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Toxicological impact of oxyfluorfen 24% herbicide on the reproductive system, antioxidant enzymes, and endocrine disruption of *Biomphalaria alexandrina* (Ehrenberg, 1831) snails

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Abstract
Oxyfluorfen (Goal 24%EC) herbicide is widely used in agriculture for weed control. *Biomphalaria alexandrina* snails can be used as bioindicator of the chemical pollution in the aquatic environment. The objective of this study was to evaluate the molluscicidal activity of this herbicide on *Biomphalaria alexandrina* snails and how it affected its biological system. The present study revealed a molluscicidal effect of oxyfluorfen 24%EC on these snails at LC_{50} 5.9 mg/l. After exposure of snails to the sub-lethal concentrations (LC_0, LC_{10}, or LC_{25}) of this herbicide, the survival rates, reproductive rate (R_0), and fecundity (M_x) of adult *B. alexandrina* snails were significantly decreased in comparison with the control group. Also, levels of testosterone and estradiol were decreased significantly. It caused alterations in the antioxidant system, where exposure to sub-lethal concentration of this herbicide caused significant increases in levels of lipid peroxide malondialdehyde (MDA), catalase (CAT), and superoxide dismutase (SOD), while it significantly decreased glutathione transferase (GST). Histopathological changes in the digestive gland included severe damage in the digestive cells, where, they lost their tips and some were degenerated, while the secretory cells increased in number. Regarding the hermaphrodite gland, there were losses of the connective tissues, irregular sperms, and the eggs degenerated. These findings concluded that *B. alexandrina* snails can be used as a bioindicator for pollution with pesticide in the aquatic environment.

Keywords Oxyfluorfen 24%EC · *Biomphalaria alexandrina* · Reproductive rate · Oxidative stress · Endocrine disruptor · Histology

Introduction

Pesticides are extensively used for agricultural purposes worldwide to reduce pests. These pesticides and their residues found their way to the aquatic environment through water drainage system and lead to toxicological effects on aquatic ecosystems (Moustafa et al. 2016).

Herbicides made up about 40% of the production of pesticides in the world and were intensively used in agriculture (Abdel-Ghaffar et al. 2016). In Egypt, these herbicides that enter the Nile River through runoff from agricultural fields result in the pollution of aquatic habitat, inducing adverse impacts on the living organisms with potential direct and indirect impacts on human health (Hassanein 2002). Globally, glyphosate is the most used herbicide where it is used for inhibition of unwanted weeds in agriculture and also used for aquatic weed control in fish ponds (Tsui and Chu 2008). The 24-h LC50 of Roundup (a glyphosate herbicide) for young Nile *Oreochromis niloticus* was 17.5 g/l and that for adult tilapia was 46.9 mg/l (Vera-Candioti et al. 2012). Also, the median lethal concentration (LC50) of this herbicide for mosquito fish (*Gambusia affinis*) was 17.82 mg/l for 72 h (Talib et al. 2018).

Oxyfluorfen (Goal 24%EC) is a diphenyl-ether herbicide and ranks as a light-dependent peroxidizing herbicide

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(LDPH) used for controlling of monocotyledonous and broadleaf weeds in fields with grapes, onion, or cotton through interference with chlorophyll production, inhibition of photosystem II, and electron transport disrupting photosynthesis (Banhawy et al. 1996). This herbicide was widely used in Egypt and its concentration reached 23.6 mg/l in some branches of the River Nile, and that concentration could adversely affect the nervous system of *Gambusia affinis* and *Oreochromis niloticus* fishes and caused reductions in the brain acetylcholinesterase activity after 6, 15, and 30 days of exposure (Hassanein 2002). Also, other studies on freshwater invertebrates as *Elliptio complanata* clam (Godfrey and Longacre 1990), Eastern oyster, *Crassostrea virginica*, *Palaemonetes pugio*, and *Daphnia magna* investigated the toxic effect of oxyfluorfen herbicide on their nervous, immune, and endocrine systems after exposure either to short- or long-term treatment that led to hyperexcitability and loss of equilibrium (EPA/OPP 2001).

Invertebrates included over 90% of the aquatic species and they were used as biomonitors of environmental pollution (Ibrahim et al. 2018). *B. alexandrina* snails are a remarkable species that serve as sensitive bioindicators for aquatic ecosystem pollution (Fahmy et al. 2014). It is the snail vector for *Schistosoma mansoni* in Egypt, distributed throughout the Nile River and in the irrigation canals that are highly polluted. Sharaf-El-Din et al. (2010) proposed that *B. alexandrina* snails as biomonitors for metal pollution and the snail oviposition have shown to be sensitive to pollution in the surrounding environment. So such snails were used in this study as bioindicators of aquatic pollution because of their abundance, wide geographical distribution, and short lifespan.

Antioxidant enzymes were used as a biomarker for metal and inorganic compound pollution in marine and freshwater organisms. The oxidative stress is the major mechanism through which several pesticides induce their effects producing pro-oxidants in cells (Tellez-Bañuelos et al. 2009). Such stress may be accompanied by developmental alterations, including reproductive effects and teratogenicity in aquatic organisms (Pašková et al. 2011). The extent of alterations depends on the cell's antioxidant network represented by the antioxidant enzymes and the non-enzymatic antioxidant molecules (Tilton et al. 2008).

Testosterone hormone is required in the gonadal development in *B. alexandrina* and might be used as a biomarker for insecticide toxicity, so *B. alexandrina* snails could be used as a bioindicator for endocrine disruption in terms of steroid levels (testosterone and 17β-estradiol), after exposure to sub-lethal concentrations of herbicides (Omran and Salama 2016).

The aim of the present study is to assess the toxic effect of oxyfluorfen 24%EC herbicide on the biological functions of freshwater *B. alexandrina* snails through studying its deleterious effects on the reproductive, oxidative enzymes, endocrine systems, and histopathology.

Materials and methods

Snails

Mature *B. alexandrina* (Ehrenberg, 1831) snails (8–10 mm) were maintained in Medical Malacology Laboratory, Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Snails were kept in plastic aquaria ($16 \times 23 \times 9$ cm). The aquaria were provided with dechlorinated aerated tap water (10 snails/l), pH 7 ± 0.2 and temperature (25 ± 2 °C) and covered with glass plates and the photoperiod was controlled (12 h/12 h) with two 48 W fluorescent lamps as the light source. Water hardness was optimized with 30 mg/l calcium carbonate and CaCO3 (small pieces of chalk) for the greatest snail fecundity, growth, and shell length (Eveland and Haseeb 2011). Water in the aquaria was changed weekly. Snails were fed with oven-dried lettuce leaves, blue-green algae (*Nostoc muscorum*), and Tetramin (Fish food). Small pieces of 3×5 cellophane sheets that float in aquaria and snails deposit egg masses on them under surface; the sheets are easily transferred to other aquaria.

Herbicide

Goal 24%EC (CAS: 42874-03-3) is a commercial herbicide and has the active constituents called oxyfluorfen 24%EC. Oxyfluorfen is a nitrodiphenyl ether herbicide [2-chloro-1-(3-ethoxy-4- nitrophenoxy)-4-(trifluoromethyl) benzene] and has a similar structure to that of nitrofen and acifluorfen (Keum et al. 2008). It is readily soluble in most organic solvents but emulsifies into water and has a high tendency to bind to soil containing organic matter. It is stable up to 50 °C and it degrades primarily via photolysis (EPA/OPP 2001). Its registration number is 190 and was purchased from Dow Agro Sciences-France.

Investigation of molluscicidal activity of oxyfluorfen 24%EC

A fresh stock solution of 1000 ppm was prepared from oxyfluorfen 24%EC on the basis of *V/V* using dechlorinated tap water. Series of concentrations (10, 8, 6, 4, 3, 2, 1 mg/l) were prepared to calculate LC_{50} and LC_{90} (WHO 1983). One hundred fifty snails were exposed to 24 h of the herbicide followed by another 24 h for recovery and then, mortality rate and lethal doses were recorded for each group. Another snail group of the same size was dipped in dechlorinated water only as control (30 snails). Three replicates were used each of 10 snails for each concentration and the control group. The percentages of observed mortalities were recorded and analyzed (Litchfield Jr and Wilcoxon 1949). LC_0 is the concentration of any toxicant, below which no measurable effects take place

(Warren [1900]), and is estimated as $1/10$ LC_{50} value (WHO [1965]).

Experimental design

One hundred *B. alexandrina* snails (8–10 mm) were used in this experiment, where 10 snails in each aquarium exposed to each sub-lethal concentration of oxyfluorfen 24%EC at LC_0 (0.016 mg/l), LC_{10} (3.14 mg/l), or LC_{25} (4.48 mg/l) for 24 h (exposure), followed by another 24 h of recovery, and this method is done for 2 weeks and followed by 2 weeks of recovery. Thirty unexposed snails (control) were assayed side by side with the treated groups, and then the following bioassays were done:

Effect on snails' egg-laying capacity of adult snails

Both the survival and reproductive rates (L_x and R_0) of *B. alexandrina* snails (8–10 mm) that were subjected to each concentration (LC_0 (0.016 mg/l), LC_{10} (3.14 ppm), or LC_{25} (4.48 mg/l)) of the herbicide and control group were weekly recorded. The egg laying capacity is expressed in the form (M_x) which is the number of eggs/snail/week, and this is determined by dividing the total number of laid eggs in any week by the total number of living snails at the beginning of this week (El-Gindy et al. [1965]). To calculate the net reproductive rate (R_0) of the snails throughout the experimental period, the following parameters will be considered:

- Time of exposure in weeks (x).
- Survivorship (L_x) which is the survived snails at any given week as a fraction of the correct one (1.0 = 100%).
- Fecundity (M_x) the mean number of eggs/snail/week.
- The net reproductive rate (R_0) at any given period was represented by the term $\Sigma L_x M_x$.

$$R_0 = \Sigma L_x M_x$$

Tissue preparation

From each treated group and the control one, snails were crushed between two slides, and their soft tissues were withdrawn from the shell using a forceps, weighed, and then homogenized in ice cold, twice-distilled water. One-gram tissue of each group/10 ml water was homogenized using a glass Dounce homogenizer. The homogenates were centrifuged at 3000 rpm for 10 min and the supernatants were stored at − 80 °C until used.

Investigation of the antioxidant system responses: the enzymatic responses (superoxide dismutase, catalase), non-enzymatic responses (glutathione), and the oxidative stress marker (malondialdehyde)

These responses were detected in the supernatant of the tissue homogenate for each group. Biodiagnostic kits (Biodiagnostic Dokki, Giza, Egypt) were used for the determination of superoxide dismutase (SOD) and catalase (CAT) (Aebi [1984]). In addition, tissue malondialdehyde (lipid peroxide) was done according to Ohkawa et al. ([1979]), and reduced glutathione (GSH) was done according to the method of (Beutler [1963]).

Hemolymph preparation

The hemolymph of some surviving snails from each group at second week of exposure was collected by removing a small portion of the shell and inserting a capillary tube into the heart to collect the hemolymph (Nduku and Harrison [1980]). Then, hemolymph was pooled in a glass vial tube (1.5 ml) and stored at − 80 °C until used for the hormonal assay (17β-estradiol and testosterone).

Investigation of testosterone and 17β-estradiol hormones in hemolymph

Hormone concentrations (T and E) were assayed in hemolymph for all groups according to the manufacturer instructions of T EIA kit (Enzo Life Science, MI, USA, ADI-900-065) and E EIA kit (Cayman Chemical Company, MI, USA, item no. 582251).

Histological studies

The digestive and hermaphrodite glands of the surviving *B. alexandrina* snails after exposure to each sub-lethal concentrations for 24 h followed by another 24 h of recovery for 2 weeks (exposure) were removed from their shells, fixed in Bouin's fluid, embedded in paraffin wax, and sectioned and stained with hematoxylin and eosin (Mohamed and Saad [1990]).

Statistical analysis

Statistical Processor System Support "SPSS" version 20 for Windows software was used for the statistics. Probit analysis was used to define the lethal concentration values (Finney [1971]). Statistical analysis was evaluated by one-way analysis of variance (ANOVA) and Duncan's test to assess the significance of differences among control and treated groups. Values were expressed as mean ± S.E.

Results

The molluscicidal activity of the tested herbicide Goal 24%EC (oxyfluorfen 24%EC) against adult *B. alexandrina* snails after 24 h of exposure followed by another 24 h for recovery is represented in (Table 1 and Fig. 1). These results show that Goal 24%EC was toxic to *B. alexandrina* snails at (LC_{50} 5.96 mg/l; LC_{90} 8.78 mg/l).

The present results (Table 2) indicated that the survival rate of snails exposed to LC_0 (0.016 mg/l) for 24 h/ week for 2 weeks followed by another 2 weeks of recovery was slightly affected, being 0.65 at the 4th week of exposure. Also, exposure of snails to LC_{10} (3.14 mg/l) considerably reduced their survival rate (L_x) to be 0.25 after the 2 weeks of recovery, i.e., at the 4th week of the experiment compared to 0.75 for the control group. Raising the concentration to LC_{25} (4.48 mg/l) caused a quick and severe death among treated snails throughout the experiment weeks.

The present data (Table 2) indicated that snails exposed for 24 h/week to LC_0 (0.016 mg/l) of Goal 24%EC negatively affected their fecundity (M_x). Thus, the snails survived at this concentration laid few eggs/snail/week through the weeks of the experiment in comparison with that of control ones. Raising the concentration to LC_{10} (3.14 mg/l) highly suppressed the snail's fecundity at the 2nd and 3rd weeks, compared with control snail. Moreover, at LC_{25}, the survived snails stopped laying egg through the 3rd week until they died at the 4th week.

In vivo exposure of *B. alexandrina* snails to sub-lethal concentrations of oxyfluorfen herbicide (LC_0, LC_{10}, or LC_{25}) exhibited a significant increase ($p < 0.05$) in malondialdehyde (MDA) and this increase was concentration-dependent. Also, there were significant increases ($P < 0.05$) in CAT and SOD activities compared with the control group. The highest increase in CAT activity was observed in the snails exposed to LC_{10} of oxyfluorfen. Referring to SOD activity, there was a significant increase in snails exposed to LC_{10} and LC_{25} but a non-significant increase following the exposure to LC_0. On the other hand, tissue GSH content was significantly ($p < 0.05$) decreased after oxyfluorfen exposure in a concentration-dependent manner (Fig. 2).

Testosterone (T) and 17β-estradiol (E) were determined in the ovotestes of oxyfluorfen-exposed snails after 2 weeks of exposure. Levels of T and E were decreased significantly ($p < 0.05$) after exposure to sub-lethal concentrations compared with control (Fig. 3).

Table 1 Molluscicidal activity of oxyfluorfen 24%EC against *B. alexandrina* snails

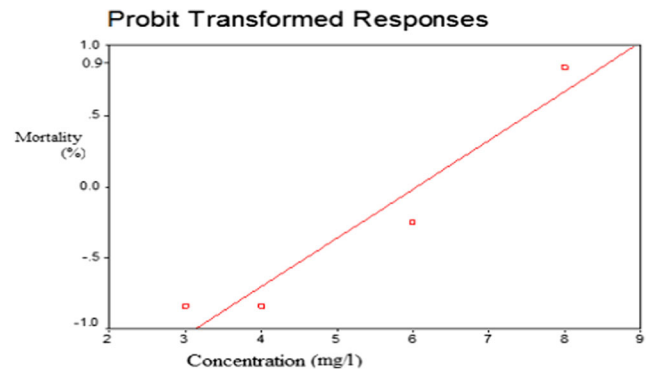| Concentration (mg/l)	LC_0	LC_{10}	LC_{25}	LC_{50}	LC_{90}	Slope
Oxyfluorfen 24%EC	0.016	3.14	4.48	5.96	8.78	1.5

Fig. 1 Molluscicidal activity of the tested herbicides against adult *B. alexandrina* snails

Regarding its histopathological changes in adult, treated snails, the normal digestive gland occupies a considerable part of the visceral hump and consists of a number of tubular glands; each tubule is lined with one layer of two cells; the digestive cells and the secretory cells (Fig. 4a). The present results showed that a moderately pathological damage in the digestive gland after exposure to LC_0 of the herbicide (Fig. 4b), while at LC_{10}, the connective tissue was broken down so the intertubular space becomes distinctive around the digestive tubules and the lumen inside tubule increased (Fig. 4c). The most destructive effect in the digestive tissue was shown with snails subjected to LC_{25} (Fig. 4d), where there is a great loss of identical shape of digestive cells (syncytium).

The hermaphrodite gland of the normal *B. alexandrina* snails is composed of a number of cub-shaped acini connected by connective tissue. The male reproductive cells are differentiated into primary and secondary spermatocytes. The fully developed sperms were observed either in the lumen or attached to Sertoli cell. While the female oogenic cells filled the acinar lumen as primary, secondary oocytes and mature ova (Fig. 5e). The treatment of *B. alexandrina* snails with oxyfluorfen 24%EC at the sub-lethal concentrations for 2 weeks has marked morphological changes to gland cells in addition to several evacuations of its tubules from gametogenic stages, but still presents a mature ovum and little sperms in snails treated with LC_0 (Fig. 5f), while in snails treated with LC_{10}, there are increases in degenerated sperms and ova (Fig. 5g). The great damage to gonadal cells occurs by subjecting snails to LC_{25} (Fig. 5h), where ova lost their shape and degenerated and sperms are reduced and degenerated. Most of the spermatogenic and oogenic stages disappeared.

Discussion

Pesticides are a double-edged sword as they have benefits for agriculture, but they can harm aquatic life and wildlife if used incorrectly. Pesticides and their residues can be easily

Table 2 Survival rate (L_x) and fecundity (M_x) of *Biomphalaria alexandrina* snails exposed for 24 h weekly to sub-lethal concentrations of oxyfluorfen 24%EC for 2 successive weeks followed by 2 weeks of recovery. L_x (survivorship): the survived snails at any given week as a fraction of the correct one (1.0 = 100%); M_x (fecundity): the mean number of eggs/snail/week, and R_0: the net reproductive rate equals $\Sigma L_x M_x$

Weeks	Control			LC$_0$ (0.016 mg/l)			LC$_{10}$ (3.14 mg/l)			LC$_{25}$ (4.48 mg/l)		
	L_x	M_x	$L_x M_x$	L_x	M_x	$L_x M_x$	L_x	M_x	$L_x M_x$	L_x	M_x	$L_x M_x$
1	1.0	1.3	1.3	1.00	0.80	0.80	0.90	0.75	0.68	0.50	1.00	0.50
2	0.9	2.5	2.25	0.90	0.10	0.10	0.80	0.10	0.10	0.25	0.77	0.19
3	0.85	4.7	3.9	0.80	0.77	0.62	0.35	0.80	0.28	0.05	0	0
4	0.75	9.5	7.1	0.65	0.15	0.10	0.25	0.70	0.17	0		
$R_0 =$	14.5			1.6			1.2			0.69		
Reduction %				− 88.9			− 91.8			− 95.3		

transferred into aquatic environments through the surface run-off and may alter the development and reproduction in various aquatic invertebrates (Pašková et al. 2011). These pollutants are known to modulate antioxidant defensive systems and to cause oxidative damage in aquatic organisms by the production of reactive oxygen species (ROS) (Fahmy and Sayed 2017).

To be a molluscicidal material, the median lethal concentration (LC$_{50}$) should not be more than 100 ppm (WHO 1993). The present investigation indicated that oxyfluorfen 24%EC has LC$_{50}$ 5.9 mg/l on *B. alexandrina* snails. This toxic effect of oxyfluorfen 24%EC herbicide was also investigated after exposure of *Crassostrea virginica*, *Palaemonetes pugio*, and *Daphnia magna* to either short- or long-term treatment, where

it caused neurotoxicity and an increase in acetyl cholinesterase concentration (EPA/OPP 2001) and has toxic effects on nervous, immune, and endocrine systems of freshwater invertebrates as *Elliptio complanata* clam (Godfrey and Longacre 1990).

The present results showed that survival rates of adult *B. alexandrina* snails were markedly reduced post their exposure to sub-lethal concentrations (LC$_0$, LC$_{10}$, or LC$_{25}$) of oxyfluorfen 24%EC herbicide. Comparable perceptions were recorded by Abdel-Ghaffar et al. (2016), who reported that survival rates of *B. alexandrina* snails were reduced after their exposure to the sub-lethal concentrations (LC$_0$, LC$_{10}$, or LC$_{25}$) butralin 48% EC, glyphosate isopropylammonium 48% SL, and pendimethalin 50% EC herbicides. They linked
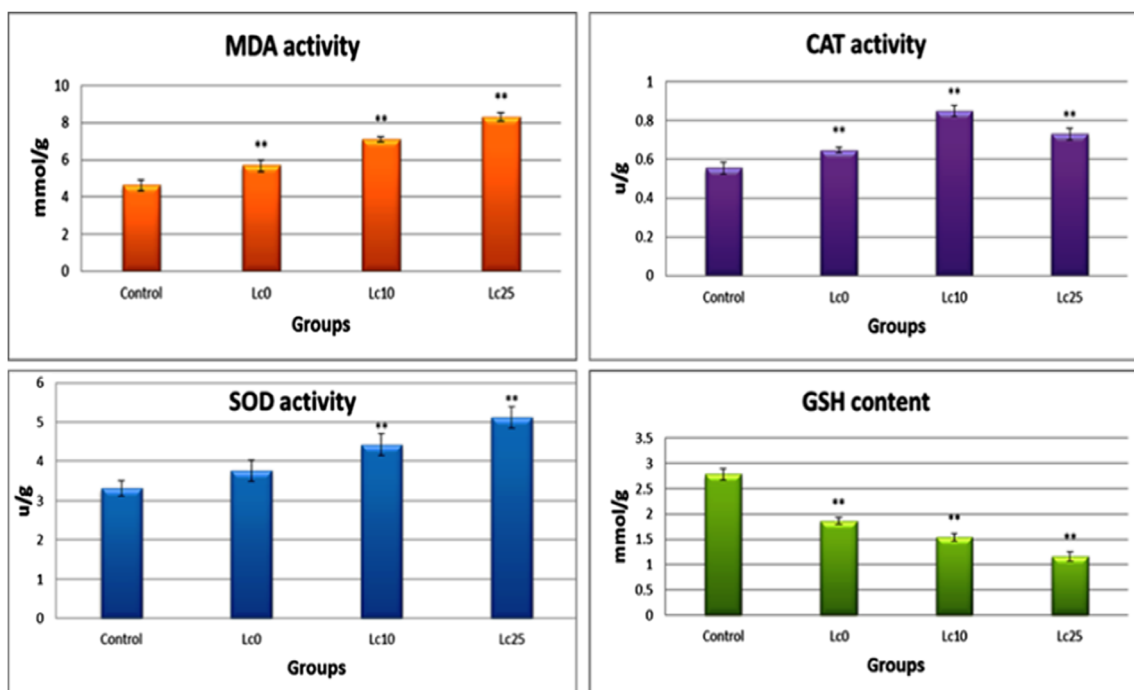
Fig. 2 Antioxidant activity changes (lipid peroxides malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and glutathione transferase (GST) in tissue of *B. alexandrina* after exposure to sub-lethal concentrations of oxyfluorfen herbicide (LC$_0$, LC$_{10}$, or LC$_{25}$). Values are mean ± S.E, $n = 5$ in each group, *$p < 0.05$: significant, **$p < 0.01$: highly significant as compared with control
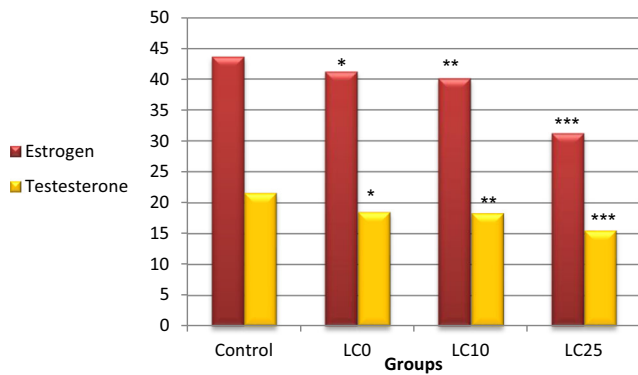
Fig. 3 Hormonal level changes (T and E) in hemolymph of *B. alexandrina* after exposure to sub-lethal concentrations of oxyfluorfen herbicide (LC$_0$, LC$_{10}$, or LC$_{25}$). Values are mean ± S.E

this reduction by histological examinations, which showed severe damage in the digestive and hermaphrodite gland cells of the treated snails. The same results obtained by Barky et al. (2012), who showed that LC$_{10}$ of two pesticides Atrazine (0.33 ppm) and Roundup (0.84 ppm) caused a considerable reduction in the survival rates and egg production of *B. alexandrina* snails. They reasoned that reduction due to snails could overcome the destructive effect of tested pesticides through discharging it to the surrounding media or by biodegrading it to non-poisonous by-products.

The reproductive rate (R$_0$) and fecundity (M$_x$) of adult *B. alexandrina* snails in the present study were significantly decreased by their exposure to the sub-lethal concentrations

(LC$_0$, LC$_{10}$, or LC$_{25}$) of oxyfluorfen 24%EC, in comparison with the control group. These low values of R$_0$ and M$_x$ of treated snails could be attributed partially to their high mortality rates and due to severe damage in the digestive and hermaphrodite gland cells of treated snails (Hasheesh and Mohamed 2011).

In the present study, the oxidative stress induced by oxyfluorfen toxicity was indicated by the significantly increased levels of tissue lipid peroxides (MDA), which is the major reactive aldehyde formed by the peroxidation of membrane lipids and one of the most damaging effects of ROS (Ohkawa et al. 1979). Lipid peroxidation was increased in all treated snails especially those exposed to LC$_{25}$ that explain a concentration-dependent effect and is an indication of the oxidative stress. The present results are in agreement with observations made by Atli and Grosell (2016) who studied the potential effects of acute copper (Cu) toxicity (2–90 g/ml for 48 h) on the antioxidant responses in three tissues—the hepatopancreas, the foot muscle, and the mantle of *Lymnaea stagnalis* snails—and they stated that all antioxidant enzymes (SOD, CAT, and glutathione peroxidase, GPx) except glutathione reductase (GR) increased after exposure to the highest Cu concentration in mantle and concluded that they could be suitable biomarkers of the metal toxicity in the aquatic environment.

The present study showed that the enzymatic defenses include catalase CAT and SOD was increased in response to the overproduction of reactive oxygen species inside the tissue.
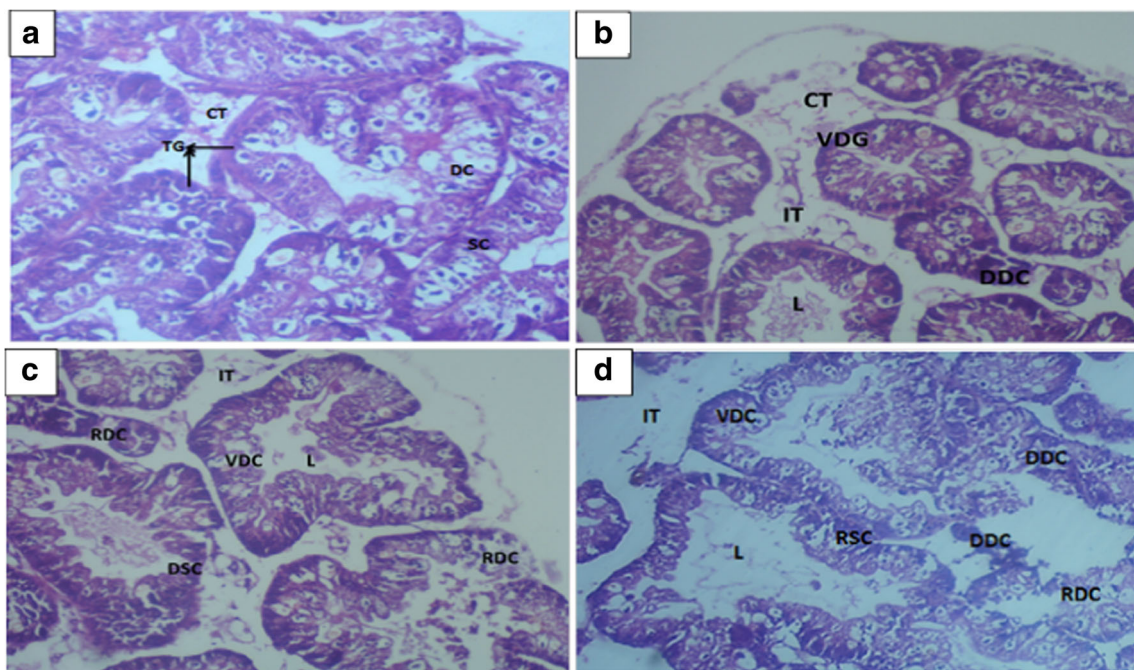

Fig. 4 Light micrograph showing sections in digestive gland of snails exposed to sub-lethal concentration of oxyfluorfen 24%EC for 2 weeks (H&E) (× 40). **a** Normal digestive gland of snails. **b** Snails subjected to LC$_0$ of tested herbicide. **c** Snails subjected to LC$_{10}$. **d** Snails subjected to LC$_{25}$ of the herbicide. DDG degenerated digestive cells, IT inter tubular space, DSC degenerated secretory cells, TG tubular gland, L lumen, SC secretory cells, VDC vacuolated digestive cell, RDC ruptured digestive cells
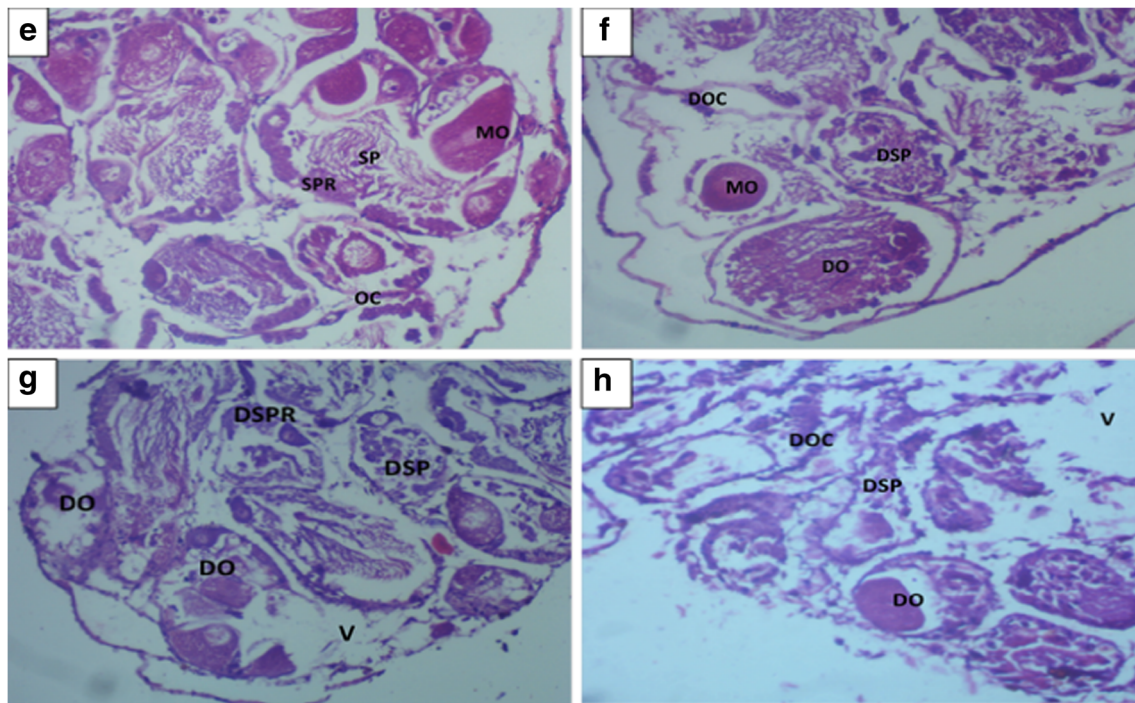
Fig. 5 Light micrograph showing sections in hermaphrodite glands of snails exposed to sub-lethal concentration of oxyfluorfen 24%EC for 2 weeks (H&E) (× 40): **e** Normal hermaphrodite gland of snails. **f** Snails subjected to LC$_0$ of tested herbicide. **g** Snails subjected to LC$_{10}$.

h Snails subjected to LC$_{25}$ of tested herbicide. MO mature ovum, SP sperms, SPR spermatocytes, OC oocyte, DO degenerated ovum, DSP degenerated sperms, DSPR degenerated spermatocyte, V vacuole

These results were consistent with (Peixoto et al. 2006), who found higher CAT activity in tilapia *Oreochromis niloticus* exposed to 0.3 and 0.6 mg/l oxyfluorfen for 7 and 14 days. In addition, catalase liver activity also showed an increase in the Neotropical *Prochilodus lineatus* fish exposed to 10 mg/l of Roundup, a glyphosate-based herbicide (Langiano do and Martinez 2008), suggesting the activation of antioxidant defenses after Roundup exposure.

GSH is a non-enzymatic antioxidant acting as a substrate for the antioxidant enzyme, glutathione transferase (GST), and an important free radical quencher. Snail exposure to different concentrations of oxyfluorfen 24%EC herbicide leads to a significant decrease in tissue GSH content which explains the high rates of free radical input. These results were in agreement with work done by Bagul et al. (2016) who showed potential toxicity of oxyfluorfen (23.5% EC) on the earthworm, *Eisenia fetida* at the concentration of 0.31 mg/kg. The increase in MDA activity accompanied by the decrease in GSH content may clarify the tissue injury occurred after the exposure to all concentrations of oxyfluorfen 24%EC herbicide. Moustafa et al. (2016) confirmed that Roundup, Stomp herbicides either individually or in combination at concentration 1/2 96 h lethal concentration 50 (LC$_{50}$) elucidated significant elevation in the levels of SOD, CAT, and GPx in Nile catfish (*Clarias gariepinus*). Meanwhile, the data depicted the reduction in levels of reduced GSH and GST. So, the induction of the SOD/CAT system provides a first-line defense

against ROS especially after the increase of lipid peroxide MDA and the diminishing of reduced glutathione GSH.

The present results determined testosterone and 17β-estradiol (T and E) levels in the gonads of oxyfluorfen-exposed snails after 2 weeks of exposure. Levels of T and E were decreased significantly after exposure to sub-lethal concentrations of oxyfluorfen 24%EC ($p < 0.05$) compared with control indicating the disrupter effect of oxyfluorfen 24%EC herbicide. This is in accordance with the findings of (Omran and Salama 2016) who found that the level of T was decreased significantly after exposure to LC$_{10}$ sub-lethal concentrations of glyphosate (4.2 ppm) and atrazine (10.1 ppm) herbicides and the level of E was decreased by nearly 50% in both glyphosate and atrazine exposed *B. alexandrina* snails compared with control. Also, these results come in accordance with Clair et al. (2012) who stated that atrazine and glyphosate acts as an endocrine disrupter, where they were toxic to Sertoli cells in mature rat fresh testicular cells from 1 to 10,000 ppm and induced apoptosis at higher doses in germ cells and in Sertoli/germ cells co-cultures.

The sub-lethal concentrations of oxyfluorfen 24%EC induced histopathological changes in the digestive gland of subjected snails and these damaged increased with increasing the concentrations. The most severe damage in the digestive cells was the presence of a great loss of identical shape of digestive cells. Some digestive cells were ruptured and most of these cells degenerated, also the secretory cells increased in number

and lumens inside tubule increased, and the connective tissue between digestive tubules shrank. These results agreed with Abdel-Ghaffar et al. (2016), who stated that butralin 48% EC, glyphosate isopropylammonium 48% SL, and pendimethalin 50% EC herbicides induced histopathological changes in the digestive glands, where the most prominent severe damage was a great loss in shape of digestive cells (syncytium) and some were ruptured or degenerated and secretory cells became denser in color and increased in number. Also, these findings were in accordance with Sharaf et al. (2015), who found that the exposure 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 of land snail *Helicella vestalis* and these alterations included severe tubular disruption, evacuation, pyknotic nuclei, and necrosis of digestive tubules.

Also, the histological examination of the treated hermaphrodite gland showed losses of connective tissues, the presence of a large number of degenerated eggs and sperms after exposure. These results were in accordance with Osman et al. (2008) who found that exposure of *B. alexandrina* snails to sub-lethal concentrations (10 ppm) of Roundup or Topik herbicides showed complete destruction of gametogenic cells. Also, Langiano do and Martinez (2008) reported that short-term exposure to sub-lethal concentration 10 mg/l of Roundup induced several histological alterations in Neotropical fish, *Prochilodus lineatus*.

Conclusion

Oxyfluorfen 24%EC, a potent molluscicidal agent, has been shown to cause alterations in the antioxidant system causing oxidative stress, reduce survival rate, and inhibit the reproduction in the freshwater snail *B. alexandrina*, in addition to endocrine disruptor effect accompanied with histopathological alterations in gonads. Further investigations are urgently needed to assess the current environmental burden of herbicides in riverine ecosystems to determine whether there is an extent need to monitor the use of herbicides in the Nile River Basin aiming to reduce water pollution and studying its harmful effects on non-target organisms.

Compliance with ethical standards

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