Insights into the ecotoxicological perturbations induced by the biocide abamectin in the white snail, *Theba pisana*

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Abstract

Abamectin (avermectin B1, ABM) has been widely used as a biocide in agriculture, veterinary and medicine worldwide. In the current study, we aimed to evaluate the acute toxicity and sub-lethal biochemical responses of ABM on the non-target land snail, *Theba pisana*. Mortality of snails increased with the dose increase, resulting 48h- LD_{50} value of 1.048 µg/snail. Sub-lethal effects were studied on the survivors of 20% and 60% LD_{50} ABM doses and the biochemical parameters were assessed for up to 7 days of exposure. The results showed a decrease in glycogen content and lipids for two sub-lethal doses after all time intervals, whereas increased the level of total proteins after exposure to 60% LD_{50} ABM. Overall, the tested sub-lethal doses significantly decreased the total energy reserves. ABM-exposure to snails elevated γ-Glutamyl transferase (γ-GT) and Lactate dehydrogenase (LDH) activities at all-time intervals. A significant increase of Glutathione-S-transferase (GST) activity was also recorded in snails exposed to 20% and 60% LD_{50} after 7 days and all time intervals, respectively. However, ABM inhibited the activity of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) after 7 days of exposure. Our investigation provides new insights into the disturbances of energy reserves and enzyme activities in *T. pisana* snails that can be used as useful sentinel organism. Indeed, these tested biochemical parameters of the snails are sensitive and may be used as biomarkers for assessing ABM toxicity.

Introduction

Growing anthropogenic activities all over the world have resulted in the subsequent contamination of the environment because of continuously loaded by different types of toxic pollutants including pesticides (Özkara et al. 2016). Consequently, pesticide pollutants have become of great concern due to the adverse effects on human health and non-target organisms (Sharma et al. 2019). Once applied to the target pests, pesticides can reach the terrestrial ecosystem and become bioavailable for assimilation by soil organisms (Sánchez-Bayo 2011). Accordingly, we need to make every effort to assess their ecotoxicological effect as a tool for the risk assessment of contaminated soils.

Abamectin (ABM) is the main avermectin group, which belongs to a 16-membered macrocyclic lactones metabolites produced by a natural fermentation of the bacterium *Streptomyces avermitilis* (Pitterna et al. 2009). ABM mixture contains more than 80% avermectin B1a and less than 20% avermectin B1b (Fisher and Mrozik 1989). ABM works as a chloride channel activator by binding δ-aminobutyric acid (GABA) receptor and glutamate-gated chloride channels disrupting nerve signals within animals (Jansson and Dybas 1998; Bloomquist 2003). Its novel mode of action, high potency and specific physico-chemical properties make this compound excellent candidate for further insecticidal, acaricidal, and molluscicidal studies (Horowitz and Ishaaya 2004; Radwan 2016).

With regard to the environmental aspects, Lumaret et al. (2012) reviewed the widespread use of ABM has led to environmental consequences for aquatic and terrestrial non-target organisms. In terrestrial ecosystems, the entry of ABM into the environment is through enriching agricultural soil with treated

animals manure or via livestock excretion on pasture soils. Its physical/chemical properties includes low water solubility, non-volatile and high affinity for lipids and for binding to organic matter in combination with a high rate of excretion of the parent compound from treated animals (Halley et al. 1989), raising concerns among many scientists that found it slowly disappears from the soil with a half-life of 2–8 weeks (Halley et al. 1989; Erzen et al. 2005).

Snails belong to the molluscan class Gastropoda, which inhabit land, freshwater and marine environments. Several herbivorous land snail species including the helicid white garden snail, *Theba pisana* is utilized as a sensitive indicator for the diagnosis of chemical pollution and climatic changes (de Vaufleury et al. 2006; Regoli et al. 2006; Radwan et al. 2010; Madejón et al. 2013; Nicolai and Ansart 2017). The potential for use of *T. pisana* as a model organism both in laboratory toxicity tests and in biomonitoring studies as bioindicator of metals and organic soil contamination is well-documented (Radwan et al. 2010; Madejón et al. 2013; El-Gendy et al. 2019).

Biomarkers are early warning tools measured in biological indicator species in response to environmental stressors in a well-known manner, and therefore can be used to assess the threat to an ecosystem in a polluted area (Hamza-Chaffai 2014). Exposure to xenbiotics can induce changes in an organism at the molecular level. Molecular biomarkers are measurable biochemical indicators of cellular effects of toxicity that can supplement the interpretation of observed organismal and population level effects (van der Oost et al. 2003). The usage of cellular and biochemical changes in the hepatopancreas and/or hemolymph of land gastropods as biomarkers of pollutant exposure and effects have been documented (Radwan et al. 2020).

The present study is a series of experiments in our laboratory to use several endpoints for investigating the ecotoxicological impacts of pesticides on the snail, *T. pisana* as a model organism (Radwan et al. 1992; El-Gendy et al. 2019). Based on the website information of the Agricultural Pesticides Committee (APC) of the Egyptian Ministry of Agriculture and Land Reclamation, the active ingredient of ABM is currently approved for use in 87 different formulated products against 8 different pests on 12 crops (APC, 2021). Despite its extensive use in Egypt, little research had been conducted into the potential adverse effects of ABM. To address the gap in data on the potential impacts of ABM on non-target terrestrial snails, our aim of the present study was to evaluate the lethal and sub-lethal toxicity of ABM on the land snail, *T. pisana*. After 1, 3 and 7 days of exposure, we analyzed the alterations of five enzyme activities; GST, γ-GT, AST, ALT and LDH along with the levels of energy reserve (lipids, glycogen and proteins) in the digestive glands of the animal to examine their utility as popular stress endpoints of pesticides.

Materials And Methods

Chemicals used

Commercial formulation of ABM (Vertimec® 18 EC) with chemical formula: $C_{48}H_{72}O_{14}$ (B1a); $C_{47}H_{70}O_{14}$ (B1b), was used in the present study and supplied by Syngenta Agro Services AG, Egypt**.** Other reagents

and chemicals used in the present work were provided by Sigma-Aldrich Company.

Animals tested

Samples of the land snail, *Theba pisana* were taken from a non-contaminated Botanic garden (Antoniades) in Alexandria Governorate, Egypt (31°12'08" *N*; 29°57'03" *E*). Before the experiments, they were caged in aerated wood boxes (30 x 25 x25 cm, with 100 snails per box) for one month at 25-28 °C, 62–65 RH and LD 12/12 h cycle. The animals were fed *ad libitum* with fresh *Lettuce sativa* leaves. Only healthy adult snail with a weight of 0.95 ± 0.01 g and a shell diameter of 14.9 ± 0.082 mm was used. The experiments were carried out according to the guidelines for animal care and handling, with the approval of the Animal Ethics Committee of Alexandria University.

Lethal toxicity experiment

To determine the contact median lethal toxicity and the sub-lethal toxicity of ABM on *T. pisana* snails, topical application method was adopted (Radwan and Mohamed 2013). Stock solution of ABM was prepared by dissolving 0.5 g of the pesticide in 500 ml of distilled water (1000 µg/ml). ABM was used at various concentrations ranging from 25 to 1000 µg/ml, after pilot experiments conducted with ABM at concentrations of 10, 100 and 1000 µg/ml. Dosage range corresponding to the concentrations described above varying between 0.25 and 10 µg ABM/snail. Three plastic boxes (each box contains ten animals) were utilized for each treatment. Each box was capped with a cloth net and tightly fixed to prevent snail escape. Using a micropipette containing 10 µL, the tested dose was gently applied once to the surface of the snail body inside the shell. Animals gained 10 µL of distilled water was considered as control. A little water was added daily to each box to ensure the moisture needed for the snail's activity. The mortality percentages were recorded 48 h after exposure. The LD_{50} values for *T. pisana* snails were determined by the Probit analysis method (Finney 1971).

Sub-lethal toxicity experiment

In a separate series of experiments, sub-lethal doses of ABM (20% and 60% 48 h-LD_{50}) were applied topically to *T. pisana* snails, as in the aforementioned procedure to examine the possible impacts of this biocide on the hepatopancreas of the animals and its biochemical alterations.

Three experimental groups of snails (30 animals in each group), were used in this study.

Group I (control): Untreated animals were considered as controls.

Group II: A single dose of 10 µl of 20% ABM -LD_{50} (i.e., 0.21 µg/snail) applied topically via injection into the shell cavity.

Group III: A single dose of 10 µl 60% ABM -LD_{50} (i.e., 0.63 µg/snail) was received by the animal via injection into the shell cavity.

The sub-lethal effects of ABM, on the survivors of snails that exposed to the sub-lethal doses, were studied on some snail biochemical attributes at 1, 3 and 7 days after treatment. Biochemical disturbances assessed by measuring the five enzymes activities; Glutathione-S-transferase (GST), γ-Glutamyl transferase (γ-GT), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Lactate dehydrogenase (LDH) along with three energy reserves; lipids, glycogen and proteins.

Sample preparation

At the end of each time, the shells of nine randomly chosen survival snails from each group were taken off. Then the hepatopancreas were excised, rinsed with 0.9% ice-cold saline and weighed. The hepatopancreas was splitted into two portions; the first portion was taken for measuring glycogen and lipids content, whereas the second portion was homogenized in 10 volumes of ice-cold saline for 30 s and centrifuged at 5000 g for 20 min at 4 ° C. The supernatant was used to measure GST, γ-GT, AST, ALT and LDH activities and total soluble proteins.

Biochemical assays

The glycogen content was assessed by the method of Van Hendel (1965). Total lipids were assessed according to Knight et al. (1972). Total soluble proteins were assessed by Lowry et al. (1951). Each biochemical assessment was determined six times. The values for glycogen, lipids and proteins were derived from standard curves plotted with known concentrations of glucose, soybean oil and bovine serum albumin, consecutively. All previous components were expressed as mg/g tissue. Total energy reserves (kilojoules per gram (kJ / g)) were calculated according to Bowen et al. (1995): 1 g of protein, lipids or glycogen is equal to an energy of 17.3, 38.9 and 16.9 kJ, respectively.

Enzymatic measurements

GST (EC 2.5.1.18) activity was determined based on Vessey and Boyer (1984) method. The enzyme activity was expressed as μmoles of CDNB conjugated/mg protein/min. γ-GT (EC 2.3.2.2) activity was measured by a commercial kit based on the method of Szasz (1974). Absorbance was recorded at 405 nm and the activity was expressed as U/L. Both AST (EC 2.6.1.1) and ALT (EC 2.6.1.2) activities were determined according to the method of Reitman and Frankel (1957) utilizing the Diamond Diagnostics kit (Diamond Co., Egypt) and their activities were expressed as U/L. LDH (EC 1.1.1.27) activity was measured by the method of McComb (1983) using Spectrum Diagnostic kit (Spectrum Co., Egypt). This enzyme activity was expressed as U/L. All enzymatic activities were determined six times.

Data analysis

All results were presented as a mean ± standard error. The data from the biochemical responses test were analyzed to assure normality and uniformity of variance (Shapiro-Wilk and Levene's tests, respectively). Subsequently, the data were analyzed by analysis of variance (Two-way ANOVA) and the means were separated according to Student–Newman–Keuls test at a significance level of $p \leq 0.05$. The statistical analysis as performed with software Costat program, Version 2.6 (2002).

Results

Impact of ABM on mortality of T. pisana snails

Following 48 h of topical application of *T. pisana* snails, the percentage mortalities of ABM treated snails are dose dependent and gradually increase with increasing dose (Table 1). No death was recorded in controls throughout the time of assay. ABM killed 100 % of test snails at 10 µg/snail. The results of Probit analysis shows that the LD_{50} value of ABM was 1.048 µg/snail.

Table 1
Lethal contact toxicity parameters of ABM against the land snail, *Theba pisana*

Doses used (µg/snail)	Mortality percentage
0.25	13.3 ± 0.33
0.5	33.3 ± 0.41
1	53.3 ± 0.33
1.5	60.0 ± 0.00
2	66.6 ± 0.88
3	73.3 ± 0.67
4	80.0 ± 0.82
5	86.6 ± 0.33
10	100 ± 0.00
Control	0.0
LD_{50} (µg/snail)	1.048 (1.21 – 0.897)
Regression equation	Y = - 0.032 + 1.55 X
Slope ± Variance	1.55 ± 0.015
χ^2 (df)	2.67 (6)
Each figure represents the mean percent mortality ± SE (n = 3).	
The 95% upper and lower confidence limits are shown in parenthesis.	

Effect of ABM on the energy reserves of T. pisana snails

The results of energy reserves (lipids, glycogen and proteins) in survivors *T. pisana* snails after exposure to sub-lethal doses; 20% and 60% LD_{50} of ABM are presented in Table 2.

Table 2
Total lipids, glycogen and total proteins (mg/g fresh tissue, ± SE) in the hepatopancreas of survivors *Theba pisana* snails treated with sub-lethal doses of ABM after different times of exposure

Time of exposure (days)	Untreated snails	Survivors from treated snails with sub-lethal doses				Mean
		20 % LD_{50}		60 % LD_{50}		
		Mean ± SE	% control	Mean ± SE	% control	
Total lipids						
1	101.6 ± 0.81	86.4 ± 0.41	85.01	85.0 ± 0.53	83.64	91.00
3	111.8 ± 0.64	82.9 ± 0.31*	74.14	81.0 ± 0.65*	72.47	91.90
7	122.0 ± 0.50	82.3 ± 0.04*	67.44	78.7 ± 0.80*	64.51	94.30
Mean	111.80	83.86		81.56		
Glycogen						
1	112.0 ± 0.65	90.9 ± 0.50*	81.10	88.6 ± 0.37*	79.11	97.16
3	113.1 ± 0.26	88.1 ± 0.03*	77.86	85.0 ± 0.19*	75.14	95.40
7	113.9 ± 1.75	83.0 ± 0.31*	72.88	81.4 ± 0.48*	71.50	92.76
Mean	113.00	87.33		85.00		
Total proteins						
1	75.8 ± 0.10	70.2 ± 0.10	92.67	88.2 ± 0.14*	116.42	78.06
3	77.0 ± 0.15	75.8 ± 0.41	98.44	82.7 ± 0.29	107.36	78.50
7	78.2 ± 0.11	67.8 ± 0.73*	86.67	90.6 ± 0.19*	115.85	78.86
Mean	77.00	71.26		87.16		
* Significantly different from the control at $p \leq 0.05$						

Table 2 shows that total lipids were significantly reduced in the survivors of treated snails after 3 and 7 days' exposure to the tested sub-lethal doses of ABM. This decrease was highest at 7 days, while the

lowest was recorded at 1 day after treatment. The data was also shown that this reduction was clearly dose- and time- dependent. However, non-significant reduction in total lipids was observed among ABM-treated snails after 1day post treatment when compared with the control.

As for the glycogen contents, ABM was caused a significant decrease at all times of exposure compared to controls. Percent reductions in glycogen after 20 % LD_{50} ABM treatment were 81.10, 77.86 and 72.00, while were 79.11, 75.14 and 71.50 after 60% LD_{50} at 1, 3, and 7 days of exposure, consecutively. Generally, these reductions appeared to be obviously dose- and time- dependent, where the marked reduction was significantly higher in the survivor's snails treated with the two sub-lethal doses after 7 days (Table 2).

The response of total proteins in ABM-treated snails was inconsistent compared to control snails. In the survivor's snails exposed to 20 % LD_{50} ABM, total proteins were non-significantly lower than of the control (92.67 %), however, significant higher total proteins were observed in 60 % LD_{50} treated snails (116.42 %) after 1 day. After 3 days of exposure, there were non-significant decreases and increases in total proteins among survivor's snails exposed to ABM at 20 % and 60% LD_{50}, respectively. After 7 days of exposure to 20 % LD_{50}, a marked reduction in total proteins in the survivors treated snails was recorded, while a significant increase in total proteins was found after exposure to 60% LD_{50} ABM (Table 2).

Overall, significant reductions in total energy reserves in the survivors *T. pisana* treated with the sub-lethal doses of ABM at all exposure times were observed (Table 3).

Table 3
Total energy reserves (kJ/g ± SE) in untreated and survivors from treated land snail, *Theba pisana* with sub-lethal doses of ABM following various time intervals

Exposure time (days)	Untreated snails	Survivors from treated snails with sub-lethal doses				Mean
		20 % LD_{50}		60 % LD_{50}		
		Mean ± SE	% control	Mean ± SE	% control	
1	7.16 ± 0.48	6.11 ± 0.36*	85.38	6.33 ± 0.47*	88.45	6.53
3	7.59 ± 0.12	6.02 ± 0.59*	79.34	6.02 ± 0.24*	79.26	6.54
7	8.02 ± 0.45	5.78 ± 0.28*	71.99	6.01 ± 0.43*	74.84	6.60
Mean	7.59	5.97		6.12		

* Significant difference from control treatment ($p \leq 0.05$).

Effect of ABM on the enzymes activities of T. pisana **snails**

Different patterns of responses were detected in the enzymes activities (GST, γ-GT, LDH, AST and ALT) in the survivors *T. pisana* exposed to sub-lethal doses of ABM (Fig. 1-5).

The GST activities in the survivor's snails treated with 20 % LD_{50} ABM were increased without significant differences when compared to the controls after 1 and 3 days' exposure, however the GST activity significantly increased after 7 days. Significant differences were also observed in 60 % LD_{50} survivors of treated snails after all times of exposure. These significant increases were 135.19 % in the 20 % LD_{50} treatment after 7 days and 153.46, 165.49 and 152.63 % in the 60 % LD_{50} treatment after 1, 3 and 7 days (Fig. 1).

Significant differences were found in the survivors treated and untreated snails in the activity of γ-GT. The activity of γ-GT was clearly induced by exposure to ABM at 20 and 60% LD_{50} compared to controls. (Fig. 2). This increase of enzyme activity was dose- dependent but not time-dependent.

LDH activity in the survivor's snails treated with either 20 or 60% LD_{50} ABM was higher than the enzyme activity of untreated snails (Fig. 3).

As shown in Fig. 4, the activity of AST in the survivor's snails was significantly decreased by 87.39 % in the 20 % LD_{50} ABM after 7 days and its activity was also decreased after 1 and 3 days of exposure without significant differences. In case of 60 % LD_{50} ABM, significant decreases in the activity of AST in the survivor's snails were recorded with values of 90.26, 86.89 and 84.49 % after 1, 3 and 7 days, respectively. Decreases of the activity were dose- and time-dependent.

Figure 5 shows that, compared to the control, there was no significant decrease in ALT activity in the survivor's snails at 1 day after treatment with each of sub-lethal dose. However, after 3 and 7 days, both sub-lethal doses caused significant decreased in ALT activity compared with that of the control.

Discussion

In recent decades, the impact of pesticides on the environment is becoming a major problem worldwide. The continuous use of pesticides is burden on the soil ecosystem and causes deterioration in its health, along with potential consequences on soil-inhabiting invertebrates, which are indicators of soil quality. Therefore, more and more detailed ecotoxicological data are needed to better understand its actual threats as pesticide use is unlikely diminish in the near future (Gunstone et al. 2021). Up to date, the ecotoxicological impacts of avermectins against land gastropods have been rarely studied. This prompted us to study the lethal and sub-lethal toxicity of ABM against *T. pisana* snails.

In current study, the acute toxicity data obviously showed that ABM has lethal action against *T. pisana* snails. Regardless to the route of administration (contact or dietary exposure), the obtained data are in a good agreement with previous results in which ABM has lethal toxic action against different land

gastropod species; *T. pisana* (Gad et al. 2016; Radwan 2016), *Massylaea vermiculata* (Syn: *Eobania vermiculata*) (Gabr et al. 2006; Kandil et al. 2014; Abdelgalil et al. 2018; Hussein and Sabry 2019), *Monacha obstructa* (Gabr et al. 2006; Kandil et al. 2014) and *Deroceras reticulatum* (Airey et al. 1989). On the other hand, ABM at 0.2% spray has high potential usefulness in protecting rape seedlings from the slug, *Arion lusitanicus*, but non-lethal to the animal (Kozlowski et al. 2010).

It is well known that pesticide sub-lethal toxicity is measured using molecular and cellular endpoints, which are also used to assess modes of action, metabolic pathways and detoxification mechanisms (van der Oost et al. 2003; Moreira et al. 2020). The digestive gland (hepatopancreas) is the main target for the toxic effects of xenobiotics, such as pesticides, that play crucial role in the accumulation, metabolism and detoxification as well as the biosynthesis of energetic macromolecules for different essential functions in molluscs (Dallinger et al. 2002). Therefore, changes in the digestive gland biochemical parameters as biomarkers due to the sub-lethal doses of the compound intoxication have been widely utilized as an indicator to assess the toxic action of xenobiotics on snails. In order to get insights into sub-lethal effects on the survivor's snails that exposed to 20% and 60% LD50 ABM doses in our study, the energy reserves (glycogen, lipids and proteins) and enzyme activities (GST, γ-GT, LDH, AST and ALT) as usual biomarkers were assessed in *T. pisana* snails.

The role of biomolecules include lipids, carbohydrates and proteins are critical in triggering different types of biochemical, physiological and behavioral responses in living organisms (Yazdani et al. 2013). These bioenergetics parameters have been suggested as useful biomarker to detect the deleterious effects and toxicological mechanisms induced by environmental pollutants. Few studies have been done to evaluate the adverse effects of pesticides including ABM on the energy reserves of land snails (Radwan et al. 2008; Radwan and Mohamed 2013; Kandil et al. 2014). Therefore, this negative impact on the energy reserves as a result of pesticide exposure needs more investigations.

Lipids play a very important role in the normal functioning of cells. They not only act as a highly reduced form of energy storage, but also play a close role in the structure of cell membranes and organelles found in cells (Kandil et al. 2014). In this investigation, total lipids were significantly decreased in the survivors of ABM- treated snails with sub-lethal doses. The decreased level of lipids after treatment may be ascribed to the impairment of lipid biosynthesis, metabolism and/or utilization as an energy source for surviving under stressful conditions (Radwan et al. 2008; Shaurub and Aziz 2015). In agreement with our results, Megahed et al. (2013) noticed that total lipids significantly decreased in hemolymph of treated 4th instar larvae of *Spodoptera littoralis* with ABM, emamectin and spinosad at 24, 48 and 72 h.

Glycogen is an important component of living cells and a source of energy for animals. In our study, there were significant decreases in the glycogen contents of the survivors of *T. pisana* snails throughout the ABM-treatment periods. This depletion indicating animals are utilizing their energy reserves to cope with toxic stress (Tendulkar and Kulkarni 2012) or for increased rate of glycogen breakdown "glycogenolysis" (Ansaldo et al. 2006). The aforementioned findings are in coincidence with those of Riaz et al. (2019) who showed that the glycogen contents were significantly deceased in 4th and 6th larval instars of two

geographically distinct *Trogoderma granarium* field populations exposed to LC_{20} of ABM, emamectin and spinosad alone and in various combinations.

Protein is an important organic constituent of animal tissue. It plays an important role in energy metabolism. Protein regulates the process of interaction between intra and extra cellular media (Remia et al. 2008). In the present study, decreases in total proteins of the survivor's snails exposed to 20 % LD_{50} of ABM were observed, however, total proteins were increased in the survivor's snails exposed to 60 % LD_{50} compared to the control. The obtained data clearly indicate that the changes in the content of proteins depends on the sub-lethal dose used. The increase in total proteins could be elucidated by increased the protein synthesis of animal in response to this stress. On the other hand, the decrease in total proteins under pesticide exposure could be due to the formation of lipoproteins usage to repair the damage of cells and/or for straight usage by cells for energy demands (Padmaja and Rao 1994; Radwan et al. 2008).

Our data confirmed the results of Kandil et al. (2014), where total protein levels in, *M. vermiculata* and *Monacha obstructa* were increased when the snails exposed to ABM as a contact poison. A single dose of 0.25 LD_{50} ABM significantly decrease the total proteins in male albino rats (El- Shafey et al. 2011). Moreover, Al-Kahtani (2011) showed that total protein levels in various organs and/or tissues in the tilapia fish (*Oreochromis niloticus*) decreased after exposure to 20 µg/L ABM for up to 96 h.

The GST enzyme is a part of the detoxification pathway II via conjugation of xenobiotics and/or endogenous compounds with glutathione (GSH) (van der Oost et al. 2003). Our data clearly indicate that ABM induced increment in GST activity of the survivors exposed snails throughout the experimental period. These data suggest that the elevation of antioxidant protection is associated with increased production of oxygen-free radicals, which can enhance antioxidant activity to prevent oxidative stress and protect cells from damage (Elia et al. 2007). An increase in GST activity is also detected in response to pollutants e.g., pesticides, resulting from their detoxification via the formation of glutathione conjugates (Saravana Bhavan and Geraldine 2000). Similar to our investigation, the activity of this enzyme was increased in the same snail species treated with 1/20 LC_{50} ABM for 2 weeks of exposure (El-Gendy et al. 2019). Enhancement of GST activity in the snail, *Physa acuta* treated with ABM during the periods of 12-48 h exposure was also observed (Ma et al. 2014).

Among the enzymes commonly used to assess hepatic function, γ-GT is considered a reliable biomarker that is closely associated with the identification of damage caused by oxidative stress (O¨zer et al. 2008). This enzyme plays a central role in the re-synthesis of glutathione. In addition, Lee et al. (2004) suggested that it is inversely proportional to the levels of many other antioxidants. It is conceivable that the pro-oxidation effect of γ-GT activity is usually balanced by its established role in facilitating the uptake of precursors by the cell to promote the re-synthesis of GSH. Thereby allowing the rebuilding of cellular antioxidant defenses (Banerjee et a1. 1999). In the current study, a significant increase in γ-GT activity in the survivor's snails was noticed due to their treatment with the two sub-lethal of ABM doses. This enzyme elevation may be attributed to the significant tissue injury provoked by pesticides, even at low

doses. These results are in line with Khaldoun-Oularbi et al. (2017) who recorded that ABM caused an increase in the activity of γ-GT in male and female rats, *Rattus norvegicus* at 14, 28 and 42 days. Likewise, there were significant increases in γ-GT activity after the isolated rat hepatocytes exposed to 10 and 100 µM of ABM, for 30, 60 and 120 min as compared to respective control (El-Shenawy 2010).

One of the ways for assessing the integrity of cell membranes is to determine the activity of LDH, an enzyme present in all organs and tissues (Kending and Tarloff 2007). LDH is an enzyme shared in the induction of anaerobic metabolism, and its assessment can be used for understanding the energy production in organisms that can occur either aerobically or anaerobically (Müller et al. 2012). In our study, ABM at sub-lethal doses caused a marked increase in the activity of LDH of the treated survivors snails, which indicates its ability to change the permeability of cell membranes, causing cell death, since an increased leakage of LDH into the serum indicates membrane degradation (Amin and Hamza 2005). Thus, it is considered a good biomarker for cell and membrane damage. The increasing energy demand of the organism during pesticide stress is achieved by using carbohydrates as the main and immediate source of energy (Umminger 1977). This may be accompanied by increased LHD levels as result of the role of LDH in converting the pyruvate into lactate. Previous studies recorded the enhancement of LDH activity in ABM-treated rats (El-Shenawy 2010; Mossa et al. 2017). However, ABM recorded no significant decrease in the LDH activity of hamsters uninfected and infected with *Schistosoma mansoni* (El-Kabbany et al. 2017).

Among the hepatocellular injury markers, transaminases enzymes ALT and AST are probably the most commonly used in both clinical diagnosis and research involving liver damage. Both enzymes are not only found in liver cells, but also in many body organs. Of the two, ALT is predominantly present in the cytosol of the liver and is present elsewhere at low concentrations and is therefore thought to be more specific for hepatic damage. Our results indicated that the activity of AST and ALT decreased in the survivor's snails treated with sub-lethal doses of ABM. The decrease in AST and ALT could indicate tissue damage in the snails as a result of the presence of ABM in their tissues. Thus, biochemical disorders and lesions of tissue and cellular functions can occur when the activity of both enzymes are deviate from the normal range (Radwan et al. 1992). In the literature, the effect of ABM on the AST and ALT activities fluctuate among activation, inhibition, and no effect. Previous studies reported that the activity of AST decreased in treated peach fruit fly, *Bactrocera zonata* than the untreated ones after treatment with ABM (Biomectin®) and spinosad (Tracer®), while the activity of ALT increased after treatment with Biomectin® and decreased after treatment with Tracer® compared to controls (Farag et al. 2017). Enhancement in ALT and AST activities were recorded in *M. vermiculata* and *T. pisana* after treatment with 0.1, 0.2 and 0.5 of LD_{50} ABM for 24 and 72 h (Hamza et al. 2020). No significant differences in the AST and ALT activities after treatment of hamsters uninfected and infected with *Schistosoma mansoni* with ABM were observed (El-Kabbany et al. 2017).

Conclusion

In this study, a prominent lethal and sub-lethal toxic effect of ABM on the land snail, *T. pisana* was detected, for energy reserves (glycogen, lipids and proteins) as well as for enzymatic activities (GST, γ-GT, LDH, AST and ALT) in the digestive glands of survivor's snails at doses of 20 and 60 % LD_{50}. The apparent changes of these biochemical parameters in the treated snails indicate that ABM may have cytotoxic and biochemical impairment and thus may be considered as good biomarkers of toxicity. All the biochemical changes that occur in ABM-treated snails in our study may be due to the reactive oxygen species (ROS) formation that leads to oxidative damage in several non-target organisms such as aquatic animals (Hong et al. 2020), terrestrial snails (El-Gendy et al. 2019), and rats (Khaldoun - Oularbi et al. 2017). In fact, changes in the tested biochemical parameters have the potential to influence on the other biological disturbances such as mucus production, movement, feeding, growth and reproduction. Our results confirmed previous studies that the land snail, *T. pisana* can be used as a bioindicator of ABM exposure. These results also confirm the importance of evaluating effects by measuring a set of biomarkers to understand the biochemical mechanisms involved in the physiology of snails in response to ABM. Further studies on other avermectin members are underway in our laboratory to verify their ecotoxicological profile in land snails.

Declarations

Ethics approval and consent to participate

The experiments were carried out according to the guidelines for animal care and handling, with the approval of the Animal Ethics Committee of Alexandria University, Alexandria, Egypt.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

MAR: conceptualized work, did formal analysis and investigation, wrote the first draft of manuscript, provided editorial comments and suggestions for draft manuscript, Resources and

Supervision. **AFG:** provided methodology, did formal analysis and investigation and wrote the first draft of manuscript. All authors read and approved the manuscript.

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Figures

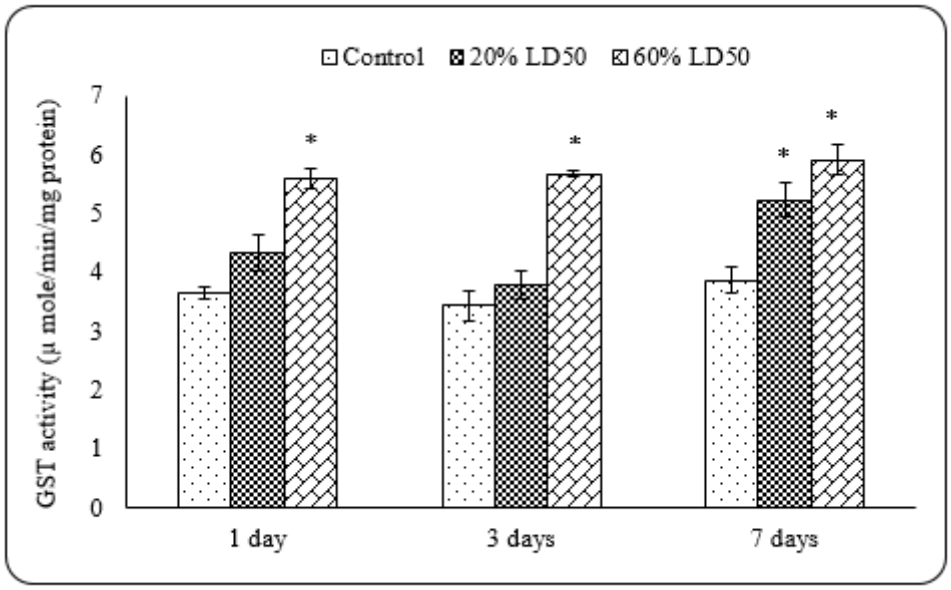

Figure 1

Glutathione-S-transferase (GST) activity in the survivors of Theba pisana treated with 20 and 60% LD50 ABM, together with their controls, for various time intervals. Bar represents mean (n = 3) and vertical line represents the standard error (SE). The star indicates a significant difference from the control ($p \leq 0.05$).

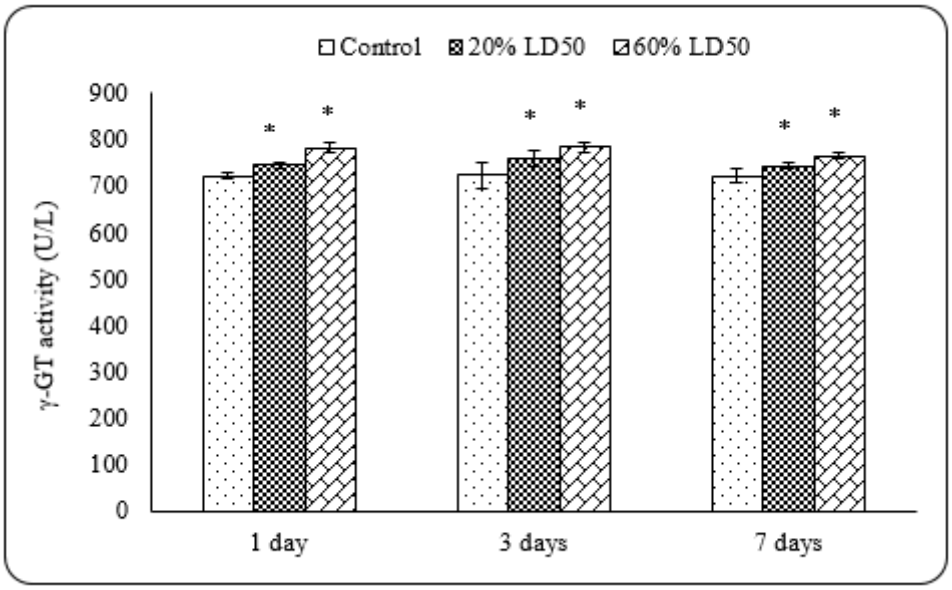

Figure 2

γ-Glutamyl transferase (γ-GT) activity in the survivors of Theba pisana treated with 20 and 60% LD50 ABM, together with their controls, for various time intervals. Bar represents mean (n = 3) and vertical line represents the standard error (SE). The star indicates a significant difference from the control ($p \leq 0.05$).

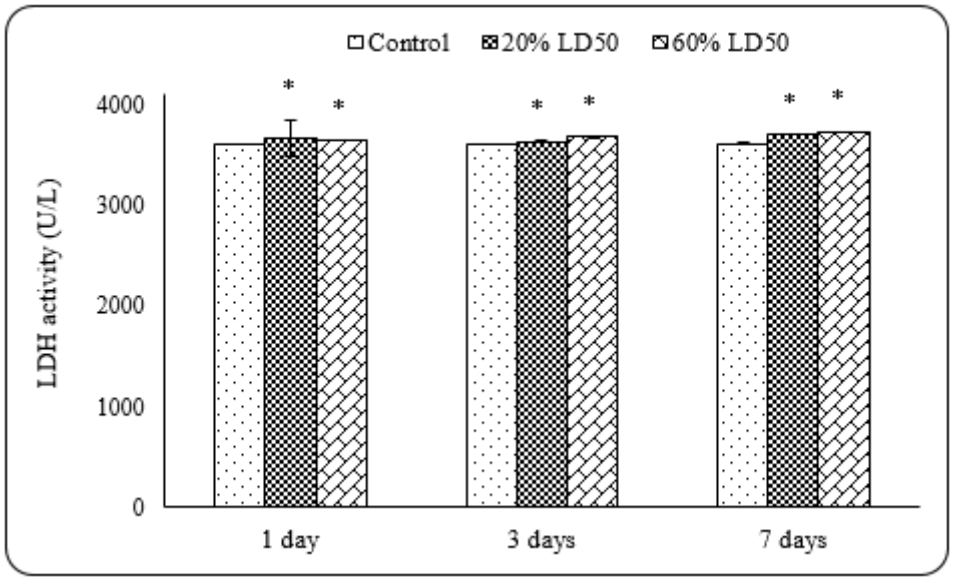

Figure 3

Lactate dehydrogenase (LDH) activity in the survivors of Theba pisana treated with 20 and 60% LD50 ABM, together with their controls, for various time intervals. Bar represents mean (n = 3) and vertical line represents the standard error (SE). The star indicates a significant difference from the control ($p \leq 0.05$).

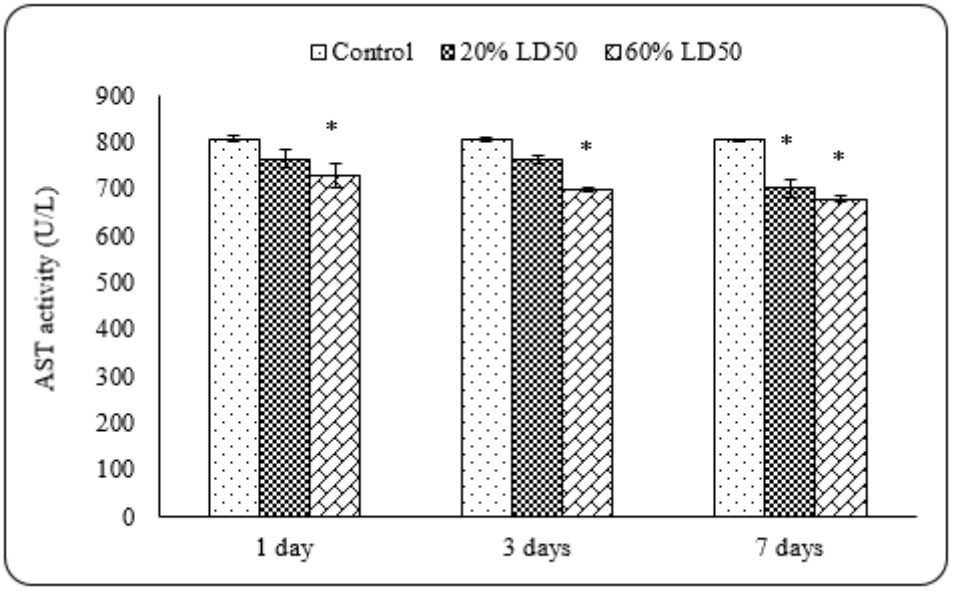

Figure 4

Aspartate aminotransferase (AST) activity in the survivors of Theba pisana treated with 20 and 60% LD50 ABM, together with their controls, for various time intervals. Bar represents mean (n = 3) and vertical line represents the standard error (SE). The star indicates a significant difference from the control ($p \leq 0.05$).

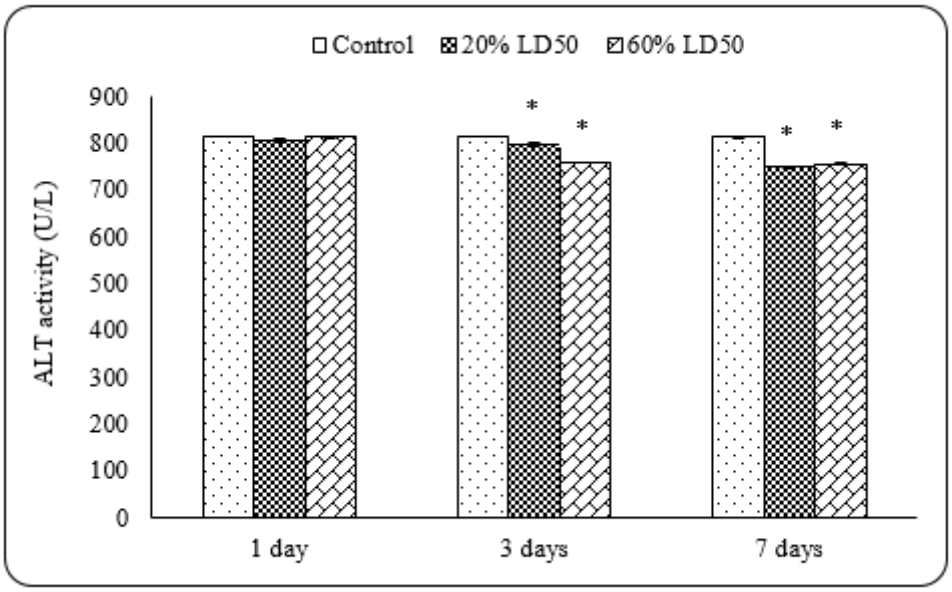

Figure 5

Alanine aminotransferase (ALT) activity in the survivors of Theba pisana treated with 20 and 60% LD50 ABM, together with their controls, for various time intervals. Bar represents mean (n = 3) and vertical line represents the standard error (SE). The star indicates a significant difference from the control ($p \leq 0.05$).

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