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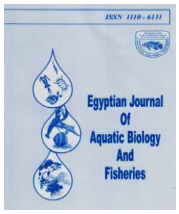


# Single and Combined Effects of Bisphenol A Exposure and *Schistosoma mansoni* Infection: Biochemical and Histological Implications on *Biomphalaria alexandrina* Snails

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## ABSTRACT

Plastic production is a huge industry all over the world. However, their release into the environment can cause unexpected impacts on the biological levels of the freshwater systems. Thus, the present study aimed to investigate the impact of bisphenol A (BPA) exposure on oxidative stress markers, sex hormones, neurotransmitters, and histology of the digestive gland in the snail, *Biomphalaria alexandrina* (infected and non-infected with *Schistosoma mansoni*). Snails were exposed to 0.1 and 1 mg $L^{-1}$ BPA for up to 4 weeks. After a sequential period of exposure (24h, 2 and 4 weeks), oxidative stress markers such as catalase (CAT), lipid peroxidase (MDA), and sex hormones (testosterone and 17β-estradiol) were determined in the ovotestis of the snails. Neurotransmitters (dopamine; DA and serotonin; 5-HT) were measured in the central nervous system (CNS) of the snails. In addition, a histological examination was done in the digestive gland after 2 and 4 weeks of infection/exposure. CAT activity, sex hormones, and neurotransmitter concentrations were significantly decreased in both exposed and infected-exposed groups, compared to the control and infected groups at all-time points. Nevertheless, MDA was markedly increased after exposure/infection at all time intervals. Histological symptoms such as the appearance of fat droplets, fibrous tissues, and vacuolization were observed in the digestive gland of exposed and/or infected-exposed snails. The present data demonstrated the risks of sublethal exposure to BPA on the snails, *B. alexandrina* as a biomonitor of freshwater contamination. In conclusion, the implications of BPA as a chemical stressor dominated the biological effect of infection alone or in combination.

## INTRODUCTION

Endocrine-disrupting chemicals include polycarbonated plastics and resins of which bisphenol A [BPA; 4,4` − (Propane −2,2 diyl) diphenol] is the main component. BPA poses an explicit threat to the environment, especially the aquatic systems **(Hernandez-Rodriguez *et al*., 2007; Reis *et al*., 2022)**. BPA is widely used in plastic products such as food packaging, bottles, can lining, thermal receipt papers, toys, CDs, and medical

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devices **(European Commission, 2018)**. Outflow and leaching from waste water treatments and landfills are the most common point sources for water contamination with BPA **(Kang *et al*., 2011)**. The dawn of the coronavirus pandemic (COVID-19) caused massive use of plastics in the form of single-use plastic products (**Alfaro-Nunez *et al*., 2021; Choi *et al*., 2022**). Environmental factors such as UV light, ageing of water, heating and water pH are the key dynamics initiating and affecting the leaching of BPA from these products **(Frenzilli *et al*., 2021)**.

*Biomphalaria alexandrina* snail is common in many Egyptian watercourses. The availability of this snail and its easy culturing suggests its usage as a bioindicator of environmental pollution with heavy metals **(Habib *et al*., 2016)**. *Biomphalaria* snail is a good and sensitive bioindicator of chemical pollution **(Morais *et al*., 2022**). *B. alexandrina* snail has a significant biomedical interest for its role in the transmission dynamics of *S. mansoni* and its increasing usage in studying the complex host-parasite interactions and innate immune responses **(Lotfy *et al*., 2005; Mohamed, 2011)**. Monitoring the changes in vital indicators such as growth, development, and reproduction or freshwater organisms is a valuable mean in the environmental risk assessment of the ecosystem. These indicators are the most prevalent measures at the level of aquatic organisms, especially invertebrate molluscs (**OECD, 2014)**.

Several studies have been conducted on the induction of oxidative stress by BPA in fish **(Hulak *et al*., 2013; Chitra & Sajitha, 2014; Kalb *et al*., 2016)**. However, little information is available about the ability of BPA to affect antioxidant defense and induce oxidative stress in other aquatic organisms. BPA induces oxidative stress in aquatic organisms by causing lipid peroxidation (LPO) and decreasing antioxidant enzyme activities such as catalase (CAT). Indeed, the activities of antioxidant-related (CAT and superoxide dismutase) in the digestive gland and gill of the mussels (*Mytilus galloprovincialis*) were increased after exposure to 10 and 100 $mgL^{-1}$ of polyethylene terephthalate microfibers for 32 days **(Choi *et al*., 2022)**. **Uçkun (2022)** mentioned that, the level of malondialdehyde (MDA) of the crayfish, *Astacus leptodactylus* was increased under exposure to 96.45 $mgL^{-1}$ BPA after 96h. Moreover, the author found decreases in the activity of the total antioxidant context (TAC) at the same time and concentration. Furthermore, exposure of the freshwater bivalve, *Unio tumidus* to 200 $ngL^{-1}$ BPA for two weeks induced oxidative damage through excessive ROS production **(Gnatyshyna *et al*., 2019**). Similarly, **El-Shenawy *et al*. (2017)** recorded an elevation in lipid peroxidation and a decrease in glutathione content in *B. alexandrina* snails following exposure to BPA at 0.097, 0.485 and 0.97 $mgL^{-1}$ for up to four weeks of exposure. The concentration of CAT activity in the ovotestis of *B. alexandrina* snails infected with *S. mansoni* fluctuated between a decrease in the $1^{st}$ week of infection and then increased after the $2^{nd}$ week of the incubation period of infection and reached its highest level at week three of infection **(Habib *et al*., 2020)**.

Molluscs gonads synthesize estrogen and testosterone-like hormones to regulate reproductive activity **(Lafont & Mathieu, 2007; Fodor & Pirger, 2022)**. Steroid hormones play an important role in the reproduction of various freshwater molluscs including *B. alexandrina* snails **(Croll & Wang, 2007; Yan *et al.*, 2011; Omran, 2012; Habib *et al.*, 2020)**. These hormones can be used as biomarkers for toxicity bioassays of endocrine disrupting chemicals (EDCs) **(Crain *et al.*, 2007)**. The estrogenic effects of BPA are linked to its interference with the release and functioning of endogenous 17β-estradiol. Indeed, **Oehlmann *et al.* (2006**) showed that BPA dislodged 17β-estradiol from binding to its receptors within cytosolic tissue extracts of the snail, *Marisa cornuarietis* suggesting that molluscs possess estrogenic receptors that are highly vulnerable to BPA activation. Moreover, **Bai and Acharya (2019)** found that testosterone was too low to be detectable in the mussels in the exposed quagga mussels, *Dreissena bugensis*, to BPA for six weeks.

The nervous system is sensitive to numerous pollutants including BPA **(Juhel *et al.*, 2017; Guo *et al.*, 2019)**. *B. alexandrina* has a relatively simple nervous system and quantifiable behaviours, promoting the snail as an excellent model for neurotoxicological investigations of environmental pollution **(Habib *et al.*, 2016; Saleh *et al.*, 2021)**. The nervous system of *Biomphalaria* spp. contains large quantities of serotonin and dopamine (**Santhanagopalan & Yoshino, 2000)**. Immunohistochemical localization of these neurotransmitters indicates their participation in important behavioral functions in this snail such as locomotion, feeding and reproduction **(Delgado *et al.*, 2012; Vallejo *et al.*, 2014)**.

Different organs have varying responses to different types of toxic compounds. In gastropods, metabolism occurs primarily in the digestive gland that serves also as the main site of xenobiotics accumulation and biotransformation **(Sheir *et al.*, 2010; Sheir and Handy, 2010; Sheir *et al.*, 2013**). Therefore, histopathology is one of the important core endpoints in assessing the pathological alterations occuring in aquatic animals when exposed to BPA **(Lajtner *et al.*, 1996)**. **Sheir *et al.* (2020)** investigated the effects of BPA on the histological and pathological signs of the freshwater clam, *Caelatura nilotica*. The signs such as necrosis, inflammation, fibrous tissue, and hyperplasia formation, fat droplets accumulations in gills, gonads, and intestine were observed at 0.25 and 2 $mgL^{-1}$ concentrations of BPA for 4 weeks. **Benjamin *et al.* (2019)** found severe changes in the soft tissue of the clams *Corbicula fluminea* after exposure to xenoestrogen as BPA. The study of **Flint *et al.* (2012)** indicated that the effects of BPA on the histology of aquatic invertebrates were due to alterations in the biochemical parameters and production of ROS. The pathological signs of increased vacuolization and hypertrophy in the tissues of clams exposed to 1 $\mu gL^{-1}$ of BPA are an adaptation to minimize the damage caused by BPA.

The present study aimed to investigate the single and/or combined effects of chemical and biological stressors (bisphenol A exposure and *S. mansoni* infection) on a battery of biomarkers in the freshwater snail, *B. alexandrina*. The current study hypothesized that BPA exposure will interfere with *S. mansoni* infection in snails. To help address this issue, oxidative stress markers (MDA and CAT), sex hormones (testosterone and 17β-estradiol), neurotransmitters (DA and 5-HT), and histology of the digestive gland were measured.

## MATERIALS AND METHODS

### Experimental animals

Adult *B. alexandrina* snails were filed-captured from watercourses in Giza Governorate during the summer of 2018. *B. alexandrina* snails were maintained in well-aerated plastic aquaria containing dechlorinated tap water. Snails were fed fresh or dried lettuce leaves at the Laboratory of Invertebrates, Faculty of Science, Menoufia University, Egypt. The snails were continuously examined (shedding process) for 4 successive weeks for natural trematode infections and acclimatized to the laboratory conditions. Laboratory conditions such as water temperature (25 ± 2ºC), pH (7.1 ± 0.2), and oxygen content (6.8 ± 0.4mg$L^{-1}$) were recorded after every water change. Snail maintenance and culturing were done according to the method of **El-Fiki and Mohamed (1978)**. The first generation of snails obtained from filed colonies, measuring 10-12 mm in shell diameter (mature) were used in the experiments.

### The tested material

Bisphenol A (4, 4'–isopropylidinedi-phenol), an opaque white granular dry powder with a purity of 97%, was used (#133027, Sigma Aldrich, USA) in the following experiments on *B. alexandrina* snails.

### The molluscicidal activity of BPA

A stock solution of BPA (100 mg$L^{-1}$) was prepared according to **Mihaich *et al.* (2012**). The resulting stock solution was continuously stirred until no un-dissolved material was found , and it was freshly prepared every week. A series of concentrations of BPA material was prepared (0.5, 1, 5, 10, and 20 mg$L^{-1}$) and used to determine $LC_{50}$ and $LC_{90}$ values for *B. alexandrina* snails. For each concentration, 30 adult snails (10-12 mm in shell diameter) were used in triplicates (10 snails/liter). Controls were also set up in triplicates using only dechlorinated tap water (25 ± 1ºC, **WHO, 1965**). Snails were exposed to BPA concentrations for 24h and then transferred to dechlorinated tap water for another 24h recovery period. Snails showing no response to the gentle stimulation with forceps were considered dead.

**Experimental design**

To study the effect of BPA on *B. alexandrina* snails, about 180 snails (10-12 mm) from the first generation of field-collected snails were used. Snails were divided into six groups, (1) the unexposed and uninfected control group, (2) snails exposed to *S. mansoni* miracidia (10 miracidia/snail) representing the infected control group, (3) snails exposed to 0.1 $mgL^{-1}$ BPA only, (4) snails exposed to 1 $mgL^{-1}$ BPA only, (5) infected snails and exposed to 0.1 $mgL^{-1}$ and (6) infected snails and exposed to 1 $mgL^{-1}$ BPA. Each group consisted of three replicates (10 snails/replicate), each of which was maintained in a tank filled with 2 liters of experimental solution. The experimental concentrations of BPA were prepared according to **Mihaich *et al.* (2009**) to detect the effects of sublethal concentrations (0.1 and 1 $mgL^{-1}$). The sublethal concentrations were chosen based on the quality control experiment to test acute and chronic concentrations of BPA on the snails. All the experimental snail groups were kept for 4 weeks at 25.0 ± 2.0°C and were daily fed dried lettuce leaves. Biochemical assays were measured at time intervals of 24h, 2 and 4 weeks. Oxidative stress markers and sex hormones were measured in the ovotestis tissues. Neurotransmitters were measured in the nervous tissues. Histology of the digestive gland was assayed after the $2^{nd}$ and $4^{th}$ weeks.

**Histological investigation**

After 2 and 4 weeks of exposure, the digestive gland was dissected from five snails from each group for histological studies. Digestive glands were immediately fixed in Bouin's solution for 24h and then transferred into 70% ethyl alcohol and processed for light microscopy according to the method of **Romeis (1989**). Sections were stained with Ehrlich's Hematoxylin and Eosin, examined and photographed using a light microscope (Optika, Italy).

**Biochemical parameters of *B. alexandrina***

**Oxidative stress markers**

***Lipid peroxidase activity***

Ovotestis (0.05 g) was homogenized in phosphate buffer solution (PBS; 50 mM potassium phosphate, 1 mM EDTA, pH 7.5) in a 1:10 weight to volume ratio using a glass homogenizer. The homogenates were centrifuged at 10,000rpm for 5min at 5°C. The resulting supernatant was used to calculate the concentration of malondialdehyde (MDA) based on the method of **Kei (1978**) using a lipid peroxidase kit (Biodiagnostic Company, Dokki, Giza, Egypt; Cat. No. MD 2528). The concentrations were measured at the wavelength of 534nm and calculated as nmol/g.

***Catalase activity***

Ovotestis (0.05 g) was homogenized in phosphate buffer solution (PBS; 50 mM potassium phosphate, 1 mM EDTA, pH 7.5) in a 1:10 weight to volume ratio using a glass

homogenizer. Ovotestis samples homogenates were centrifuged at 10,000rpm for 5min at 5°C. Catalase (CAT) activity was calculated using a catalase assay kit (Biodiagnostic Company, Dokki, Giza, Egypt; Cat. No. CA 2516) according to the method of **Aebi (1984)**. The concentrations were measured at the wavelength of 510nm and calculated as µg/ml.

**Steroid sex hormones**

***Testosterone***

Testosterone level in the ovotestis was measured using testosterone kit (Lifespan Bioscience, Inc. North America. Catalog No. LS-F10538) according to **De Longcamp *et al.* (1974)**. Ovotestis was weighed (0.05) and then homogenized in PBS on ice. The homogenate was centrifuged at 10,000rpm for 5min at 5°C. At the end, the supernatant was collected for the assay using microplate reader (Elisa reader, Bio Tek KL-800). The concentrations were measured at the wavelength of 450nm and calculated as ng/ml.

***17β-estradiol***

17β-estradiol concentration in the ovotestis was measured using a commercial kit (Abnova. Taipei, Taiwan Cat. No. KA0297) according to **Tietz (1986**). Ovotestis samples (0.05 g) were prepared as above using microplate reader (Elisa reader, Bio Tek KL-800). The concentrations were measured at the wavelength of 405nm and calculated as ng/ml.

**Neurotransmitters**

Snails were dissected and CNS were collected and weighed (0.03 g), then homogenized in PBS on ice. The homogenate was centrifuged at 10,000rpm at 5 °C, and the supernatant was collected for the assay. Dopamine (DA) was quantified using a solid phase Enzyme-Linked Immunosorbent Assay (ELISA), based on the sandwich principle, with a Dopamine ELISA kit (GenWay Biotech Inc.; San Diego CA 92121) according to **Yoshioka *et al.* (2002)**. Serotonin (5-HT) was measured in duplicate with a commercial enzyme immunoassay technique (Serotonin ELISA, DLD Diagnostika GmbH, REF EA 630/96, Hamburg, Germany) according to **Harenberg *et al.* (2000)**. The concentrations of DA and 5-HT were measured at the wavelengths of 405 and 450nm, respectively, and calculated as ng/ml.

**Statistical analysis**

Data were analyzed using Statgraphics 18 (Statgraphics Technologies, Inc., Virginia, USA). All data were expressed as means ± SD. Mortality rates ($LC_{50}$ & $LC_{90}$) were calculated using simple regression between mortality versus concentrations. One-way ANOVA was used for variance analysis between the control and exposed and/or infected groups. When ANOVA could not be applied, a non-parametric ranking test was used (Kruskal-Wallis). Difference (LSD) post-hoc test was used to set the significant difference at $P < 0.05$.

## RESULTS

### 1. Effect of BPA exposure on biochemical parameters of *B. alexandrina* snails

#### *1.1. Oxidative stress markers*

MDA concentration was significantly increased ($p$ = 0.03, Kruskal-Wallis) in the ovotestis tissues of 0.1 and 1 $mgL^{-1}$ BPA-exposed *B. alexandrina* snails after 24h, 2 and 4 weeks (7 ± 02, 9.05 ± 0.35, 18.95 ± 0.25 nmol/g) and (8.24 ± 0.05, 11.35 ± 0.15, 26.05 ± 1.05 nmol/g), respectively, compared to the unexposed control group at the same time intervals (4.21 ± 0.09, 5 ± 0.1, 4.7 ± 0.2 nmol/g). While, MDA concentration was significantly increased ($P$ = 0.03, Kruskal-Wallis) in 1 $mgL^{-1}$ BPA after 24h, 2 and 4 weeks (8.24 ± 0.05, 11.35 ± 0.15, 26.05 ± 1.05 nmol/g), compared to 0.1 $mgL^{-1}$ BPA at the same time intervals (7 ± 02, 9.05 ± 0.35, 18.95 ± 0.25 nmol/g), respectively (Table 1).

On the other hand, CAT activity was significantly decreased ($P$ = 0.03, Kruskal-Wallis) in 0.1 and 1 $mgL^{-1}$ BPA exposed groups after 24h, 2 and 4 weeks with values of (40 ± 1, 29.5 ± 2.5, 18 ± 1 U/g) and (42 ± 1, 25.5 ± 0.5, 12.5 ± 1.5 U/g), respectively, compared to (48 ± 1, 52.5 ± 1.5 and 52 ± 1 U/g) for the unexposed control group at the same time interval of exposure. While, CAT activity was significantly increased ($P$ = 0.03, Kruskal-Wallis) after 24h of exposure to 1 $mgL^{-1}$ BPA (42 ± 1 nmol/g), compared to 0.1 $mgL^{-1}$ BPA (40 ± 1 nmol/g) at the same interval.Whereas, CAT activity was significantly increased ($P$ = 0.03, Kruskal-Wallis) in o.1 $mgL^{-1}$ BPA exposed groups after 2 and 4 weeks (29.5 ± 2.5, 18 ± 1 U/g), compared to 1 $mgL^{-1}$ BPA exposed groups at the same time intervals (25.5 ± 0.5, 12.5 ± 1.5 U/g), respectively (Table 1).

In the ovotestis tissue of *S. mansoni* infected *B. alexandrina* snails, MDA concentrations (nmol/g) were significantly increased ($P$ = 0.02, Kruskal-Wallis) in 1 $mgL^{-1}$ BPA infected group after 24h, 2 and 4 weeks, with values of (8.5 ± 0.1, 12.3 ± 0.2, 17.1 ± 0.8 nmol/g), compared to (8 ± 0.1, 9.1 ± 0.2, 9.45 ± 0.35 nmol/g) and (8.05 ± 0.15, 10.75 ± 0.45, 15.15 ± 0.65 nmol/g) for unexposed infected control groups and 0.1 $mgL^{-1}$ BPA infected group, respectively, at the same time intervals (Table 1). While, MDA concentrations in 0.1 $mgL^{-1}$ BPA infected group was significantly increased ($P$ = 0.02, Kruskal-Wallis) only after 2 and 4 weeks, with values (10.75 ± 0.45, 15.15 ± 0.65 nmol/g), compared to (9.1 ± 0.2, 9.45 ± 0.35 nmol/g), respectively, for unexposed infected control groups at the same time intervals (Table 1).

For CAT, the enzyme activities were significantly decreased ($P$ = 0.02, Kruskal-Wallis) for 1 $mgL^{-1}$ BPA infected groups after 2 and 4 weeks at values (26 ± 2, 18.16 ± 2.02 U/g), compared to the unexposed infected control group (32.5 ± 1.5, 23.5 ± 0.5 U/g), at the same time intervals. Additionally, CAT activities were significantly decreased ($P$ = 0.02, Kruskal-Wallis) in 0.1 $mgL^{-1}$ BPA infected group after 4 weeks only at values (20 ± 1 U/g), compared to the unexposed infected control group (23.5 ± 0.5 U/g) at the same time intervals (Table 1). Comparing between the two infected exposed groups of 0.1 and

1 $mgL^{-1}$ BPA, it was found that CAT activity was significantly decreased only ($P$ = 0.02, Kruskal-Wallis) in 1 $mgL^{-1}$ BPA infected exposed group (26 ± 2 U/g) after 2 weeks, compared to 0.1 mg $L^{-1}$ BPA infected exposed group (30 ± 1 U/g) at the same time interval (Table 1).

**Table 1.** Levels of oxidative stress markers in the ovotestis (OT) tissues of *B. alexandrina* snails following exposure to BPA and/or infection with *S. mansoni*

| Experimental group | Lipid peroxide (MDA) | | | CAT | | |
|---|---|---|---|---|---|---|
| | 24 h | 2 weeks | 4 weeks | 24 h | 2 weeks | 4 weeks |
| **Unexposed control** | 4.21 ± 0.09 | 5 ± 0.1 | 4.7 ± 0.2 | 48 ± 1 | 52.5 ± 1.5 | 52 ± 1 |
| **0.1 $mgL^{-1}$** | 7 ± 0.2*$ | 9.05 ± 0.35*$ | 18.95 ± 0.25*$ | 40 ± 1*$ | 29.5 ± 2.5*$ | 18 ± 1*$ |
| **1 $mgL^{-1}$** | 8.24 ± 0.05* | 11.35 ± 0.15* | 26.05 ± 1.05* | 42 ± 1* | 25.5 ± 0.5* | 12.5 ± 1.5* |
| **Unexposed infected control** | 8 ± 0.1 | 9.1 ± 0.2 | 9.45 ± 0.35 | 33 ± 2 | 32.5 ± 1.5 | 23.5 ± 0.5 |
| **0.1 $mgL^{-1}$ + infection** | 8.05 ± 0.15$ | 10.75 ± 0.45*$ | 15.15 ± 0.65* | 37 ± 2 | 30 ± 1$ | 20 ± 1* |
| **1 $mgL^{-1}$ + infection** | 8.5 ± 0.1* | 12.3 ± 0.2* | 17.1 ± 0.8* | 33.5 ± 1.5 | 26 ± 2* | 18.16 ± 2.02* |

**Note**: $n$ = 10**;** MDA was expressed as nmol/g and CAT as µg/ml; (*) denotes significant difference between the unexposed/ infected control group and exposed groups, and ($) indicates significant difference between exposed groups ($P \leq 0.03$, Kruskal-Wallis)

### *1.2. Sex hormones*

Exposure of *B. alexandrina* snails to 0.1 or 1 $mgL^{-1}$ BPA deteriorated the concentrations of the two steroid sex hormones investigated in the ovotestis tissues (Table 2). Exposure of uninfected *B. alexandrina* snails to 0.1 or 1 $mgL^{-1}$ BPA significantly decreased ($P$ = 0.03, Kruskal-Wallis) the levels of testosterone hormone after 24h, 2 and 4 weeks of exposure at values of (2.01 ± 0.10, 1.78 ± 0.09, 0.82 ± 0.01 ng/ml) and (1.85 ± 0.10, 1.12 ± 0.03, 0.75 ± 0.01 ng/ml) respectively, when compared to the unexposed control group (2.96 ± 0.06, 3.15 ± 0.05, 4.22 ± 0.08 ng/ml) at the same time intervals. However, testosterone levels were significantly increased ($P$ = 0.03, Kruskal-Wallis) in 0.1 $mgL^{-1}$ BPA after 2 and 4 weeks of exposure record (1.78 ± 0.09 ng/ml) and (0.82 ± 0 .01 ng/ml), compared to (1.12 ± 0.03) and (0.75 ± 0.01 ng/ml) for 1 $mgL^{-1}$ BPA, respectively, for the two time intervals (Table 2).

Similarly, the exposure of uninfected *B. alexandrina* snails to 0.1 and 1 $mgL^{-1}$ BPA caused a significant decrease ($P$ = 0.03, Kruskal-Wallis) in the levels of estrogen (17β-estradiol) after 24h, 2 and 4 weeks with concentrations of (0.24 ± 0.00, 0.11 ± 0.00, 0.06 ± 0.00 ng/ml) and (0.22 ± 0.00, 0.09 ± 0.00, 0.05 ± 0.00 ng/ml), respectively, compared to the unexposed control group at the same time intervals (0.29 ± 0.00, 0.25 ± 0.00, 0.26 ± 0.00 ng/ml). While, BPA at 0.1 $mgL^{-1}$ caused a significant increase ($p$ = 0.03, Kruskal-Wallis) in the levels of estrogen after 24h, 2 and 4 weeks with values of (0.24 ± 0.00, 0.11 ± 0.00, 0.06 ± 0.00 ng/ml), compared to (0.22 ± 0.00, 0.09 ± 0.00, 0.05 ± 0.00 ng/ml) for 1 $mgL^{-1}$ BPA, respectively, at the three time intervals (Table 2).

The exposure of infected *B. alexandrina* snails to 0.1 or 1 $mgL^{-1}$ BPA influenced the activity of the two examined steroid sex hormones in the ovotestis tissue (Table 2). Exposure to 0.1 or 1 $mgL^{-1}$ BPA significantly decreased ($P$ = 0.02, Kruskal-Wallis) the levels of testosterone hormone after 24h, 2 and 4 weeks, showing values of (2.26 ± 0.05, 2.095 ± 0.005, 1.35 ± 0.05 ng/ml) and (2.2 ± 0.2, 1.68 ± 0.03, 1.145 ± 0.045 ng/ml), respectively, compared to unexposed infected control group (2.855 ± 0.055, 2.635 ± 0.045, 3.115 ± 0.025 ng/ml) for the same time interval of exposure. However, infected snails exposed to 0.1 $mgL^{-1}$ BPA showed a significant increase ($P$=0.03, Kruskal-Wallis) in testosterone levels after 2 and 4 weeks, withlevels of (2.095 ± 0.005, 1.35 ± 0.05 ng/ml), compared to infected snails exposed to 1 $mgL^{-1}$ BPA at the same time intervals (1.68 ± 0.03, 1.145 ± 0.045 ng/ml), respectively, for 2 and 4 weeks (Table 2).

In the ovotestis tissues of infected snails, exposure to 0.1 and 1 $mgL^{-1}$ BPA caused a significant decrease ($P$ = 0.02, Kruskal-Wallis) in the level of estrogen after 24h, 2 and 4 weeks and recorded values of (0.238 ± 0.003, 0.1605 ± 0.0015, 0.0985 ± 0.0035 ng/ml) and (0.1985 ± 0.0015, 0.12 ± 0.001, 0.132 ± 0.003 ng/ml), respectively, compared to unexposed infected control group at the same time intervals (0.20 ± 0.0045, 0.26 ± 0.003, 0.249 ± 0.002 ng/ml) (Table 2). However, infected *B. alexandrina* snails exposed to 0.1 $mgL^{-1}$ BPA showed a significant increase ($P$ = 0.02, Kruskal-Wallis) in the level of estrogen after 24h, 2 and 4 weeks, recording values of (0.238 ± 0.003, 0.1605 ± 0.0015, 0.0985 ± 0.0035 ng/ml), compared to infected snails exposed to 1 $mgL^{-1}$ BPA (0.1985 ± 0.0015, 0.12 ± 0.001, 0.132 ± 0.003 ng/ml), respectively, at the same time intervals (Table 2).

**Table 2.** Levels of sex hormones in the ovotestis of *B. alexandrina* snails following exposure to different concentrations of BPA and/or infection with *S. mansoni*

| Experimental group | 17β-estradiol | | | Testosterone | | |
|---|---|---|---|---|---|---|
| | 24 h | 2 weeks | 4 weeks | 24 h | 2 weeks | 4 weeks |
| **Unexposed control** | 0.29 ± 0.00 | 0.25 ± 0.00 | 0.26 ± 0.00 | 2.96 ± 0.06 | 3.15 ± 0.05 | 4.22 ± 0.08 |
| **0.1 $mgL^{-1}$** | 0.24 ± 0.00*$ | 0.11 ± 0.00*$ | 0.06 ± 0.00*$ | 2.01 ± 0.10* | 1.78 ± 0.09*$ | 0.82 ± 0.01* |
| **1 $mgL^{-1}$** | 0.22 ± 0.00* | 0.09 ± 0.00* | 0.05 ± 0.00* | 1.85 ± 0.10* | 1.12 ± 0.03* | 0.75 ± 0.01* |
| **Unexposed infected control** | 0.21 ± 0.005 | 0.26 ± 0.003 | 0.25 ± 0.002 | 2.86 ± 0.055 | 2.64 ± 0.045 | 3.12 ± 0.025 |
| **0.1 $mgL^{-1}$ + infection** | 0.24 ± 0.003*$ | 0.16 ± 0.002*$ | 0.098 ± 0.004*$ | 2.26 ± 0.05* | 2.09 ± 0.005*$ | 1.35 ± 0.05*$ |
| **1 $mgL^{-1}$ + infection** | 0.19 ± 0.0015* | 0.12 ± 0.001* | 0.132 ± 0.003* | 2.20 ± 0.20* | 1.68 ± 0.03* | 1.15 ± 0.04* |

**Note**: $n \geq 4$; Both 17β-estradiol and testosterone were expressed as ng/ml; (*) indicates significant difference between the unexposed/ infected control group and exposed groups at different concentrations, and ($) denotes significant difference between exposed groups at $P \leq 0.05$) (Kruskal-Wallis)

### *1.3. Neurotransmitters*

Results presented in Fig. (1a) indicate that the exposure of *B. alexandrina* snails to 0.1 and 1 $mgL^{-1}$ BPA affected the neurotransmitter contents in the CNS of snails after 24h, 2 weeks and 4 weeks of exposure. The levels of DA and 5-HT were significantly low ($P$ = 0.03, Kruskal-Wallis) in the exposed groups, compared to their values in the unexposed control group. DA values were (199.5 ± 1.50, 114.00 ± 1.00, 46.50 ± 2.50

ng/ml) for 0.1 $mgL^{-1}$ exposed group and (163.50 ± 1.50, 87.00 ± 2.00, 33.00 ± 2.00 ng/ml) for 0.1 $mgL^{-1}$ exposed group compared to (212.00 ± 2.00, 230.00 ± 1.00, 200.00 ± 5.00 ng/ml) for the unexposed control group, respectively, after 24 h, 2 weeks and 4 weeks of exposure (Fig. 1a). Regarding 5-HT, its values in 0.1 and 1 $mgL^{-1}$ BPA exposed groups after 24h, 2 weeks and 4 weeks were (62.50 ± 0.50, 57.50 ± 1.50, 27.00 ± 1.00 ng/ml) and (60.00 ± 1.00, 46.00 ± 1.00, 14.00 ± 1.00 ng/ml), respectively, compared to the unexposed control group at the same time intervals (97.00 ± 1.00, 130.00 ± 1.00, 114.00 ± 1.00 ng/ml) (Fig. 1b). Comparing between the two exposed groups, a significant increase ($p$ = 0.03, Kruskal-Wallis) was observed in DA and 5-HT levels in 0.1 and 1 $mgL^{-1}$ BPA exposed groups for 24h, 2 weeks and 4 weeks. The level of DA exposed to 0.1 $mgL^{-1}$ BPA increased significantly ($P$ = 0.03, Kruskal-Wallis) at 24h, 2 and 4 weeks (199.50 ± 1.50, 114.00 ± 1.00, 46.50 ± 2.50 ng/ml), compared to the exposed 1 $mgL^{-1}$ BPA group (163.50 ± 1.50, 87.00 ± 2.00, 33.00 ± 2.00 ng/ml), respectively, at the same time intervals (Fig. 1a). The levels of 5-HT in the CNS of snails exposed to 0.1 $mgL^{-1}$ BPA were significantly increased ($P$ = 0.03, Kruskal-Wallis) at 24h, 2 weeks and 4 weeks (62.50 ± 0.50, 57.50 ± 1.50, 27.00 ± 1.00 ng/ml), compared to its values in snails exposed to 1 $mgL^{-1}$ BPA (60.00 ± 1.00, 46.00 ± 1.00, 14.00 ± 1.00 ng/ml), respectively, at the same time intervals (Fig. 1b).

The results provided in Figs. (1c, d) reveal that the exposure of infected *B. alexandrina* snails to BPA affected the neurotransmitters contents in the CNS of snails after 24h, 2 and 4 weeks of exposure. The levels of DA and 5-HT were significantly low ($P$ = 0.02, Kruskal-Wallis) in the infected exposed groups at 0.1 and 1 $mgL^{-1}$ BPA, compared to their values in the unexposed infected control group. The DA levels in the infected snails exposed to 0.1 and 1 $mgL^{-1}$ BPA were significantly decreased ($P$ = 0.02, Kruskal-Wallis) after 24h, 2 and 4 weeks of exposure to record (173.00 ± 2.00, 145.00 ± 24.00, 130.50 ± 1.50 ng/ml and 149.50 ± 2.50, 124.50 ± 0.50, 122.50±4.50 ng/ml), respectively, for 0.1 $mgL^{-1}$ and 1 $mgL^{-1}$ exposed groups, compared to (203.50 ±5 .50, 205.00 ± 3.00 and 187.00 ± 2.00 ng/ml) for the unexposed infected control group at the same time intervals (Fig. 1c). Also, the concentrations of 5-HT in infected snails exposed to 0.1 and 1 $mgL^{-1}$ BPA were significantly decreased ($P$ = 0.02, Kruskal-Wallis) after 24h, 2 and 4 weeks of exposure. The concentrations of 5-HT in 0.1 and 1 $mgL^{-1}$ BPA exposed snails were (81.00 ± 1.00, 84.00 ± 2.00, 56.50 ± 1.50 ng/ml) and (70.50 ± 0.50, 70.50 ± 1.50, 50.50 ± 1.50 ng/ml), respectively, at 24h, 2 weeks and 4 weeks of exposure, compared to the unexposed infected control group at the same time intervals (110.00 ± 1.00, 93.00 ± 2.00, 122.00 ± 3.00 ng/ml, Fig. 1d). Nevertheless, exposure of infected snails to BPA at 0.1 $mgL^{-1}$ caused a significant increased ($p$ = 0.02, Kruskal-Wallis) in the levels of DA and 5-HT at 24 h, 2 weeks and 4 weeks, compared to 1 $mgL^{-1}$ BPA at the same time intervals. In the DA of the infected snails exposed to 0.1 $mgL^{-1}$, the BPA was significantly increased ($P$ = 0.02, Kruskal-Wallis) after 24h, 2 and 4 weeks at (173.00 ± 2.00, 145.00 ± 24.00, 130.50 ± 1.50 ng/ml), compared to the infected snails exposed to 1

$mgL^{-1}$ BPA group (149.50 ± 2.50, 124.50 ± 0.50, 122.50 ± 4.50 ng/ml), respectively (Fig. 1c). The level of 5-HT in the infected snails exposed to 0.1 $mgL^{-1}$ BPA was significantly increased ($P$ = 0.02, Kruskal-Wallis) after 24h, 2 and 4 weeks with values of (81.00 ± 1.00, 84.00 ± 2.00, 56.50 ± 1.50 ng/ml), compared to the infected snails exposed to 1 $mgL^{-1}$ BPA group (70.50 ± 0.50, 70.50 ± 1.50, 50.50 ± 1.50 ng/ml), respectively (Fig. 1d).

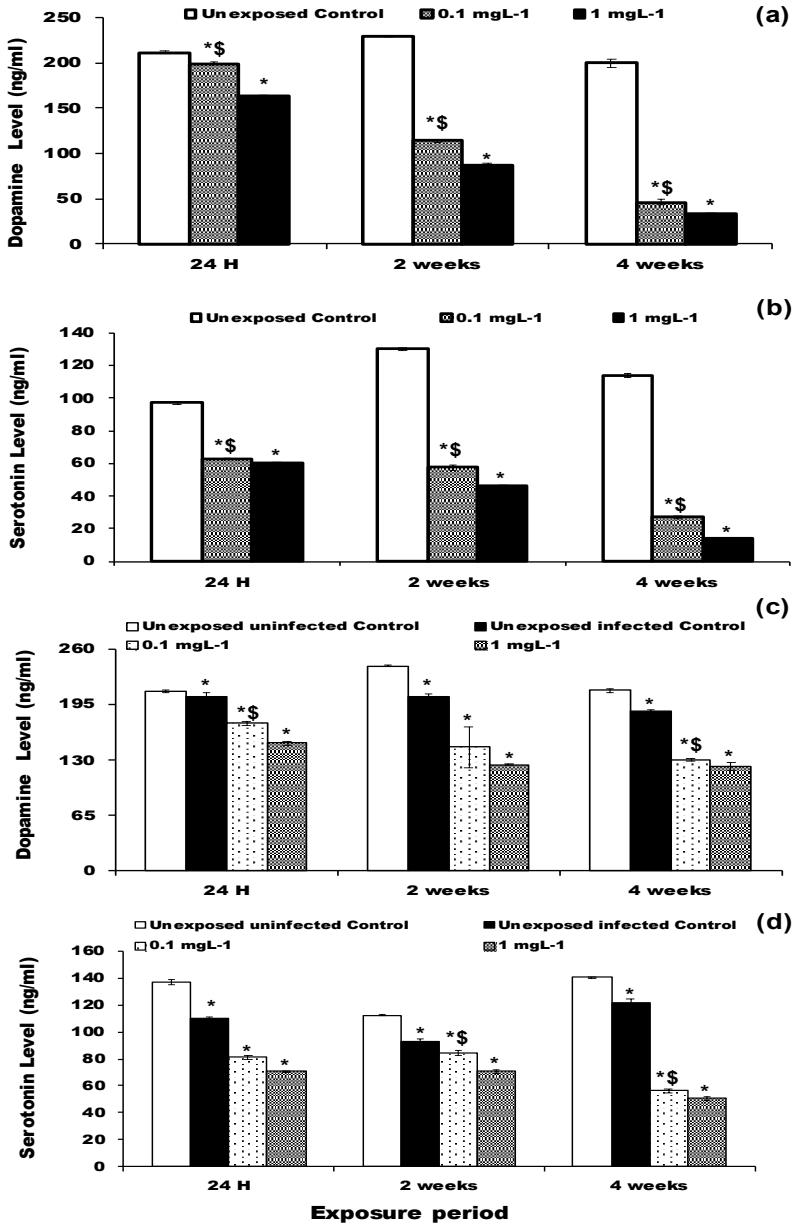


**Fig. 1.** Concentrations of dopamine and serotonin in the CNS of *B. alexandrina* snails after 24 h, 2 and 4 weeks of exposure to different concentrations of BPA and infection with *S. mansoni*. **(a**, **c)** Exposed snails, **(b, d)** Exposed and infected snails.

(*) denotes significant difference between the unexposed control group and exposed groups; ($) indicates significant difference between exposed groups at $P$ = 0.03 (Kruskal-Wallis).

### *2. Histopathological effects of BPA on the digestive gland*

The digestive gland of *B. alexandrina* snails consists of several tubular glands. Unexposed control showed normal architecture of tubular glands (Figs. 2A, B). After 2 weeks of the exposure *B. alexandrina* snails to 0.1 $mgL^{-1}$, the BPA resulted in the connective tissue appeared necrotic, thus the intertubular space became distinctive around the digestive tubules. Other digestive tubules fused to form one large and irregular-shaped tubule (Fig. 2C). While, in the 1 $mgL^{-1}$ BPA exposed snails, the digestive gland became compacted, with more than two tubules connected to one larger lumen. In addition, darkening of secretory cells stain was observed with an increased number of the secretory cells. In addition, fibrosis started to appear in the connective tissue between the tubules (Fig. 2E).

Observations on a 4- week exposure to 0.1 $mgL^{-1}$ BPA showed the presence of vacuolization, necrosis and degeneration in the digestive tubule epithelia. The tubular lumens became narrow and a degeneration was detected in the connective tissue between tubules (Fig. 2D). While, exposure to 1 $mgL^{-1}$ BPA showed necrotic changes in the digestive gland. Cell remains/debris were recorded in the lumens of the tubules, as well as granules from secretory cells. On other hand, the processes of atrophy of connective tissue took place **(**Fig. 2F).

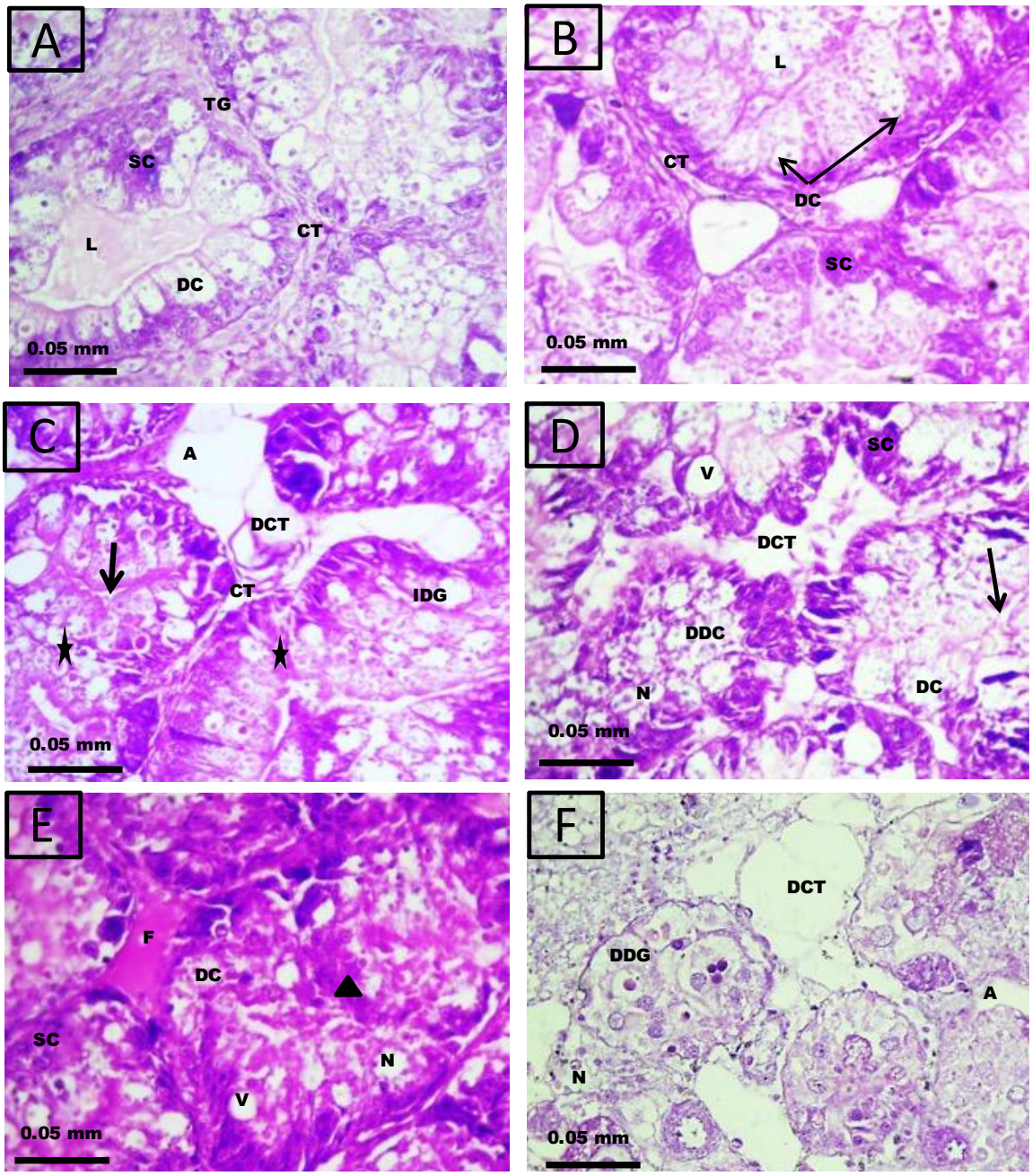


**Fig. 2.** Light photomicrographs through digestive gland of *B. alexandrina* snails stained with H & E after 2 (left panel) and 4 (right panel) weeks of exposure to different concentrations of BPA. **(A, B):** Unexposed control snails; **(C, D):** 0.1 $mgL^{-1}$ BPA exposed snails, and **(E, F):** 1 $mgL^{-1}$ BPA exposed snails

**A,** atrophy; **CT**, connective tissue; **DC**, digestive cell; **DCT**, degenerated connective tissue; **F**, fibrosis; **IDG**, irregular digestive gland; **IT**, inter tubular space; **L**, Lumen; **N**, necrosis; **SC**, secretory cells; **V**, vacuolization; **arrow head**, Exudation in the lumen of tubules; **Large arrow**, narrowing of the tubular lumen; **Stars**, fusion.

The histological structure of the digestive glands was examined in the infected unexposed and exposed snails. The histological examination of the digestive gland of the unexposed infected control snails after 2 weeks showed some digestive tubules adherent together with no intertubular space; however, others were observed with an increased number of digestive cells with a decreased lumen size of the tubule. While, one or two digestive tubules were separated with a connective tissue with dark secretions in the epithelial cells of the digestive tubules. In addition, the digestive gland had obvious developmental stages of sporocysts. After 4 weeks, it showed a completely degenerated architecture. Moreover, the developing cercariae as sporocysts filled the digestive gland (Figs. 3A, B). In the infected exposed snails to 0.1 $mgL^{-1}$ BPA, the epithelial cells of the digestive tubules incorporated with more dark secretions than the infected snails only. Furthemore, the digestive tubules showed vacuolization in the epithelial cells, and the lumen became narrow (Fig. 3C). Whereas, the infected exposed snails to 1 $mgL^{-1}$ BPA showed complete necrosis in the connective tissue, vacuolization in the epithelial cells, as well as congested lumen and several cysts (Fig. 3E).

On the other hand, alterations were detected in the digestive tubules of snails infected and exposed to 0.1 $mgL^{-1}$ BPA as vacuolization of the tissue; the presence of substances obstructing the lumens of the tubules and showing the different stages of the sporocysts. Moreover, the sporocysts fused in a bizarre shape (Fig. 2D). Furthemore, the histopathological examination in the digestive gland of infected snails exposed to 1 $mgL^{-1}$ BPA for 4 weeks showed similar alteration present in 0.1 $mgL^{-1}$ BPA but the different stages of sporocysts were bigger in size than the unexposed infected control group and infected snails exposed to 0.1 $mgL^{-1}$ BPA (Fig. 3F). In addition, major cytological changes in the epithelial cells and their nuclei were recorded.

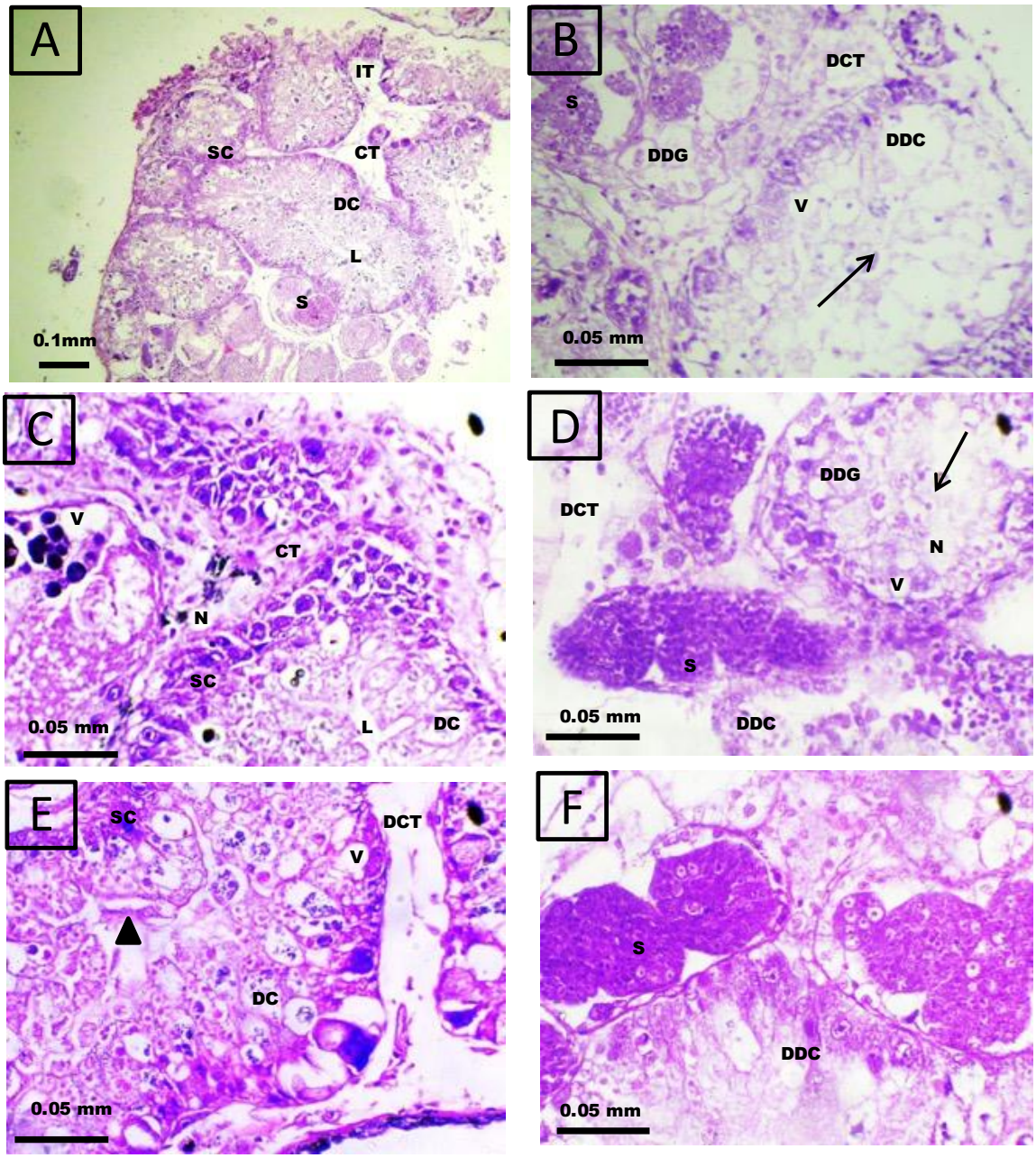


**Fig. 3.** Light photomicrographs for the digestive gland of infected *B. alexandrina* snails stained with H **&** E after 2 (left panel) and 4 (right panel) weeks of exposure to different concentrations of BPA; **A** & **B:** Unexposed infected control snails; **C** & **D:** 0.1 $mgL^{-1}$ BPA infected-exposed snails**,** and **E** & **F:** 1 $mgL^{-1}$ BPA infected-exposed snails

**CT**: Connective tissue; **DC**: Digestive cell; **DCT**: Degenerated connective tissue; **DDC**: Degenerative digestive cell; **DDG**, Degenerative digestive gland; **IT**: Inter tubular space; **L**: Lumen; **N**: Necrosis; **SC**: Secretory cells; **S**: Sporocyst; **V**: Vacuolization; **arrow head**: Narrowing of the tubular lumen, and **Large arrow**: Exudation in the lumen of tubules.

## DISCUSSION

### 1. Effect of BPA on the biochemical parameters of *B. alexandrina* snails

#### *1.1. Oxidative stress markers*

Oxidative stress biomarkers are a good ecotoxicological tool to evaluate the impacts of xenobiotics on aquatic organisms. In the present study, exposure of *B. alexandrina* snails to 0.1 and 1 $mgL^{-1}$ BPA resulted in a significant increase in malondialdehyde (MDA) activities in the ovotestis tissues. Enhancement of intracellular ROS is one important general pathway of toxicity of many environmental pollutants, which modulates the occurrence of cell injury through the initiation and proliferation of lipid peroxidation **(Gutteridge, 1995; Regoli *et al.*, 2002)**. The measurement of MDA content (an index of LPO) provides a relative measure of the potential oxidative stress caused by BPA. **El-Shenawy *et al.* (2017)** detected increased levels of LPO in *B. alexandrina* snails tissues after 2 and 4 weeks of exposure to 0.097, 0.485 and 0.97 $mgL^{-1}$ BPA. Similar to the present finding, **Abu El Einin *et al.* (2019)** depicted an increase in MDA levels in the ovotestis of *B. alexandrina* after 2 and 4 weeks of exposure to 0.3 and 1 $mgL^{-1}$ 17β-estradiol. *B. alexandrina* snails exposed to sub-lethal concentrations of different pesticides (diazinon and profenfose) showed a similar increase in MDA levels in their tissues after 4 weeks of exposure **(Bakry *et al.*, 2016)** and 2 weeks of exposure to oxyfluorfen 24% EC herbicide **(Ibrahim & Sayed, 2019)**. The mechanism of lipid peroxidation (MDA production) is linked to an increase in the production of highly reactive oxygen species and other reactive metabolites in the chain of biochemical reaction pathways.

The present study showed a significant decrease in CAT activity in the ovotestis of *B. alexandrina* snails following the exposure to sub-lethal concentrations (0.1 and 1 $mgL^{-1}$) of BPA. The response of CAT activity to pollution is variable from one organism to another depending on several factors. In some species, CAT exhibited increased activity; while in others, it shows suppressed activity **(Doyotte *et al.*, 1997; Regoli *et al.*, 1998)**. The present decrease in CAT activity may be due to a malfunction in the antioxidant defense mechanism of *B. alexandrina* snails that would be due to the inactivation of enzymes by overproduction of ROS as discussed in the stdy of **Pigeolet *et al.* (1990).** This result agrees with previous results that demonstrated that prolonged exposure to pesticides such as diazinon and profenfose induced oxidative stress conditions in tissues of *B. alexandrina* snails, as evidenced by a decrease in CAT activity **(Bakery *et al.*, 2016)**. Moreover, **Abu El Einin *et al.* (2019)** reported a similar decrease in CAT activity in the ovotestis tissues of *B. alexandrina* following exposure to 0.3 and 1 $mgL^{-1}$ 17β-estradiol for 2 and 4 weeks. However, **Ibrahim and Sayed (2019)** found that the activity of CAT increased in the tissues of *B. alexandrina* following the exposure to oxyfluorfen 24% EC herbicide. On the contrary, **San Juan *et al.* (2020)** found no change in CAT

activity in the freshwater snail *chilina parchapii* after exposure to 0.1 and 10 $mgL^{-1}$ of pyrethroid cypermethrin (GYP, organic insecticide) for 10 days. The change in the response of CAT activity could be explained as a regulatory mechanism to ROS production depending on the toxic exposure conditions **(Regoli & Giuliani, 2014)**.

In the present study, the oxidative stress induced by BPA toxicity was evidenced by the significant increase in the levels of MDA because of the peroxidation of lipid membranes, which is one of the most damaging effects of ROS **(Ohkawa *et al.*, 1979)**. The present results indicated that infected *B. alexandrina* snails exposed to 0.1 and 1 $mgL^{-1}$ BPA showed a significant increase in MDA levels and a significant decrease in CAT activities after 24h., 2 and 4 weeks of exposure. This indicates an overproduction of ROS in infected-exposed snails and a state of oxidative stress in snails. Similarly, *B. alexandrina* snails exposed to both *S. mansoni* and 0.3 ppm of Topas (fungicide) showed an increase in MDA levels starting after 24 h of exposure and continued until the $4^{th}$ week of exposure **(Osman *et al.*, 2010)**. However, the previous authors recorded an increase in CAT activity during infection and exposure beginning from 24h to 4 weeks. Then, a remarkable decline in CAT activity was observed after 4 weeks of infection and exposure to 0.3 ppm of Topas **(Osman *et al.*, 2010).** According to the authors, the increment indicated that the enzymes are not capable of controlling and keeping the normal level under the stress condition. afaurthermore, they attributed the elevation in the CAT activity to the presence of the parasite itself or the stimulated immune system.

### *1.2. Sex hormones*

In the present study, exposure to different concentrations of BPA resulted in a decrease in testosterone concentrations. A significant decrease was also reported in *B. alexandrina* snails following exposure to 0.3 $mgL^{-1}$ 17β-estradiol after 2 and 4 weeks **(Abu El Einin *et al.*, 2019)**. In addition, the exposure of *B. alexandrina* snails to sublethal concentrations of oxyfluorfen 24% EC herbicide for 2 weeks caused a significant decrease in testosterone levels (**Ibrahim & Sayed, 2019)**. The decrease in the testosterone level of *B. alexandrina* snails recorded in the present work agrees with the results of **Janer *et al.* (2006),** who found a significant decrease in the esterified testosterone concentrations in males *M. cornuarietis* snails exposed to 125-500 $ngL^{-1}$ tributyltin (TBT). They interpreted the decrease as the interference of TBT with the activity of esterases or the availability of cofactors that regulate the equilibrium between free and esterified steroids. **Sheir *et al.* (2020)** found that the testosterone of the freshwater clam, *C. nilotica* was decreased after exposure to the endocrine disruptor BPA at 0.25 and 2 $mgL^{-1}$ for 4 weeks. Similarly, these data agree with those of **Bai and Acharya (2019)** who reported that testosterone was very low and undetectable in the quagga mussels, *Dreissena bugensis* after exposure to 2,163 $ngL^{-1}$ BPA for six weeks. The clams may have released testosterone back into the water or esterified it with fatty acids to stabilize the exogenous steroid inside their bodies. **Giusti *et al.* (2013)** explained

that the exposure of *L. stagnalis* snails to 19.6 µgL$^{-1}$ Chlordecone (CLD, pesticide) tends to decrease the level of esterified testosterone.

For the 17β-estradiol levels, the present results showed a significant decrease following exposure to different concentrations of BPA after 4 weeks. These results coincide with those of **Sheir *et al.* (2020)** who reported that, 17β-estradiol concentrations of the freshwater clam, *C. nilotica* were significantly decreased after exposure to 0.25 and 2 mgL$^{-1}$ BPA for 4 weeks. In the same context, **Abu El Einin *et al.* (2019)** found that 17β-estradiol was significantly decreased in *B. alexandrina* snails exposed to 0.3 mgL$^{-1}$ EDCs for 2 and 4 weeks. Moreover, the exposure of *B. alexandrina* snail to the herbicide oxyfluorfen 24% EC for 2 weeks caused a significant decrease in 17β-estradiol levels **(Ibrahim & Sayed, 2019)**. Additionally, **Bai and Acharya (2019)** reported that, 17β-estradiol was undetectable in the mussel, *Dreissena bugensis* after 42 days of exposure to BPA at 2,163 ngL$^{-1}$. Generally, the decrease in endogenous sex steroid hormones is a well-known phenomenon after any exogenous/synthetic treatment of hormones which suppresses the natural-excreted hormone **(Waye & Trudeau, 2011; Zoeller *et al.*, 2012)**.

In the present study, exposure of *B. alexandrina* snails infected with *S. mansoni* to low (0.1 mgL$^{-1}$) and high (1 mgL$^{-1}$) concentrations of BPA caused a significant decrease in the levels of 17β-estradiol and testosterone after 2 and 4 weeks. Identically, **Habib *et al.* (2020)** reported a decrease in the concentrations of testosterone and 17β-estradiol in the ovotestis of *B. alexandrina* snails during the course of *S. mansoni* infection. Exposure to exogenous testosterone elicited the appearance of male secondary sex characteristics in the castrated slugs *Euhadra peliomphala* **(Takeda, 1980)**. In other molluscs such as the land snail *Theba pisana* and sea scallop, *Placopecten magellanicus*, exogenous exposure to testosterone, estradiol, and progesterone stimulated spermatogenesis and oogenesis in their gonads **(Sakr *et al.*, 1992; Wang & Croll, 2004)**.

### *1.3. Neurotransmitters*

The exposure of *B. alexandrina* snails to 0.1 and 1 mgL$^{-1}$ BPA in the current study led to a significant decrease in the levels of DA and 5-HT in the nervous system of exposed snails. This decrease in neurotransmitters may be due to BPA targeting the dopamine and serotonin receptors in exposed snails leading to downregulation of their production. A significant decline in DA and 5-HT was also observed in the CNS tissues of *B. alexandrina* snails following exposure to sublethal concentrations of the veterinary antibiotics, oxytetracycline and trimethoprim-sulphadiazine **(Saleh *et al.*, 2021)**. **Miglioli *et al.* (2021)** reported that exposure to 0.05, 0.5 and 5 µM BPA for up to 48h resulted in a reduction in the number of serotoninergic neurons at the early larval stages of the bivalve, *Mytilus galloprovincialis*. The authors postulated that this reduction was associated with developmental delay and downregulation of the 5-HT receptor-5-HTR. In addition, they reated the dysregulation of organic matrix deposition and calcification to the exposure to

BPA that may directly affect the metabolism of 5-HT and other monoamines like DA. **Boscolo *et al.* (2018)** found a decrease in DA levels in the brain of the male Nile tilapia (*Oreochromis niloticus*) exposed to herbicides and diuron metabolites at 100 $ngL^{-1}$ for 10 days. Additionally, **Badruzzaman *et al.* (2021)** recorded a significant decrease in DA of the freshwater catfish, *Mystus cavasius* exposed to Rotenone, pesticide 2.5, 25 and 250 $\mu gL^{-1}$ for 2 days. They attributed these effects to the lipophilic nature of rotenone (pesticide), which freely crosses the blood-brain barrier, as well as cell membranes, and destroys specific dopaminergic neurons in the brain, resulting in motor deficits causing neurotoxicity **(Xiong *et al.*, 2012). Jia and Pittman (2015)** also recorded a reduction in the striatal dopamine transporter and a decrease in the hippocampal 5-HT content of the male zebrafish exposed to 30 and 50 $mgL^{-1}$ of BPA for 14 days. Furtherore, the embryonic exposure of zebrafish to the endocrine disruptor pesticide, chlorpyrifos, decreased dopamine and serotonin in *Danio rerio* larvae **(Weis, 2014)**. Therefore, any changes in the expression of these neurosubstances will culminate the disturbances in vital physiological functions in the snails including reproduction **(Manger *et al.*, 1996; Muschamp & Fong, 2001)**.

In the present investigation, chronic exposure of infected *B. alexandrina* snails to 0.1 and 1 $mgL^{-1}$ BPA caused a decrease in the levels of DA and 5-HT in the CNS of snails. Given the function of these amines in the reproduction of *Biomphalaria* spp., it is anticipated that the decline in their concentrations will add (additive effect) to the disturbance in the reproductive capacity of infected snails. Previous studies showed that these two monoamines were reduced in the hemolymph and CNS of *B. glabrata* snails following infection with *S. mansoni* **(Manger *et al.*, 1996)**. Specifically, an infection-induced decrease in the concentration of 5-HT may be linked to parasitic castrations caused by schistosome infection **(Crews & Yoshino, 1989; Manger *et al.*, 1996)**. The down production of DA and 5-HT in the CNS of *B. alexandrina* snails observed in the present study could be due to the additive effect of *S. mansoni* infection and BPA exposure. In this case, the invading larvae deplete the DA and 5-HT of the snails for subsequent development, and BPA intoxication may target specific receptors involved in the production of these monoamines in snails. **Webster *et al.* (2006)** found that inhibition of dopamine through the application of 1.5 mg/kg/d of haloperidol (psychotic drug) in infected rats with *Toxoplasma gondii* reduces their parasite-induced behavior. **Cutler (2021)** reported that the slugs, *Deroceras invadens* infected with *Phasmarhabditis hermaphrodita* and fed 10 μM of cyproheptadine (reduces serotonin levels) were no longer attracted to the side with *P. hermaphrodita*. In addition, the application of 10 μM of haloperidol to infected slugs (which should graduate to the nematode side) was no longer attracted to the nematodes; presumably, dopamine signalling was antagonized.

**2. Histopathological effects of BPA on the digestive gland of *B. alexandrina* snails**

In *Biomphalaria alexandrina* snails exposed to 0.1 or 1 $mgL^{-1}$ BPA for up to 4 weeks, degeneration in the connective tissue, necrotic changes in digestive tubules, cellular swelling and vacuolization in the secretory cells were recorded. The digestive gland of molluscs has a crucial role in contaminant uptake, metabolism and intracellular food digestion. Thus, it is a main target of pollutant impacts **(Marigómez *et al.*, 2002; Usheva *et al.*, 2006)**. BPA and its metabolites were detected at higher concentrations in the lumen of the digestive gland tubules of the bivalve, *Corbicula japonica* causing histological damages to this organ **(Hayashi *et al.*, 2008)**. Moreover, **Kanapala and Arasada (2013)** indicated that the high sensitivity of the digestive gland is directly attributed to its role in homeostasis, contaminant uptake, digestion, metabolism as well as detoxification process **(Hayashi *et al.*, 2008; Kanapala and Arasada, 2013)**. The primary adaptive response of tissues exposed to toxic chemicals such as BPA is hypertrophy. Cells that died as a result of BPA exposure were compensated for by increasing in size and producing new ones (**Goss, 1966).** When exposed to a toxic chemical, necrosis causes a proinflammatory response in nearby cells as mentioned by **Zong and Thompson (2006)**.

The intestinal epithelia of the bivalve, *C. nilotica* showed a hyperplastic response in the intestine of the clam after exposure to BPA for 4 weeks **(Sheir *et al.*, 2020)**. They explained the appearance of hyperplastic tissue due to defense mechanism of the animal against pollution. Also, **Faheem *et al.* (2016)** found that hyperplasia was a sign of exposure to BPA, in the Indian major carp, *Catla catla* gill mucous cells. According to **Ibrahim and sayed (2019)**, the sub-lethal concentrations of oxyfluorfen 24% EC induced histological damage in the digestive gland of subjected *B. alexandrina* snails. Based on their studies, herbicides are often directed particularly to the digestive cells that found ruptured and degenerated, also the secretory cells increased in number and lumens inside the tubules dilated, and the connective tissue between digestive tubules shrank in size. Moreover, exposure of the freshwater bivalve, *Corbicula fluminea* to BPA showed pathological signs in the adductor muscles as the occurrence of necrosis, loss of surface adherence and loss of muscle organization. **(Benjamin *et al.*, 2019)**. Our results agreed with **Abdel-Ghaffar *et al.* (2016)** who mentioned that butralin 48% EC, glyphosate isopropylammonium 48% SL, and pendimethalin 50% EC herbicides induced histopathological signs in the digestive glands of *B. alexandrina* snails. The most prominent severe damage was a great loss in the shape of digestive cells (syncytium), ruptured and/or degenerated and the secretory cells became denser in colour and increased in number. According to **Sharaf *et al.* (2015)**, exposing the land snail *Helicella vestalis* to sub-lethal concentrations ($LC_{25}$) of both methiocarb (4.4 ppm) and chlorpyrifos (0.005 ppm) pesticides caused many histological changes in the digestive gland, including severe tubular disruption, evacuation, pyknotic nuclei, and necrosis of digestive tubules.

Furthermore, the presence of xenobiotics has a significant impact on the membrane stability of lysosomal molluscan digestive cells **(Ringwood *et al.*, 1998)**.

The digestive gland of gastropod molluscs is known to be the key organ of metabolism, as well as the primary site of xenobiotic accumulation and biotransformation. A study revealed that the mode of action of Topik on plants might be similar in the snails. Topik is used to control weeds by inhibiting acetyl Co-enzyme A carboxylase (ACC ase) which is essential for the production of fatty acids needed for plant growth **Zimdahl (1999).** The digestive gland of *S. mansoni*-infected *B. alexandrina* snails exposed to a sublethal concentration of fungicides, Topas (0.3 ppm) resulted in complete deformation and degeneration of the gland architecture at 4 weeks **(Osman *et al.*, 2010)**. According to Vega et al. (1989), the decrease in digestive gland epithelium thickness with increasing lumen size could be due to apical cytoplasm losses during the detoxification process. Furthermore, there were severe histological changes in the digestive gland of the land snail, *Helix aspersa*, exposed to fungicide, such as a decrease in the height and area of digestive gland epithelium **(Snyman *et al.*, 2003)**.

## CONCLUSION

The exposure of BPA as a single stressor or combined with the infection of *S. mansoni* caused a disturbance in the biological systems of *B. alxeandrina* snails represented in some oxidative stress markers, sex hormones and neurotransmitters, in addition to pathological symptoms of the digestive gland tissue. This can lead to the interruption of metabolic efficiency of freshwater organisms and modification of animal survival and consequently disease transmission and epidemiology of schistosomiases.

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