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 around the world. Yet, there is still a lack of knowledge about the ecotoxicological effects of ABM. In this study, we investigated the acute toxicity and sub-lethal (20% and 60% LD50) biochemical responses of ABM on the non-target land snail, *Theba pisana*. Mortality of snails increased with the dose increase, resulting 48 h-LD50 value of 1.048 μg/snail. The biochemical 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% LD50 ABM. Overall, the tested sub-lethal doses significantly decreased the total energy reserves. ABM-exposure to snails elevated γ-Glutamyl transferase and Lactate dehydrogenase activities at all-time intervals. A significant increase of Glutathione-S-transferase activity was also recorded in snails exposed to 20% and 60% LD50 after 7 days and all time intervals, respectively. However, ABM inhibited the activity of Aspartate aminotransferase and Alanine aminotransferase after 7 days of exposure. Our investigation provides new insights into the disturbances of energy reserves and enzyme activities in *T. pisana* that are sensitive and may be used as biomarkers for assessing ABM toxicity.

**GRAPHICAL ABSTRACT**

**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 emerging pollutants.[1] Among the emerging pollutants in the environment are veterinary pharmaceuticals and/or pesticides that have become of great concern due to the adverse effects on human health and non-target organisms.[2,3] Meanwhile, these chemicals can easily enter the terrestrial ecosystem and become bioavailable for assimilation by soil organisms possibly resulting in toxic effects and/or exposure of organisms higher up the food chain.[4] Accordingly, soil contamination with these chemicals has emerged as a serious global environmental concern that has necessitated more research on their potential impacts on soil organisms.

Abamectin (ABM), the main avermectin group, is a broad-spectrum parasiticide/anthelmintic drug used in veterinary medicine against external and internal parasites in commercial livestock and companion animals as well as against gastrointestinal nematodes.[5] It is also used for crop protection against agricultural pests as insecticide, acaricide, nematicide and molluscicide.[6–8] It belongs to the chemical class of a 16-membered macrocyclic lactones metabolites produced by a natural fermentation of the bacterium *Streptomyces avermitilis.*[9] ABM mixture contains more
than 80% avermectin B1a and less than 20% avermectin B1b.\(^{10}\) ABM works as a chloride channel activator by binding δ-aminobutyric acid (GABA) receptor and glutamate-gated chloride channels disrupting nerve signals within animals.\(^{11}\)

With regard to the ecotoxicological aspects, Lumaret et al.\(^{12}\) 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 include low water solubility, non-volatility 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,\(^{13}\) raising concerns among many scientists that found it slowly disappears from the soil with a half-life of 2–8 weeks.\(^{13,14}\) Owing to its high toxicity to non-target organisms, it is often persistent and may have a potential bio-accumulate, resulting in robust evidence that ABM causes environmental risks.\(^{15}\) Therefore, it is critical to formulate more effective regulations to manage the use of this compound, pollution discharge restrictions, and disposal measures, and establish environmental safety thresholds applicable to the terrestrial environments in order to protect terrestrial ecosystems. In this regard, European regulations require an environmental risk assessment for this chemical before it is approved for use, and there are guidelines on how risks should be assessed.\(^{16,17}\)

Snails belong to the molluskan class Gastropoda inhabit land, freshwater and marine environments. Several herbivorous land snail species including the helicid white garden snail, \textit{Theba pisana} is utilized as a sensitive indicator for the diagnosis of chemical pollution and climatic changes.\(^{18–21}\) The potential for use of \textit{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.\(^{22,23}\)

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.\(^{24}\) Exposure to xenobiotics 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.\(^{25}\) The usage of cellular and biochemical changes in the digestive gland and/or hemolymph of land gastropods as biomarkers of pollutant exposure and effects have been documented.\(^{26}\)

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.\(^{27}\) Despite its extensive use in Egypt, little research had been conducted into the potential adverse effects of ABM. The present study is a series of experiments in our laboratory to use several endpoints for investigating the ecotoxicological impacts of pesticides on the land snail, \textit{T. pisana} as a model organism.\(^{23,28–30}\) Our first investigation observed that \textit{T. pisana} shows oxidative stress, genotoxicity and immunotoxicity in response to ABM and these multiple parameters may be considered for use as biomarkers to identify and estimate the sublethal ABM effect.\(^{23}\) Nevertheless, there are still gaps that need to be clarified. The application of more sensitive and simpler assays such as energy reserves and some metabolic enzymes as endpoints give a comprehensive picture and provide better insights to investigate the potential impacts of ABM on non-target terrestrial snails, all of the above have motivated the present work.

Our aim of this study was designed to evaluate the lethal and sub-lethal toxicity of ABM on the land snail, \textit{T. pisana}. After 1, 3 and 7 days of exposure, we analyzed the alterations of the levels of energy reserve (lipids, glycogen and proteins) along with five enzyme activities; Glutathione-S-transferase (GST), \(\gamma\)-Glutamyl transferase (\(\gamma\)-GT), Lactate dehydrogenase (LDH), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in the digestive gland of the animal to examine their utility as popular endpoints for exploring the metabolic and biochemical defects involved in the physiology of snails in response to ABM stress.

**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, \textit{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 × 25 x 25 cm, with 100 snails per box) for one month under controlled conditions; temperature (25–28°C), relative humidity (62–65%) and photoperiod (12/12 h light/dark regime). The animals were fed \textit{ad libitum} with fresh \textit{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.

**Experimental framework**

Two experiments were conducted in the present study; one for lethal effect, and the other for sub-lethal effect.
**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. Stock solution of ABM was prepared by dissolving 0.5 g of the pesticide in 500 mL of distilled water (1000 µg mL⁻¹). ABM was used at various concentrations ranging from 25 to 1000 µg mL⁻¹, after pilot experiments conducted with ABM at concentrations of 10, 100 and 1000 µg mL⁻¹. 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 provide the moisture needed for the snail’s activity. The mortality percentages were recorded 48 h after exposure. The LD₅₀ values for *T. pisana* snails were determined by the Probit analysis method.

**Sub-lethal toxicity experiment**

In a separate series of experiments, sub-lethal doses of ABM (20% and 60% 48 h-LD₅₀) were applied topically to *T. pisana* snails, as in the aforementioned procedure to examine the possible impacts of this biocide on the digestive gland of the animals and its biochemical alterations.

Three experimental groups of snails (30 animals in each group, n = 3), 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-LD₅₀ (i.e., 0.21 µg/snail) applied topically via injection into the shell cavity.
- Group III: A single dose of 10 µL 60% ABM-LD₅₀ (i.e., 0.63 µg/snail) was received by the animal via injection into the shell cavity.

The sub-lethal effects of ABM on some snail biochemical attributes studied 1, 3 and 7 days after treatment. Biochemical disturbances assessed by measuring the five enzymes activities; GST, γ-GT, LDH, AST and ALT 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. The digestive gland was excised, rinsed with 0.9% ice-cold saline, weighed and homogenized in 10 volumes of ice-cold saline for 30s. Then the homogenate was divided into two portions; the first portion was taken for measuring glycogen and lipids content, whereas the second portion was centrifuged at 5000 g for 20 min at 4°C. The supernatant was used to measure GST, γ-GT, LDH, AST, and ALT activities and total soluble proteins.

**Biochemical assays**

The glycogen content was assessed by the method of Van Hendel. Stock solution of ABM was prepared by dissolving 0.5 g of the pesticide in 500 mL of distilled water (1000 µg mL⁻¹). ABM was used at various concentrations ranging from 25 to 1000 µg mL⁻¹, after pilot experiments conducted with ABM at concentrations of 10, 100 and 1000 µg mL⁻¹. 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 provide the moisture needed for the snail’s activity. The mortality percentages were recorded 48 h after exposure. The LD₅₀ values for *T. pisana* snails were determined by the Probit analysis method.

**Statistical analysis**

All results were presented as a mean ± standard deviation. Data from the biochemical responses tests were analyzed to...
assure normality and uniformity of variance (Shapiro–Wilk and Levene’s tests, respectively). Subsequently, data were analyzed by the two-way analysis of variance and means were compared according to Student–Newman–Keuls test at a significance level of $p \leq 0.05$. The statistical analysis was performed with software Costat version 2.6.  

Results

**Impact of ABM on mortality of *T. pisana* snails**

Following 48 h from topical application of ABM on *T. pisana* snails, the percentage mortalities of ABM treated snails are dose dependent and gradually increased with increasing the dose (Table 1). No death was recorded in the snails of the control group 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.

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

The results of energy reserves (lipids, glycogen and proteins) in *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, ± SD) in the digestive gland of *Theba pisana* snails treated with sub-lethal doses of ABM after different times of exposure. |
|---|---|---|---|---|
| Time of exposure (days) | Untreated snails | 20% $LD_{50}$ | Mean ± SD | % control | Mean ± SD | % control |
| Total lipids | 1 | 101.6 ± 1.40 | 86.4 ± 0.71 | 85.01 | 85.0 ± 0.92 | 83.64 |
| Glycogen | 2 | 111.8 ± 1.11 | 82.9 ± 0.54* | 74.14 | 81.0 ± 1.12* | 72.47 |
| Total proteins | 3 | 122.0 ± 0.86 | 82.3 ± 0.07* | 67.44 | 78.7 ± 1.38* | 64.51 |

Table 2 shows that total lipids were significantly reduced in the treated snails after 3 and 7 days’ exposure to the tested sub-lethal doses of ABM. The decrease was the highest at 7 days, while the lowest was recorded at 1 day after treatment. The data also showed that this reduction was clearly dose- and time-dependent. However, non-significant reduction in total lipids was observed among ABM-treated snails after 1 day post treatment when compared with the control.

As for the glycogen contents, ABM 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 72.88, while they were 79.11, 75.14 and 71.50 after 60% $LD_{50}$ at 1, 3, and 7 days of exposure, respectively. Generally, these reductions appeared to be obviously dose- and time-dependent, where the marked reduction was significantly higher in snails treated with the two sub-lethal doses after 7 days (Table 2).

By comparing treated snails to the control group (untreated snails) in Table 2, several variance patterns were observed. The mean of total lipids and total proteins did not change significantly at 1 and 3 days of exposure for both doses, respectively. The variance of total lipids showed consistent differences compared to the control at all exposure times except at 3 days of exposure for 60% $LD_{50}$, respectively. After 7 days of exposure to 20% $LD_{50}$, a marked reduction in total proteins in treated snails was recorded, while a significant increase in total proteins was found after exposure to 60% $LD_{50}$ ABM (Table 2).

The response of total proteins in ABM-treated snails was inconsistent compared to control snails. In snails exposed to 20% $LD_{50}$ ABM, total proteins were non-significantly lower than that of the control (92.67%), however, significant increase in total protein was observed in 60% $LD_{50}$ treated snails (116.42%) after 1 day. After 3 days of exposure, there was non-significant effect in total protein among 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 treated snails was recorded, while a significant increase in total proteins was found after exposure to 60% $LD_{50}$ ABM (Table 2).
were found at 7 days (in control group) and a lower one at 3 days (in 20% LD50 ABM treated snails).

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

**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 *T. pisana* exposed to ABM (Figs. 1–5).

The GST activities in the snails treated with 20% LD50 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% LD50 treated snails after all times of exposure. These significant increases were 135.19% in the 20% LD50 treatment after 7 days and 153.46, 165.49 and 152.63% in the 60% LD50 treatment after 1, 3 and 7 days (Fig. 1).

Significant differences were found in treated and untreated snails in the activity of γ-GT. The activity of γ-GT was significantly increased by exposure to ABM at 20 and 60% LD50 compared to controls (Fig. 2). This increase of enzyme activity was dose-dependent but not time-dependent.

LDH activity in snails treated with either 20 or 60% LD50 ABM was significantly higher than the enzyme activity of untreated snails (Fig. 3).

As shown in Fig. 4, the activity of AST was significantly decreased by 87.39% in the 20% LD50 ABM after 7 days and its activity was also decreased after 1 and 3 days of exposure without significant differences. In case of 60% LD50 ABM, significant decreases in the activity of AST were recorded with values of 90.26, 86.89 and 84.49% after 1, 3 and 7 days, respectively. Decrease of activity was dose- and time-dependent.

Fig. 5 shows that, compared to the control, there was no significant decrease in ALT activity after treatment with two sub-lethal doses after 1 day. 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.[42] 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, 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* [7,29], *Massylaea vermiculata* (Syn: *Eobania vermiculata*) [43-46], *Monacha obstructa* [43,44] and *Deroceras reticulatum*. [47] 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. [48]

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. [25,49] The digestive gland 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 mollusks. [50] 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 in our study, the energy reserves (glycogen, lipids and proteins) and enzyme activities (GST, \( \gamma \)-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. [51] 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 on the energy reserves of land snails. [31,44,52] However, the effect of ABM on energy reserves of *T. pisana* has not yet been reported in the literature, therefore, the negative impact on energy reserves resulting from exposure to ABM needs to be studied.

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. [44] In this investigation, total lipids were significantly decreased in 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. [52,53] In agreement with our results, Megahed et al. [54] 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 the present study, the glycogen contents were significantly decreased in treated snails throughout the experimental periods. This depletion indicating that animals are utilizing their energy reserves to cope with toxic stress [55] or for increased rate of glycogen breakdown "glycogenolysis". [56] The aforementioned findings are in coincidence with those of Riaz et al. [57] 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 LC20 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. [58] In our study, decrease in total protein of snails exposed to 20% LD50 of ABM was observed, however, total protein was increased in snails exposed to 60% LD50 compared to the control. The obtained
data clearly indicated 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 use to repair the damage of cells and/or for straight usage by cells for energy demands.[52,59]

Our data confirmed the results of Kandil et al.[44], where total protein levels in, M. vermiculata and M. obuctra were increased when the snails exposed to ABM as a contact poison. A single dose of 0.25 LD₅₀ ABM significantly decrease the total proteins in male albino rats.[60] Moreover, Al-Kahtani[61] 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 96h.

Since the magnitude of inter-individual variability is often an ecotoxicological response, variance among individuals in end-point responses to a contaminant stress should not be ignored.[62] In the present study, we used the mean and variance to predict and interpret the response pattern to ABM stress. The overall pattern of variance changes of the energy reserves end-points appears to be a rapid response to ABM exposure. Moreover, testing variance changes is important because variance change can indicate the impact of the toxicant and can provide additional relevant information about the effect of the toxicant even when the mean does not predict any impact.[63]

The GST enzyme is a part of the detoxification pathway II via conjugation of xenobiotics and/or endogenous compounds with glutathione (GSH).[25] Our data clearly indicate that ABM induced increment in GST activity of 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.[64] 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.[65] Similar to our investigation, the activity of this enzyme was increased in the same snail species treated with 1/20 LC₅₀ ABM for 2 weeks of exposure.[23] Enhancement of GST activity in the snail, Physa acuta treated with ABM during the periods of 12–48h exposure was also observed.[66]

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.[67] This enzyme plays a central role in the re-synthesis of glutathione. In addition, Lee et al.[68] 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 allowed the rebuilding of cellular antioxidant defenses.[69] In this study, a significant increase in γ-GT activity was noticed due to the treatment of T. pisana snails with ABM. This enzyme elevation may be attributed to the significant tissue injury provoked by pesticides, even at low doses. These results are in line with Khaloud-Oulabri et al.[70] 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.[71]

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.[72] 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.[73] In our study, ABM caused a marked increase in the activity of the LDH enzyme, 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.[74] 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.[75] This may be accompanied by increased LHD levels as a result of the role of LDH in converting the pyruvate into lactate. Previous studies recorded the enhancement of LDH activity in ABM-treated rats.[71] However, ABM recorded no significant decrease in the LDH activity of hamsters uninfected and infected with Schistosoma mansoni.[76]

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 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 deviated from the normal range.[24] 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.[77] 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 LD₅₀ ABM for 24 and 72 h.[78] No significant differences in the AST and ALT activities after treatment of hamsters
uninfected and infected with Schistosoma mansoni with ABM were observed.[26]

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 at doses of 20% and 60% LD₅₀. 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,[79] terrestrial snails,[23,29] and rats.[70] 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.

Disclosure statement

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Data availability statement

The data that support the findings of this study are openly available in Research Square at https://doi.org/10.21203/rs.3.rs-995720/v1

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