 30 days.

Results also declared that there was a marked inhibition of AChE in African catfish after BPA-exposure for 30 days. Our results agreed with **Hamed and Abdel-Tawwab (2017)**. CSNPs supplementation up regulated the AChE level to near the normal levels and this enhancement was more clear in fish received the high CSNPs dose (0.66ml/L) compared to the fish exposed to BPA only.

Proteins are important macromolecules which play an essential role in cellular metabolism of living beings **(Mommsen and Walsh, 1992; Bakhshwan *et al.*, 2009)**. Catfish exposed to BPA exhibited hypoproteinaemia, hypoalbuminaemia, and hypoglobulinaemia compared to the control ones. These results agreed with that documented in African catfish after pesticide exposures **(Hamed, 2016; Marzouk *et al.*, 2012; Sayed and Hamed, 2017; Abdel-Tawwab and Hamed, 2018)**. The depletion of serum total protein, albumin, and globulin in the BPA-exposed fish may be a result of dysfunctions of the liver and kidneys **(Giron-Pérez *et al.*, 2007)**. In a similar study, **Qiu *et al.* (2016)** recorded a significant reduction in albumin and C-reaction protein levels in the serum of common carp, *Cyprinus carpio* after BPA exposure.

The observed hypoproteinaemia could be attributed to the inhibition of liver protein synthesis **(Fontana *et al.*, 1998; Bakhshwan *et al.*, 2009)** or may be from damaged renal tissue **(Gad, 2005)**. Whereas, co-administration of CSNPs at (0.33 ml/L and 0.66 ml/L) restored total protein, albumin and globulin to about normal levels and this enhancement was more apparent in group (V) exposed to BPA and treated with CNPs (0.66ml/L) in comparison to the fish exposed to BPA alone (group III).

The present investigation showed that the antioxidant profile of BPA-exposed fish altered through the elevation in renal MDA and SOD levels and the reduction in CAT, GSH-PX, and TAC enzymes. The results are supported with **Ibrahim (2015) and Abdel-Wahhab *et al.* (2016)** who recorded marked increase in MDA levels and significant decrease in TAC levels of hepatorenal tissues of Nile tilapia and African catfish, respectively. Administration of CSNPs at low and high doses returned the level of renal MDA to the normal levels, particularly in group (V) administrated with the higher dose of CSNPs. Furthermore, SOD enzyme level in renal tissues of BPA-exposed fish was elevated markedly compared to the control ones as shown in table (3).

Antioxidant enzymes (SOD, CAT, and TAC) may be elevated or declined under chemical stress to transform ROS into harmless metabolites **(Clasen *et al.*, 2014; Hamed *et al.*, 2021)**. Depletion in GSH-Px values is measured as an early consequence of BPA prompted oxidative stress. The decline in GSH-Px levels in renal tissues of catfish exposed to BPA for 30 days, may reflect its use to oppose oxidative damage causing from ROS production due to toxicant exposure **(Abdel- Wahhab *et al.*, 2016; Hamed and Osman, 2017; Hamed and El-Sayed, 2019; Xie *et al.*, 2020)**.

This could be attributed to the cellular damage which produced by interactions of ROS with lipids, protein, and, nucleic acids. Treatment with CSNPs even at the low or high dose markedly improved renal antioxidant enzymes and TAC levels in catfish after

exposure to BPA compared to the groups (I and II). However, the enhancement in the renal antioxidant biomarkers was more pronounced particularly at the high dose (0.66ml/L) of CSNPs.

CONCLUSION

The results confirmed BPA toxicity on behavior, biochemical profile and oxidative stress biomarkers of African catfish. On the other hand, our investigation revealed that co-administration of CSNPs has a protective role against the negative impacts of BPA in catfish.

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