Salicylic acid against the disruptive effects of cadmium induces oxidative stress and biochemical and histopathological changes in the ovary of the freshwater leech Limnatis nilotica (Savigny, 1822)

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ABSTRACT

In this study, we investigated the effect of Cd on the ovaries of the freshwater leech Limnatis nilotica and the ability of salicylic acid (SA) treatment to alleviate the Cd harmful effects. Our results showed that Cd treatment increased the level of oxidative stress leading to severe alterations in histopathological and biochemical indexes. Cd exposure was found to induce a substantial increase in malondialdehyde (MDA) and hydrogen peroxidase (H2O2) contents as well as an impairment in catalase (CAT) and Glutathione peroxidase (GPx) activities. These findings are thoroughly supported by histopathological evidence which showed several modifications in the ovary cord organization. However, SA addition reduced the toxic effect of Cd-exposure through decreasing MDA and H2O2 levels, the regulation of enzymatic and non-enzymatic antioxidant mechanism pathways leading to the alleviation of the histopathological lesions. These results suggest that SA can prevent damage under Cd toxicity in the leech ovaries.

1. Introduction

Over the past years, heavy metal pollution in the aquatic environment has gained increasing issues [1], due to the increasing anthropogenic activities including industrial chemicals, fertilizers, heavy metals, and pesticides [2]. Heavy metals are considered as one of the most harmful pollutants given their persistent and bioaccumulative properties [3]. Among them, Cadmium (Cd) is a non-essential heavy metal of concern due to its considerable biological half-life (20–30 years) and non-biodegradable nature [4]. It is mainly derived from natural sources such as volcanic eruption, forest fire, and anthropogenic sources since it is known as a by-product of refining, mining, and smelting of lead, zinc, and copper [5]. Given its specific hydrochemical properties, Cd is characterized by its high mobility in groundwater which promotes the contamination level in the aquatic ecosystem and causes a serious threat to the growth and the survival of aquatic species [6]. According to World Health Organization (WHO), recommended level of Cd in groundwater is 0.003 mg/l. In drinking water, Cd concentration should not exceed 0.001 mg/l [7,8]. Cd is a biotoxic heavy metal with a destructive impact on physiologic and biochemical functions. Particular interest is being devoted to its effects on reproductive function. Importantly, reproductive organs are sensitive targets to Cd accumulation and thus, it is mainly associated with several disruptions of reproductive functions both in males and females [9,10]. In fact, Cd is reported to induce upset in the histological structure of the ovary, a decrease in the volume of growing follicles, an augmentation of atretic follicles percentage, and a decrease in the number of follicles at different stages of development [11,12]. Furthermore, Cd is known as an endocrine disruptor resulting in the perturbation of the endocrine ovary hormone secretion and steroid biosynthesis process [13].

Organisms are endowed with a complex antioxidant defense system including enzymatic and non-enzymatic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, ascorbic acid, and thiols. These antioxidant molecules play a pivotal role to contend against oxidative stress and they act collectively to scavenger free radicals induced by environmental stressors [14]. Interestingly, it has been reported that Cd exerts its harmful effect through the impairment of the antioxidant defense system and evidently increases the generation of reactive oxygen species (ROS) including hydrogen peroxide and
hydroxyl radicals [6,15]. Herein, in recent years, natural dietary substances have received much interest in preventing pollutants toxicity and enhancing antioxidant defense systems. Salicylic acid (SA), is a phenolic compound derived from dietary plants that plays a relevant role in the occurrence of local and systemic defense responses against stressors attack [16,17]. SA arises from various ranges of vegetables, fruits, and spices [18]. It has been established that SA has an antioxidant property and ameliorates environmental attack resistance, both in animals and plants since it is known as a powerful scavenger of free radicals [19].

Leeches constitute an appropriate bioindicator for water and sediment monitoring and they are considered as a suitable tool for physiological and biological process assessment [20]. Thus, the present study aimed to examine the adverse influence of Cd on the 1) ovarian histopathological structure and the antioxidant balance in an aquatic invertebrate leech model L. nilotica, and 2) the beneficial effect of SA addition to reduce Cd-toxicity of the leech ovary. This study is novel in exhibiting the role of SA treatment in enhancing the antioxidant defense system and repairing the ovaries’ histopathological damage in invertebrates.

2. Materials and methods

2.1. Sample collection

Specimens of L. nilotica having a length ranging between 80 and 110 mm were collected during the breeding season (May to September 2021), from Tamerza and Chebika waterfalls, situated in the southwest of Tunisia (Figure 1). Leeches were sexually mature and some of the specimens have a well-defined clitellum. Leeches were maintained in laboratory conditions in aquaria containing aerated and pure water for 15 days before experiments for the physiological adjustment to new environmental changes. The leeches were fed weekly with calves’ blood using nitrile rubber gloves.

2.2. Experimental design

Healthy and active leeches were chosen and used as animal models to perform the bioassay. Leeches were divided into five groups (n = 10). The first group served as a control, the second group contained leeches exposed to 50 µg/l of Cd, the third group was treated with 50 µg/l of Cd and received concomitantly 25 µg/l of SA, the fourth group was treated with 50 µg/l and 50 µg/l of SA and the last group were treated with 50 µg/l of Cd and 100 µg/l of SA.
2.3. Evaluation of oxidative stress biomarkers

Five specimens of *L. nilotica* from each group were dissected and ovaries were removed and homogenized in 2 ml ice-cold Tris-buffered saline and the homogenates were centrifuged at 5000 g for 15 min and 4°C. Obtained supernatants were maintained at 4°C for biochemical assays.

Lipid peroxidation was measured based on the reaction of MDA with the thiobarbituric acid (TBA) according to Buege and Aust (1978) [22]. Briefly, 125 µl of supernatant was mixed with 15 µl of 20% trichloroacetic containing 1% butyl-hydroxyl-toluene. After centrifugation at 1000 g for 10 min at 4°C, 200 µl of the sample was mixed with Hydrochloric acid (HCl) and 160 µl Tris-thiobarbituric acid and the samples were mixed at 8°C for 10 min. The absorbance was measured at 530 nm. MDA level was expressed in nmol of MDA/ mg of protein.

H$_2$O$_2$ level was measured based on the oxidation of ferrous to ferric ions. The absorbance was measured spectrophotometrically at 530 nm and H$_2$O$_2$ was expressed nmol g$^{-1}$ FW. Glutathione (GSH) level was evaluated following the method of Ellman [23] that was slightly modified by Jollow et al. [24]. Briefly, 0.5 ml of the tissue homogenate in phosphate buffer was mixed with 3 ml of sulfosalicylic. After there, centrifugation at 3000 g for 15 min was effectuated and 0.5 ml of the supernatant was added to Ellman’s reagent. The absorbance was evaluated after 10 min of reaction at 412 nm. The GSH level was expressed as µmol g$^{-1}$ FW.

Oxidized glutathione (GSSG) level was determined following Hissin and Hilf’s (1976) protocol [25]. 50 µl of the samples were added to 20 µl of NEM (0.04 M), the mixture was then kept for 30 min at room temperature. 1.68 ml of Na2HPO4 (0.3 M) and 250 µl of DTNB reagent were added to the homogenate. The absorbance was measured at 412 nm and the GSSG level was expressed as µmol g$^{-1}$ FW.

The total thiol (total sulphhydryl groups) level was evaluated as previously described by Sedlak and Lindsay (1968) [26]. Briefly, 50 µl from the samples were mixed with 0.6 ml of Tris EDTA buffer and 40 µl of 10 mM DTNB in methanol. Me OH was added to make the mixture up to 1 ml and the mixture was kept at room temperature for 20 min. The absorbance was measured at 412 nm and results were expressed as µmol g$^{-1}$ FW.

Ascorbic acid (AsA) was determined following the method of Mukherjee and Choudhuri (1983) [27]. Ovaries were removed and homogenized in liquid nitrogen and extracted in 6% (w/v) trichloroacetic acid (TCA), 2% (w/v) dinitrophenyl-hydrazine in 50% H$_2$SO$_4$ and 10% (v/v) thiourea in 70% ethanol. The homogenate was kept for 15 min in boiling water and after being cooled at room temperature, it was centrifugated at 1000 g for 10 min at 4°C. The resulting pellet was dissolved with 80% H$_2$SO$_4$. The absorbance was monitored at 530 nm and the AsA level was expressed as µmol g$^{-1}$ FW.

Superoxide Dismutase (SOD) level was measured as described previously by Beyer and Fridovich (1987) [28]. One unit of SOD was defined as the amount of enzyme that caused the inhibition of NBT by 50% at 25°C. SOD level was expressed as U mg$^{-1}$ protein.

CAT was assayed according to Aeberli (1984) [29] based on the dismutation of the H$_2$O$_2$ at 240 nm. CAT level was expressed as µmol H$_2$O$_2$ min$^{-1}$ mg$^{-1}$ protein.

GPx content was determined as previously described by Flohé and Gündler (1984) [30]. Based on the measure of the subsequent oxidation of NADPH at 340 nm. GPx level was expressed as nmol min$^{-1}$ mg protein.

2.4. Histopathological assessment

Five specimens from each group were used for histopathological assessment according to Bancroft and Steven [31]. Ovaries of tested specimens were removed and fixed in neutral buffered formalin (NBF) 4% for 24 h. After dehydration in a series of alcohols, the ovaries were incorporated in paraflin wax. Sections 6 µm thick were stained with eosin and hematoxylin and examined under Leica Dm500 light microscopy. To evaluate the morphology alterations of treated ovaries microphotographs were imported into ImageJ software, and then, degenerating oocytes were manually contoured and counted.

2.5. Statistical analysis

To determine the statistical significance of the differences in the mean values between the treated groups and the control group, the data were analyzed using GraphPad Prism version 9. The basic statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison. Differences were considered statistically significant at P < 0.05.

3. Results

3.1. SA treatment mitigated MDA and H$_2$O$_2$ levels

We assayed the oxidative stress in leech ovaries in terms of an increase in MDA and H$_2$O levels as a relevant biomarker resulting from membrane peroxidation. Compared to control, Cd-exposure induced
a noteworthy increase in MDA and H$_2$O$_2$ contents (p < 0.05) showing the generation of oxidative stress (Figure 2(a)). The increase in MDA level was about 59.13% when compared to the control group. Concomitantly, the H$_2$O$_2$ level exhibited a significant increase (p < 0.05) in Cd-exposed leeches as compared with controls by about 103.69% (Figure 2(b)). These effects were significantly reduced in Cd-exposed leeches by the addition of SA. Of note, MDA and H$_2$O$_2$ levels showed a significant decrease in all the groups treated with SA (p < 0.05). However, as compared to Cd-free leeches, MDA and H$_2$O$_2$ levels were still higher and the most prominent effect was observed in the leeches treated with 100 µg/l of SA and 50 µg/l of Cd in which MDA and H$_2$O$_2$ levels drastically decreased by about 25.08% and 37.67% respectively.

3.2. Effect of SA treatment on non-enzymatic antioxidant activities under Cd-stress

A substantial decrease in GSH level was shown in Cd-stressed leeches as compared to the control group (Table 1). Cd-exposure decreased GSH by about 56.7% when compared to the control group. In contrast, SA addition enhanced GSH level showing a significant increase notably in the group treated with 100 µg/l of SA and 50 µg/l of Cd for which GSH level increased by about 63.58% compared to the group of leeches treated with Cd alone. No significant differences were detected in the groups treated with 25 µg/l and 50 µg/l of SA when compared to the Cd-treated group (p > 0.05).

Conversely, GSSG exhibited a significant increase in ovaries of Cd-stressed leeches with respect to the control group (p < 0.05) by about 90.35% (Table 1). However, these data were reversed by SA treatment and GSSG showed a significant decline as compared to the group treated with Cd-alone (p < 0.05). This effect was particularly noted in the group treated with 100 µg/l of SA and 50 µg/l of Cd in which the GSSG level marked a decline by about 23.04%. No significant difference was noted between the group treated with Cd alone and after 25 µg/l SA-addition (p > 0.05).

Exposure to Cd dramatically decreased total thiols level (p < 0.05), that was 44.42% lower than in the control group (Table 1). SA addition counteracted the effect induced by Cd-stress and enhanced total thiols activities. A substantial increase was apparent in the groups treated with 50 µg/l and 100 µg/l of SA by about 31.9% and 49.90% respectively (p < 0.05). No significant change was noted in the group treated with 25 µg/l as compared to the group treated with Cd alone (p > 0.05).

In Cd-exposed leeches, the ascorbic acid level had an increased tendency with respect to the control group by about 66.99% (Table 1). Upon SA treatment, ascorbic acid level marked a significant decrease over

![Figure 2](image-url)

**Figure 2.** Effect of SA treatment on malondialdehyde (MDA) (a) and hydrogen peroxide (H$_2$O$_2$) (b) levels in the ovaries of Cd-stressed leeches. All data are expressed as the means ± SDs. **p < 0.005; ***p < 0.0005; ****p < 0.00001.

| Treatment | GSH (µmol g$^{-1}$ FW) | GSSG (µmol g$^{-1}$ FW) | Total thiols (µmol g$^{-1}$ FW) | AsA (µmol g$^{-1}$ FW) |
|-----------|-------------------------|--------------------------|---------------------------------|-------------------------|
| As (µg/l) | Cd (µg/l) | 42.50 ± 0.72a | 3.42 ± 0.45a | 180.30 ± 1.03a | 6.18 ± 0.80a |
| 0 | 0 | 18.40 ± 0.70b | 6.51 ± 0.47b | 100.20 ± 0.10b | 10.32 ± 0.10b |
| 25 | 50 | 19.20 ± 0.81b | 5.72 ± 0.41bc | 117.20 ± 0.11b | 9.02 ± 0.10c |
| 50 | 50 | 22.04 ± 0.84b | 5.15 ± 0.44d | 132.25 ± 0.14c | 9.34 ± 0.11c |
| 100 | 50 | 30.10 ± 0.92c | 5.01 ± 0.47e | 150.20 ± 0.12d | 8.05 ± 0.11d |

Table 1. Effect of SA treatment on Glutathione (GSH), Glutathione disulfide (GSSG), thiols, and ascorbic acid (AsA) contents in the ovaries of Cd-stressed leeches. Data are noted as means of n = 5 ± SE from three independent experiments. Means marked by a different letter (a–e) indicate significant differences between treatments p < 0.05.
Cd-stressed leeches. Of note, the most pertinent effect was observed in the group treated with 100 µg/l SA and 50 µg/l of Cd which marked a decrease by about 21.99% when compared to the Cd-stressed group (p < 0.05).

3.3. Effect of SA treatment on antioxidant enzymes

The activities of antioxidant enzymes are shown in Figure 3. It was revealed that Cd exposure afforded a perceptible increase in SOD content in ovaries of Cd-treated leeches (Figure 3(a)) by about 41.5% with respect to the control group (p < 0.05). Meanwhile, this effect was considerably counteracted by the addition of SA resulting in a noticeable decline in SOD level in all SA concentrations (25, 50, and 100 µg/l) by about 7.8%, 14.04%, and 23.4% respectively.

Under Cd-exposure, CAT level was reduced by about 38.95% (p < 0.05) as compared to unstressed leeches (Figure 3(b)). By contrast, in Cd-exposed leeches, supplemented SA influenced CAT content and we observed a marked increase of CAT level notably in 100 µg/l of SA by about 37.03% as compared to the group of leeches treated with Cd alone (p < 0.05).

Exposure to Cd drastically declined GPx level in leech ovaries (p < 0.05). This level is 57.04% less than the controls (Figure 3(c)). SA supplementation significantly enhanced GPx content which showed a perceptible rise particularly in 50 and 100 µg/l SA by about 27.11% and 38.28% respectively (p < 0.05). No significant effect was noticed in GPx content in leeches treated with 25 µg/l SA (p > 0.05).

3.4. SA treatment alleviated the histopathological damage in the ovary of Cd-induced leech

Under non-stressed conditions, light microscopy findings identified typical ovary cord architecture and developing oocyte morphology (Figure 4(a), (b), and (c)). Cd-induced leeches displayed various morphological alterations in their ovaries resulting in the vacuolation and the degenerescence of germ cells (oogonia, nurse cells, and previtellogenic oocytes), and the loss of the typical organization of the ovary cord due to the dissolved and the broken intercellular bridges between germ cells (Figure 4(d) and (e)). Moreover, an increase in the number of degenerating oocytes was detected (Figure 4(f)). Indeed, some freely floating oocytes showed compromised morphology that is generally characterized by an outline of the nuclear membrane that is irregular in some areas (Figure 4 inset b), and a less compact cytoplasm with large vacuolation that was noted especially in vitellogenic oocytes (Figure 4(f) and inset b).

Figure 3. Effect of SA treatment on (a) Superoxide- dismutase (SOD), (b) Catalase (CAT) and (c) Glutathione Peroxidase (GPx) activities in the ovaries of Cd-stressed leeches. All data are expressed as the means ± SDs. **P < 0.005; ***P < 0.0005.
Figure 4. Hematoxylin and eosin (H&E) staining of paraaffinized ovisac sections of Limnatis nilotica from the control group. (a): Histological section of spheroid ovisac showing normal structure as evidenced by the well-organized ovary cord and by the normal morphology of developing oocyte. Note the several portions of the long and convoluted central part of the ovary cord that are clearly visible inside the ovisac (asterix); numerous oocytes in advanced stages of oogenesis floating freely in ovisac lumen occur (arrows) (H&E x 100), (b) details of a portion from the long and convoluted central part of the ovary cord (cp) (H&E x 400). (c) Morphology of vitellogenic (v) (inset) and late vitellogenic oocyte (lv) (H&E x 400). (d), (e) and (f): Effect of a 50 µg/l Cd exposure on L. nilotica ovisacs indicating extensive alteration and vacuolar degeneration of the germ cells cysts forming the ovary cord integrity (asterix) in (d) (H&E x 100) and in (e) (H&E x 400) arrows mark degenerative growing oocytes. (f): Morphology of damaged vitellogenic (v) (inset a) and late vitellogenic oocyte (lv) in the treated group; note the irregular nuclear envelope (inset b) (H&E x 400). (g), (h) and (i) ovisac from Animals in group III which were treated with 50 µg/l of Cd and 25 µg/l of SA. Note the presence of normal cysts (asterix) and of a growing oocyte inside the ovisac (arrow) (H&E x 400). (h) and (i) details from parts of the ovary cords and vitellogenic oocyte (v) (H&E x 400). (j), (k), and (l) ovisac from Animals in group IV which were treated with 50 µg/l of Cd and 50 µg/l of SA. Notice normal cysts inside the ovary cord (asterix) and the increased number of normal growing vitellogenic oocytes (arrows) (H&E x 400). (k) and (l) details from parts of the ovary cords and vitellogenic oocyte (v) (H&E x 400). (m), (n), and (o) ovisac from Animals in group IV which were treated with 50 µg/l of Cd and 100 µg/l of SA. Remark the almost complete restoration of the normal architecture of the ovary cord (asterix) and of the normal morphology of vitellogenic oocytes (arrows) (H&E x 100). (n) and (o) details from parts of the ovary cords and vitellogenic oocyte (v) (H&E x 400).
Ovaries appearance from leeches treated with SA revealed pertinent improvement evidenced by the reappearance of normal ovaries structure and the prevention of histopathological injuries (Figure 4g-o), particularly in the group treated with 100 µg/l SA (Figure 4m-o).

4. Discussion

In aquatic ecosystems, Cd cannot be biologically degraded which endangers all forms of aquatic life. Cd-exposure has become a controversial issue since it can cause severe damage to the tissues and organ functions including liver, neurons, and gonads [32,33]. Leeches have been considered as an appropriate model organism in many fields of biology including phenology, environmental sciences, and pharmacology since they exhibited an important scope of diversity of feeding, morphology, physiology, and reproductive behavior [34,35]. They are sensitive and easily bred under laboratory conditions which facilitate the analysis of collected data parameters as well as the monitoring of various physiological and behavior changes. Many previous studies have documented the adverse effect of several other environmental pollutants on leech ovaries [36,37]. Although, Cd toxicity is well reported in many aquatic species, to our knowledge there is no previous study that illustrated the oxidative stress induced by Cd-stress in leech ovaries.

As it has been previously established in the literature, Cd toxicity could be ascribed to oxidative stress triggering and free radicals production [38,39]. Interestingly, Cd exposure led to an unbalance in cellular redox homeostasis through disturbance of the antioxidant system [40]. These disruptions are caused by the contribution of several factors notably reactive oxygen species generation (ROS), DNA methylation, alteration of calcium intracellular level, and cell kinases [41]. In the present study, we found that Cd-exposure crucially increased MDA level in the ovaries of *L. nilotica* exposed to 50 µg/l of Cd for 7 days implying a possible ovarian ROS accumulation. MDA is recognized as one of the major oxidation products derived from polyunsaturated fatty acids of biological membranes [42]. The marked increase of MDA in stressed leeches is a pertinent indicator of reactive oxygen species generation and the extent of oxidative stress which may lead to the loss of membrane integrity and functionality [43]. Moreover, our results showed a substantial increase of H$_2$O$_2$ in the ovaries of stressed leeches, which represents the main contributor to free radical generation through Fenton and Haber-Weiss reaction [43]. Our results substantiate previous findings which reported MDA level increase and ROS overproduction under Cd-stress in ovaries [4,9,44].

On the other hand, our data showed a marked increase in SOD level in ovaries of Cd-exposed leeches with a drastic decrease of CAT and GPx activities. It is well known that CAT and GPx react completely with SOD and they are implicated in H$_2$O$_2$ elimination [14]. The impairment of the antioxidant enzyme CAT and GPx activities under Cd-stress led to the increased level of intracellular H$_2$O$_2$ as demonstrated in this study. Our results are in agreement with previous studies which reported that heavy metals, including Cd, are pro-oxidant and cause the impairment of antioxidant defense systems [3]. In fact, Cd exposure compromises the cellular redox homeostasis leading to an overproduction of ROS which overwhelms repair capacity [45]. Likewise, our data revealed a disruption in non-enzymatic antioxidant activities, and the redox state was avidly altered showing a significant decrease in GSH, thiols, and AsA levels along with the increase of GSSG level following Cd-stress. It has been reported that Cd is characterized by a high affinity for sulfhydryl groups of enzymes and proteins which, consequently, result in their structure disruption and dysfunctions [46]. Intracellular thiols are known to be a major target of ROS and they are particularly vulnerable to ROS oxidation due to their high nucleophilicity [47].

However, treatment with SA substantially counteracted Cd-induced toxicity and prevented oxidative stress in the ovaries of Cd stressed leeches resulting significant decrease of MDA, H$_2$O$_2$, SOD in addition to an increase in CAT, and GPx as well as GSH, thiols, AsA, and decrease in GSSG. Consequently, SA-treatment offered protection against Cd-oxidative stress attack. This protective effect could be attributed to its ability to scavenge and eliminate ROS and its iron chelation properties since it has been reported that SA has a potential efficiency against intracellular free radicals [48]. Indeed, SA could act directly as a free radical scavenger or indirectly by maintaining cellular redox homeostasis [16,49]. The decrease of SOD and the increase of CAT and GPx as well as non-enzymatic antioxidant regulation were related to a marked decrease in MDA and H$_2$O$_2$ production which can be explained by the antiperoxidative effect conferred by SA treatment. Previous studies highlighted the ability of SA to upregulate the antioxidant system and to upset cell injuries through HSP (heat shock protein) expression [16,50]. It has been reported that the induction of HSP was strongly associated with protection against environmental stimuli such as heavy metals, thermal stress thereby repairing damaged proteins and assisting the resumption of normal state [51]. The regulation of antioxidant system activity and the enhanced HSP represents an integrative defense against heavy metals, including Cd and oxidative stress [52,53]. However, this suggestion needs further investigation, especially in the invertebrates.
These biochemical findings were confirmed by the histopathological study. Indeed, the ovaries of exposed leeches showed several alterations either in the organization of the ovary cord due to the vacuolation and loss of the connection between germ cells to the cytophore, and the degeneration and the necrosis of the freely floating vitellogenic oocytes. These alterations could be due to the overproduction of free radicals resulting from enhanced lipid peroxidation in the ovaries of Cd-exposed leeches. Similar ovary injuries were also described in several Cd-exposed species. It has been reported that subchronic exposure to Cd-induced degenerated oocyte, atretic follicles, and distorted Graafian follicle in the female rat [9,44,54]. In aquatic organisms, several histopathological changes have been described in the female gonad of the freshwater mollusks *Lamellidens marginalis* under Cd-stress including disruption of the ovarian follicle, damage in connective tissue, and germinal cells vacuolization [55]. Ovarian damage and deformed follicles were further noted in the *Tilapia Oorechomis niloticus* that were exposed to Cd [56]. Lamellar hypertrophy, immature oocyte, extensive vacuolization, and yolk globule reduction were observed in the ovary of the freshwater prawn *Macrobrachium rosenbergii* [57]. A relative recovery of typical histological features of leech ovaries following SA treatment was observed, showing normal architecture of the ovary cord by the establishment of the syncytial cysts and by the reappearance of the cord integrity. As well the morphology of vitellogenic oocytes remained normal and similar to the control group [58]. The ameliorative effect conferred by SA treatment against Cd-induced tissue injuries is broadly consistent with many previous studies. Indeed, it has been reported that SA addition attenuated histopathological injuries in renal of the gentamicin-treated rat [16]. A potential protection was observed after SA treatment of sciatic nerve fibers of rats that were exposed to acrylamide, showing a decrease in apoptotic cell death and recovery of typical nerve fiber state [59]. In addition, a decrease in histopathological damage was revealed in chicken myocardial cells following SA treatment [53].

In summary, this paper highlighted the beneficial effect of SA treatment in Cd-ovarian toxicity alleviation in the freshwater leech *L. nilotica*. The mitigation of Cd-induced ovaries toxicity conferred by SA treatment was evidenced by a marked remission of many biochemical and histological indexes. SA treatment upregulated the antioxidant defense system, maintained the redox homeostasis, and attenuated histopathological injuries due to its free radical scavenging and iron-chelating properties. This study is the first attempt to demonstrate the counteractive effect of SA against Cd-induced toxicity in the leech ovary. However, further investigations on the current topic are therefore required in order to better understand the role of phenolic compounds to upset environmental stressor effects in the invertebrates.

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