# Potential Utility of Protein Content as A Biomarker of Xenobiotic Toxicity in the Land Snail, *Theba pisana*

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## ABSTRACT

**Agricultural and industrial chemicals are currently receiving great attention due to their possible risks to non-target species, including land gastropods. Protein is a key component for living organisms to maintain their vital lives.The present investigation looked at protein content changes in the hemolymph, hepatopancreas, head-foot, and mantle of the land snail, *Theba pisana* subjected to an artificial diet spiked with sub-lethal concentration (0.05 $LC_{50}$) of abamectin, thiamethoxam, and acrylamide for two weeks of treatment followed by a week recovery. The obtained results showed a marked increase in the protein content in all tested tissues of *T. pisana* after two weeks of treatment with abamectin, thiamethoxam, and acrylamide. After one-week post recovery, protein contents in treated animals were slightly repaired, but still higher than those of untreated animals. Abamectin seemed to be more harmful to the protein content of *T. pisana* followed by thiamethoxam and acrylamide. Additionally, the protein content (%) in the hepatopancreas was more affected by the tested compounds. Our study suggests that the protein content response to xenobiotic may prove to be a useful biomarker for biomonitoring terrestrial ecosystem health and providing important information on the mechanisms underlying xenobiotic toxicity.**

**Key words: Land snails, Protein content, Abamectin, Acrylamide, Thiamethoxam.**

## INTRODUCTION

Undoubtedly, increased industrialization, urbanization, and the misuse of agrochemicals have led to a significant release of chemicals and their byproducts in the natural environment, which serve as reservoir for various pollutants. Numerous organic and inorganic substances, including pesticides, industrial chemicals, metals and their nanoparticles, contaminate natural ecosystems, and thus may have harmful impacts on non-target organisms (Pathak *et al.*, 2022).

Among the organic chemicals extensively utilized in both the agricultural and industrial sectors across the globe, are abamectin, thiamethoxam, and acrylamide. These compounds play a crucial role in various applications, contributing to the advancement and efficiency of agricultural practices as well as industrial processes.

Abamectin, a member of macrocyclic lactones belonging to the avermectin family, possesses insecticidal, nematicidal, acaricidal, and anthelminthic activities (Jansson & Dybas, 1998). This biocide is made of a combination of avermectins B1a and B1b (~ 80% and 20%, respectively) with the same toxicological and biological characteristics (Pitterna *et al.*, 2009). Avermectins are extremely toxic to a variety of pest species and can be quite hazardous to mammals (Bai and Ogbourne, 2016).

Thiamethoxam, a second-generation neonicotinoid insecticide, is used to control a wide variety of pest species worldwide. This pesticide has distinctive chemical and biological characteristics; it is effective against a wide range of insects, has a low application rate, and efficient plant uptake and translocation. Neonicotinoids have often demonstrated negative impacts on a variety of non-target invertebrate species in terrestrial and aquatic ecosystems at realistic pollution levels (Pisa *et al.*, 2015).

Acrylamide, identified as an industrial substance, is a primary component of polyacrylamide polymer, that is extensively used in agriculture processing, wastewater and sewage treatments, textile manufacturing, cosmetics, petroleum industries, plastic products, food packaging, paper and grout production, as well as soil conditioners. Acrylamide is also produced when a range of foods rich in carbohydrates are cooked to temperatures above 120 °C. Human beings may be exposed to acrylamide in the environment or at the work (Friedman, 2003). It exhibits genotoxicity, neurotoxicity, carcinogenicity and reproductive impacts in different animals (Krishna and Muralidhara, 2015). Moreover, the International Agency for Research on Cancer (IARC) has determined that the acrylamide monomer is probably carcinogenic to humans as well (Raju *et al.*, 2011).

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Terrestrial snail species including, *Theba pisana* have the potential to play a significant role in assessing the risk of soil contamination and serve as proper sentinel animals "bioindicators" for the health status of ecosystems (Regoli *et al.,* 2006; Radwan *et al.,* 2010 and Louzon *et al.,* 2023). These organisms are characterized by their quick response to contaminants, tolerance to pollutants, ability to accumulate toxic substances in their tissues, suitability for eco-toxicological testing, and enable them to directly transfer contaminants to higher levels of food chains, playing a vital role in the process (Notten *et al.,* 2005 and de Vaufleury *et al.,* 2006).

Over the past few decades, the necessity for assessing the possible risk of exposure to contaminants has prompted the evolution of biomarkers as diagnostic tools for the early-warning detection of pollution (Lionetto *et al.,* 2019). A biomarker is an alteration in an organism's biological response which could be detected in tissues, body fluids, or at the organismal level. It is frequently applied to the evaluation of pollution (Galloway *et al.,* 2002).

Certain contaminants accumulate in the tissues of individuals without causing toxic effects, but others are characterized by increased toxicity even at low exposure levels. Their toxic impacts on an organism may occur either *via* direct action on specific tissues in the body or, in general, *via* affecting biological mechanisms. Most pollutants are certainly produced their toxicity by the increase of reactive oxygen species (ROS) which frequently occur before the onset of changes like protein dysfunction, lipid peroxidation, enzyme inhibition and DNA damage (Winston, 1991). Organisms can adapt a wide range of strategies to reduce the negative impacts of chemical stress. The anti-stress responses originate at the subcellular level and typically entail the perturbation of normal metabolic mechanisms. These responses suggest an energetic cost that depletes the energetic budget needed for other essential processes like growth, defense strategies and reproduction (Widdows and Donkin, 1992). Protein content is one of the indicators that shows the energy reserves status of an organism. Moreover, protein is used rapidly when organisms are under stress, and its content has been proposed as a potential biomarker of general stress. It is critical to the metabolism of energy, building of tissue, architecture of cells and controls how intracellular and extracellular media interact (Remia *et al.,* 2008).

Although a change in protein content has been assumed as a biomarker of xenobiotic-mediated pro-oxidant stress in a range of organisms, there are few studies about using protein content in land snails as a biomarker for xenobiotic toxicity. Therefore, the present investigation aimed to ascertain the protein content in various tissues of *T. pisana* subjected to an artificial diet spiked with sub-lethal concentration (0.05 $LC_{50}$) of abamectin, thiamethoxam, and acrylamide for two weeks of treatment followed by a week recovery, validate its use as a biomarker, and confirm that this snail might serve as a bioindicator.

## MATERIALS AND METHODS

**Tested chemicals:**

Abamectin at a purity of 95% and thiamethoxam at a purity of 97% were supplied by Hailir Pesticides and Chemicals Groups, Egypt. Acrylamide at a purity of 98.5% was obtained from BDH Chemicals Ltd., England. Additional chemicals and reagents for this investigation were acquired from Sigma Chemical Company.

**Tested animals:**

The land snails, *Theba pisana* were gathered from an untreated park in the Alexandria Governorate of Egypt. After collection, the snails were acclimatized in the laboratory (12:12 h light/dark photoperiod, and 25° C) for 14 days and were fed on *ad libitum* with lettuce leaves and fasted for 48 h before trials.

**Experimental layout:**

An artificial food was made based on the method of El-Gendy *et al.* (2011), using 5 g rabbit meal, 3 g sucrose, and 2 g agar with 100 ml of water. Four petri dishes, each received 25 ml of medium, were evenly divided. After being cooled, petri dishes were placed in the refrigerator. Snails were given artificial food discs that had been cut off with a cork borer.

The adult acclimatized snails of approximately similar size were chosen for investigation. The snails were divided into four groups. Snails in group ‘A’ were used as controls. Snails in group ‘B’ were exposed to sub-lethal concentration 0.05 $LC_{50}$ (0.046 µg/g) of abamectin in artificial food. Snails in group ‘C’ were exposed to 0.05 $LC_{50}$ (15.69 µg/g) of thiamethoxam, and group ‘D’ were exposed to 0.05 $LC_{50}$ (2.28 µg/g) of acrylamide that was previously acquired by Gad *et al.* (2016), up to two weeks' exposure followed by toxicant-free diet for a week recovery. Each treatment consisted of three replications (15 animals/replicate). Snails were checked every day, the dead animals were removed, and fresh food was provided as needed.

At the end of the trial period (either two weeks of exposure or one week recovery), the hemolymph, hepatopancreas, head-foot, and mantle were acquired from the treated and untreated snails. The hemolymph (500 µl) was centrifuged at 3000×g for 20 min at 4 °C, and the supernatant (plasma) was obtained to determine protein content. Other tissues (0.5 g) were homogenized using a polytron homogenizer (Tekmar tissumizer) for

60 s in 10 volumes of ice-cold saline. The homogenates were centrifuged in a Janetzki K23 cooling centrifuge set to 5000×g for 20 min at 4 °C. The supernatants were utilized for the quantification of protein content.

**Protein content estimation:**

Total protein content was assessed according to the method described by Lowry *et al.* (1951). Five µl of the supernatant of hemolymph, hepatopancreas, head-foot and mantle were mixed with 0.3 ml of Folin-Ciocalteau phenol reagent. After 30 min, the developed blue color was measured at 750 nm against the blank using a T-80 + UV/VIS spectrometer PG Instrument Ltd. Protein values were obtained from a standard curve by plotting bovine serum albumin at the concentrations ranged from 20 to120 mg/ml.

**Statistical analysis:**

All values are presented as a mean ± standard error. The statistical analysis was carried out using Costat program (2002). ANOVA was used, along with the Student-Newman Keuls test, and a *p*-value of 0.05 was used to compare the means.

## RESULTS AND DISCUSSION

### RESULTS

As shown in Tables (1 and 2), sub-lethal concentration of abamectin, thiamethoxam, and acrylamide were evaluated *in vivo* on the protein content of the hemolymph, hepatopancreas, head-foot, and mantle of *T. pisana* after two weeks of treatment, followed by a week recovery.

As a result of two weeks' exposure to a sub-lethal concentration of abamectin, hemolymph, hepatopancreas, head-foot and mantle protein contents were significantly increased by 130.64, 135.30, 132.78, and 124.37 %, respectively. The treatment of the snails with a sub-lethal concentration of thiamethoxam caused a significant elevation in protein content in all tested tissues compared to the control value. Moreover, the protein content in hemolymph (120.18 %), hepatopancreas (121.78 %), head-foot (121.73 %) and mantle (115.75 %) were significantly increased after exposed the snails to acrylamide compared to controls (Table 1).

The obtained results also clearly indicate that after one-week recovery, abamectin treatment caused significant elevation in protein content in hemolymph, hepatopancreas, head-foot and mantle with values of 126.92, 127.03, 125.16 and 116.86%, respectively. Furthermore, the snails treated with thiamethoxam and acrylamide exhibited significant increases in protein content in all tested tissues except in mantle. Generally, the protein content of all treated snails after a week recovery was lower than after two weeks' exposure, but still higher than that of untreated snails (Table 2).

Overall, abamectin is the most efficient chemical for increasing protein content followed by thiamethoxam and acrylamide. Moreover, the hepatopancreas was more impacted by the tested chemicals than the other organs.

**Table 1. *In vivo* effect of sub-lethal concentration (0.05 $LC_{50}$) of abamectin, thiamethoxam and acrylamide on the protein content (µg/100 mg wet tissue) in various tissues of *Theba pisana* after two weeks of exposure**

| Treatment | Hemolymph | | Hepatopancreas | | Head-foot | | Mantle | |
|---|---|---|---|---|---|---|---|---|
| | Mean ± SE | % of Control | Mean ± SE | % of Control | Mean ± SE | % of Control | Mean ± SE | % of Control |
| Control | 53.62 ± 2.6 $^{a}$ | 100 | 80.81 ± 0.7$^{a}$ | 100 | 83.78 ± 5.4$^{a}$ | 100 | 90.89 ± 2.8$^{a}$ | 100 |
| Abamectin | 70.05 ± 1.0$^{b}$ | 130.64 | 109.34±1.5$^{b}$ | 135.30 | 111.25±5.6$^{b}$ | 132.78 | 113.04±4.0$^{b}$ | 124.37 |
| Thiamethoxam | 67.92 ± 1.4$^{b}$ | 126.67 | 105.92±2.2$^{b}$ | 131.06 | 107.36±3.2$^{b}$ | 128.14 | 107.10±5.7$^{b}$ | 117.83 |
| Acrylamide | 64.44 ± 2.4$^{b}$ | 120.18 | 98.36±1.9$^{b}$ | 121.78 | 101.90±5.6$^{b}$ | 121.73 | 105.21±3.4$^{b}$ | 115.75 |

Means having the same letter in each column are not significantly differences from control ($p \leq 0.05$).

**Table 2. Protein content (µg/100 mg wet tissue) in various tissues of *Theba pisana* exposed to sub-lethal concentration (0.05 $LC_{50}$) of abamectin, thiamethoxam and acrylamide after one- week recovery**

| Treatment | Hemolymph | | Hepatopancreas | | Head-foot | | Mantle | |
|---|---|---|---|---|---|---|---|---|
| | Mean ± SE | % of Control | Mean ± SE | % of Control | Mean ± SE | % of Control | Mean ± SE | % of Control |
| Control | 54.51±2.6$^{a}$ | 100 | 83.06±1.6$^{a}$ | 100 | 84.81±5.2$^{a}$ | 100 | 90.52±5.3$^{a}$ | 100 |
| Abamectin | 69.19±3.9$^{b}$ | 126.92 | 105.52±2.0$^{b}$ | 127.03 | 106.16±2.6$^{b}$ | 125.16 | 105.78±0.6$^{b}$ | 116.86 |
| Thiamethoxam | 65.83±4.9$^{b}$ | 120.75 | 103.23±3.6$^{b}$ | 124.29 | 100.44±1.7$^{b}$ | 118.42 | 102.35±7.8$^{a}$ | 113.07 |
| Acrylamide | 64.50±3.0$^{b}$ | 118.31 | 99.12±6.1$^{b}$ | 119.33 | 97.98±1.8$^{b}$ | 115.51 | 98.44±5.8$^{a}$ | 108.76 |

Means having the same letter in each column are not significantly differences from control ($p \leq 0.0\ 5$).

## DISCUSSION

The impacts of pollution on ecosystems have been a subject of concern and exploration for more than a century. It is important to monitor the eco-toxicological effects of xenobiotic on the environment with biomarkers as early-warning tools. The impact of a pollutant can be partially understood by its effect on the protein content, a key component of the organisms (Radwan *et al.*, 2008).

Protein content is one of the essential components of body and a proximal constituent of the protoplasm, and serve as a growth material for living organisms. It is a key factor in the architecture, physiology and metabolism of the cell. It also serves as a buffer in the acid-base balance, a source of fast tissue protein replacement during tissue depletion, and a transporter of blood components such as vitamins, lipids, hormones, copper, iron, and some enzymes (Warnick and Cartar, 1972). Furthermore, it is one of the energy reserves which used as fuel for organism's numerous physiological and metabolic functions (Calow & Forbes, 1998 and McLoughlin *et al.,* 2000). Any alterations in protein content from its normal level can be considered as a stress biomarker which then influences physiological activity.

In the present study, it was obviously shown that the protein contents in different tissues increased after dietary exposure to sub-lethal concentration of abamectin, thiamethoxam, and acrylamide. This elevation might be due to the induction of protein synthesis under the effect of stress induced by exposure to the tested xenobiotic or due to the increased energy demand to cope with the stress condition.

Our data are consistent with earlier findings of Radwan and Gad (2022) who found that after 1 and 7 days, *T. pisana* snails subjected to 60% $LD_{50}$ of abamectin had a substantial increase in total protein in their hepatopancreas. However, after 3 days of exposure, there was non-significant effect in total protein. Also, a significant increase in total protein was indicated in *T. pisana* snails treated with 20 and 60% of emamectin benzoate for 1, 3 and 7 days compared with untreated snails (Gad, 2022). Kandil *et al.* (2009 and 2014) found that the total proteins in *Monacha obstructa and Eobania vermiculata* were increased when snails exposed to abamectin as a baiting technique or contact poison up to 7 days. Mobarak (2014) reported that the $LC_{50}$ of thiamethoxam-based formulation caused a fluctuating response to the total protein content of *E. vermiculata* where it elevated 1-day post exposure then it diminished 3 days' post treatment, but increased again compared to the control after 7 days' post treatment. As for *M. obstructa*, total protein content increased after 1 day of the administration, and then decreased after 3 and 7 days of exposure. Indeed, total protein levels in the hepatopancreas of *Helix aspersa* snails treated with sub-lethal doses of imidacloprid (0.2 and 0.6 $LD_{50}$) were higher than those in untreated controls after 1, 3, and 7 days of exposure (Radwan and Mohamed, 2013). Furthermore, Zouaghi *et al.* (2020) noted a dose-dependent increase in the total protein contents in two target organs (hepatopancreas and kidney) of *H. aspersa* subjected to the mixtures of acetamiprid and imidacloprid compared to the control. In contrary, total proteins were considerably reduced at doses of 100 and 200 mg/L of thiametoxam after six weeks of *H. aspersa* exposure by ingestion or contact with fresh lettuce leaves (Hamlet *et al.,* 2012).

## CONCLUSION

Numerous contaminants have the potential to negatively impact gastropod mollusc species, which are regarded as bioindicators of environmental biomonitoring. The biomarkers of land gastropod have grown in significance for evaluating the effects of chemicals on terrestrial ecosystems. In fact, change in the protein content has the potential to affect various other biological disorders such as growth, defense mechanisms and reproduction. Our data revealed that sub-lethal concentrations of the tested xenobiotics; abamectin, thiamethoxam and acrylamide have significant impacts on protein content in the hemolymph, hepatopancreas, head-foot and mantle of the land snail, *T. pisana* after two weeks of treatment followed by a week recovery. So, from our findings it can be deduced that assessing the protein content in different tissues of *T. pisana* exposed to tested chemicals might be efficient endpoint and would consider a helpful biomarker and a useful tool for identifying chemical stress in terrestrial ecosystems. Furthermore, *T. pisana* can be utilized as a useful bioindicator animal for the diagnosis of environmental contamination and eco-toxicological impacts of various pollutants. Additionally, this research also served to consolidate the use of *T. pisana* snail as an excellent model organism for ecotoxicology investigations. Finally, our study suggests that further studies based on protein profiles, Western blots of protein patterns, and application of novel proteomic techniques should be conducted on the tested animal to analyze the entire set of proteins and identify changes in the proteins that may be induced by chemicals.

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## الملخص العربي

## أمكانية استخدام محتوى البروتين كمؤشر حيوي لسمية المركبات في القوقع الأرضي *Theba pisana*

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تحظى المواد الكيميائية الزراعية والصناعية حاليًا باهتمام كبير نظرًا لمخاطرها المحتملة على الكائنات غير المستهدفة بما في ذلك بطنيات الأقدام البرية. البروتين هو عنصر أساسي للكائنات الحية للحفاظ على حياتها الحيوية. تهدف الدراسة الحالية إلى اختبار التغير في محتوى البروتين في الليمف الدموي والقناة الهضمية والرأس– قدم والوشاح في القوقع الارضي *Theba pisana* بتعريضه لإقراص البيئة المحتوية على ٠،٠٥ من التركيز النصف مميت من الأبامكتين والثيامثوكسام والأكريلاميد لمدة أسبوعين متبوعا بأسبوع من الاستعادة. وقد أظهرت النتائج التي تم الحصول عليها حدوث زيادة ملحوظة في محتوى البروتين في جميع أنسجة القوقع والتي تم اختبارها بعد أسبوعين من التعرض للأبامكتين والثيامثوكسام والأكريلاميد، وبعد أسبوع من الاستعادة، تم إسترجاع محتوى البروتين في القواقع المعاملة قليلاً ولكن مستواه ظل أعلى من مستواه في القواقع غير المعاملة. بشكل عام، ظهر أن الأبامكتين أكثر ضررًا على محتوى البروتين في قوقع الحدائق الأبيض من الثيامثوكسام والأكريلاميد. بالإضافة إلي أن محتوي البروتين في القناة الهضمية أكثر تأثرا بالمبيدات المختبرة – وتشير دراستنا إلي أن استجابة محتوى البروتين للملوثات قد تكون علامة حيوية مفيدة للمراقبة الحيوية لصحة النظام البيئي الأرضي وتوفير معلومات مهمة عن الآليات الكامنة وراء سمية الملوثات المختلفة.