International Journal of Zoological Investigations

Contents available at Journals Home Page: www.ijzi.net

ISSN: 2454-3055

Effects of Abamectin on Glutamate Decarboxylase, Alanine Aminotransferase and Aspartate Aminotransferase of Land Snails

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Received: 20th June, 2020

Accepted: 24th July, 2020

Published online: 1st August, 2020

<https://doi.org/10.33745/ijzi.2020.v06i02.010>

Abstract: The objective of this study was to examine the effect of abamectin on glutamate decarboxylase (GAD), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in land snails, *Eobania vermiculata* (BGS) and *Theba pisana* (WGS). We investigated the *in vivo* effects of abamectin on glutamate decarboxylase (GAD) of snails which was determined by HPLC. Snails (*Eobania vermiculata* and *Theba pisana*) were exposed to abamectin at 0.1, 0.2 and 0.5 of LD₅₀ concentrations for 24 and 72 h. The most effective dose of abamectin on GAD activity in both snails was 0.5 of LD₅₀ after 24 and 72 h which caused maximum elevation of GAD activity. In *Eobania vermiculata* ALT activity significantly increased with an increase in the abamectin dose. Elevation of ALT activity reached 737 and 750% at 0.5 of LD₅₀ after 24 and 72 h, respectively, followed by 516 and 567% activity at 0.2 of LD₅₀ value after 24 and 72 h and 333 and 400% at 0.1 of LD₅₀ after 24 and 72 h, respectively. In *Theba pisana* maximum ALT activity was 478 and 500% at 0.5 of LD₅₀ value after 24 and 72 h, respectively. Abamectin at 0.2 of LD₅₀ concentration provoked 322 and 333% ALT activity in *Theba pisana* after 24 and 72 h exposure.

Increased AST activity (n=5, p <0.05) has been recorded in *Eobania vermiculata* after abamectin at 0.5 of LD₅₀ concentration after 24 (540%) and 72 h (560%) whereas after 0.2 of LD₅₀ abamectin concentration the AST values at 24 and 72 h were 400 and 420% respectively. Abamectin exposure to *Eobania vermiculata* at 0.1 of LD₅₀ concentration increased AST activity after 24 (340%) and 72 h (360%). AST activity in *Theba pisana* was increased by 500 and 580% after 0.5 LD₅₀ abamectin exposure at 24 and 72 h, respectively. The values of AST activity in *Theba pisana* after 0.2 of LD₅₀ abamectin concentration were 340 and 360% after 24 and 72 h, respectively whereas after 0.1 of LD₅₀ abamectin concentration 180 and 190% after 24 and 72 h, respectively.

Keywords: Alanine aminotransferase, Aspartate aminotransferase, Glutamate decarboxylase, Snails, Abamectin, *Eobania vermiculata*, *Theba pisana*

Citation: Hamza S.A., Abdelgalil G.M., Abdallah E.A.M., Kassem F.A. and Asran A.A.: Effects of abamectin on glutamate decarboxylase, alanine aminotransferase and aspartate aminotransferase of land snails. Intern. J. Zool. Invest. 6 (2): 311-320, 2020. <https://doi.org/10.33745/ijzi.2020.v06i02.010>

Introduction

Land snails are considered as serious economic pests as they cause damage to several types of plants. These snails attack leaves, flowers, roots, buds, and even the trunk of trees causing great damage to the cultivated plants. In Egypt, terrestrial snails are most dangerous pests to most crops such as field crops, vegetables, ornamental plants and orchards (Kassab and Daoud, 1964; El-Okda, 1979; Abo-Bakr, 1997, 2004; Abdallah *et al.*, 1992, 1998, 2015; Ibrahim, 1995; Mohamed, 1995; Abdelgalil, 2011, 2016, 2018). Damage caused by snails is mainly due to feeding and to contamination with their bodies, faeces or slime, leading to deterioration of the product quality besides, the financial loss (Lglesias *et al.*, 2003). The importance of terrestrial snails, as pests has definitely expanded in the previous decades. (Godan, 1983; Gathwaite and Thomas, 1996).

There are only few pesticides having molluscicidal activity therefore it is needed to search more pesticides having molluscicidal activity. Avermectins are neurotoxins which act through the glutamate or potentially γ -amino butyric corrosive (GABA)-gated chloride channel (Bai and Ogbourne, 2016). Abamectin, a blend of avermectins, is isolated by fermentation of *Streptomyces avermitilis*, bacterium (Strong and Brown, 1987; Lasota and Dybas, 1991; Bai and Ogbourne, 2016; He *et al.*, 2009; Khalil and Darwesh, 2019). It acts on the GABA receptor/chloride ionophore complex and glutamate-gated chloride channel (Huang and Casida, 1997; Kane *et al.*, 2000; Hussein and Sabry, 2019). Recently abamectin-based formulations have been studied on antioxidant and detoxifying enzymes in *Daphnia magna* (Moriena *et al.*, 2020).

In this study the effects of abamectin on glutamate decarboxylase (GAD), alanine transaminase (ALT) and aspartate transaminase (AST) activity of two species of terrestrial snails, brown garden snail (BGS) *Eobania vermiculata* and white garden snail (WGS) *Theba pisana* have been studied.

Materials and Methods

Chemicals:

Abamectin (avermectin B1a > 80% and avermectin B1b < 20%)(95%) Technical was supplied by Agrochem Co. Egypt. Dimethyl sulfoxide (DMSO), potassium phosphate, L-glutamic acid (L-Glu), pyridoxal 5'-phosphate (PLP), water and methanol (HPLC grade) were supplied by National Institute of Oceanography and Fisheries (NIOF), Central Laboratories Unit (CLU), Alexandria, Egypt. Other chemicals used were analytical grade including sodium acetate, sodium hydroxide, 3-mercaptopropionic acid (3-MPA), o-phthaldialdehyde (OPA), boric acid and glacial acetic acid (all from Sigma Chemical Co. St. Louis, MO, USA). Alanine transaminase (ALT) and aspartate transaminase (AST) enzymes were assayed with Diamond Diagnostic kits (Diamond Co. Egypt).

Experimental snails:

Adults of the brown garden snail, *Eobania vermiculata* (Müller) and the white garden snail, *Theba pisana* (Müller) having approximately the same age and size were collected for laboratory experiments. These snails were collected from El-Maamoura garden during spring, Alexandria, Egypt. The snails were transferred to glass boxes (50x30x40 cm) containing 10 cm soil at the bottom. Boxes were covered with cloth

netting to prevent snails from escaping. These boxes were maintained under laboratory conditions at 27 C and 65% R.H. The snails were daily fed on lettuce leaves (*Lettuce sativa*) and acclimatized to laboratory conditions for 14 days.

Topical application procedure was used according to Abdallah *et al.* (1992). Abamectin technical was dissolved in dimethyl sulfoxide (DMSO). DMSO has been demonstrated to be the most suitable for topical application as it makes little distress to snails (Young and Wilkins; 1989). *Eobania vermiculata* (BGS) and *Theba pisana* (WGS) were exposed to three sub-lethal doses of abamectin at 0.1, 0.2 and 0.5 of LD₅₀ values for 24 and 72 h. The LD₅₀ values for 24 and 72 h for *Eobania vermiculata* were 0.379 and 0.250 and for *Theba pisana* 0.199 and 0.125 µg/g body weight, respectively (Abdelgalil *et al.*, 2018). A control group of each species of snails was also run in same laboratory conditions.

Sample preparation:

Abamectin treated and control (untreated) snails of both species were procured after 24 and 72 h. Snail shells were evacuated and the soft bodies were homogenized in 1:10 (w/v) 200 mM potassium phosphate buffer, pH 6.8 utilizing Polytron Kinemetica homogenizer. The homogenate was centrifuged at 5000 rpm for 30 min at 4 C utilizing IEC-CRU 5000 under cooling centrifuge. Supernatant was utilized as the source of enzyme for the determination of enzymes.

Glutamate decarboxylase (GAD) activity:

Enzyme estimation was done by the technique of Allen and Griffiths (1984) with certain modifications. 10 µl of source of enzyme, 140 µl (200 mM potassium phosphate buffer, pH

6.8), 400 µl (50 mM L-Glu) and 55 µl (0.2 mM pyridoxal 5'- phosphate, PLP) were added. After brooding for 20 min at 37 C, 605 µl methanol was added at room temperature to end the response and at a similar minute deproteinization operator. The suspension was centrifuged at 1500 rpm (10 min, 0 C). L- glutamic acid present in the supernatant was estimated as indicated by Abdelgalil *et al.* (2018) with certain modifications.

Standard calibration curve for OPA- glutamic acid linear response over the concentration range of 0.15, 0.3, 0.6, 1.2 and 2.4 n mole as shown in Figure 1 was determined by applying the similar derivatization procedure. The corresponding optical density was recorded as previously mentioned. Glutamic acid was calculated as µg/mg protein.

Calculate Limit of Detection (LOD) for each standard by formula: $3.3 \times \text{standard error/slope}$.

Calculate of Limit of Quantitation (LOQ) for each standard by formula: $10 \times \text{standard error/slope}$.

Alanine transaminase (ALT) and aspartate transaminase (AST) activities:

Alanine transaminase (ALT) and aspartate aminotransferase (AST) were estimated according to the method of Reitman and Frankel (1957). Absorbance was measured at 546 nm.

Statistical analysis:

The data were presented as the mean $\pm$ S.D. Analysis of variance (ANOVA) and differences among the means were determined for significance at 0.05 using (Fisher LSD test) by using the SAS software programme.

Fig. 1: Standard calibration curve for OPA-glutamic acid linear response over the concentration range of 0.15-2.4 n mole.

Fig. 2 : Chromatogram illustrating the separation of OPA-derivatized L-glutamic acid (L-GLU).

Results and Discussion

The effects of abamectin on glutamate decarboxylase activity of snails *Eobania vermiculata* and *Theba pisana* have been shown in Table 1. After 24 h following abamectin exposure the glutamate decarboxylase activity in *Eobania vermiculata* was increased which was maximum in 0.5 of LD₅₀ abamectin (134.50%) followed by 0.2 of LD₅₀ abamectin (124.9%) and 0.1 of LD₅₀ abamectin (120.6%). In *Eobania vermiculata* the glutamate decarboxylase activities recorded at 72 h were 147.4% at 0.5 of LD₅₀ abamectin, 143.4% at by 0.2 of LD₅₀ abamectin and 135.6% at by 0.1 of LD₅₀ abamectin (Table 1).

In *Theba pisana* GAD activities noticed after 24 h were 133.6%, 131.4% and 124.4% after 0.5 of LD₅₀, 0.2 of LD₅₀ and 0.1 of LD₅₀ abamectin exposure, respectively. After 72 h following abamectin exposure the glutamate decarboxylase activity in *Theba pisana* was increased (Table 1) which was maximum in 0.5 of LD₅₀ abamectin (144.7%) followed by 0.2 of LD₅₀ abamectin (142.8%) and 0.1 of LD₅₀ abamectin (135.2%).

Glutamate decarboxylase (GAD, EC 4.1.2.5) play a significant role in brain excitability through the combination of γ -aminobutyric acid (GABA), the major inhibitory synapse in the central nervous system (CNS) and is considered as a particular marker for GABAergic neurons and their procedures (Wu *et al.*, 1981; Rossetti and Lombard, 1996). Therefore the evaluation of GAD activity is very important in neurochemical as well as toxicological research (Bedair, 2010). However, GABA has been appeared to evoke both inhibitory and excitatory activities in the central neurons (Walker, 1986, Yarovsky and Carpeneter, 1978) in mollusca -- *Limax maxima* (Cooke and Gelperin, 1988) and *Helix pomatia* (Hernadi, 1994). Figure 2 illustrates the chromatogram of the separation of OPA-derivatized L-glutamic acid (L-GLU) which appeared with retention time at 7.76 min. while Figure 3 illustrates chromatogram of the separation of L-glutamic acid derivatives HPLC separation due to abamectin treatments of snails -- (A) *Eobania vermiculata* and (B) *Theba pisana* after 24 h at (0.5 of LD₅₀ value) with retention time at 7.533 and 7.534 min, respectively. The spectrums indicated the same values of retention time as indicated with standard OPA derivatized L-glutamate.

Table 1: Effects of *in vivo* abamectin on *Eobania vermiculata* (BGS) and *Theba pisana* (WGS) glutamate decarboxylase (GAD) activities

	<i>Eobania vermiculata</i>			<i>Theba pisana</i>		
Exposure time	Sub-lethal Doses $\mu\text{g/g b.w.}$	Specific activity (nM glutamic/ mg protein/min) $\pm$ SD	Activity %	Sub-lethal Doses $\mu\text{g/g b.w.}$	Specific activity (nM glutamic/ mg protein/min) $\pm$ SD	Activity %
24 h	0.1	7.34 $\pm$ 0.11	120.6	0.1	6.99 $\pm$ 0.22	124.4
	0.2	7.61 $\pm$ 0.20	124.9	0.2	7.385 $\pm$ 0.100	131.4
	0.5	8.18 $\pm$ 0.030	134.5	0.5	7.505 $\pm$ 0.10	133.6
LSD 0.05		0.226		0.255		
72 h	0.1	8.25 $\pm$ 0.05	135.6	0.1	7.599 $\pm$ 0.104	135.2
	0.2	8.72 $\pm$ 0.11	143.4	0.2	8.022 $\pm$ 0.054	142.8
	0.5	8.97 $\pm$ 0.09	147.4	0.5	8.129 $\pm$ 0.105	144.7
LSD 0.05		0.146		0.155		

Specific activity of untreated snail (GAD) 6.086 $\pm$ 0.0211 and 5.619 $\pm$ 0.046 (nM glutamic/ mg protein/min) $\pm$ SD for *Eobania vermiculata* and *Theba pisana*, respectively, $n = 5$ animals; LSD_{0.05} (least significance difference test at 5%); b. w. (body weight)

Fig. 3: Chromatogram illustrating the separation of L-glutamic acid derivatives HPLC separation due to abamectin treatments after 24 h at 0.5 of LD₅₀ value). **(A)** *Eobania vermiculata* (BGS); **(B)** *Theba pisana* (WGS), with retention time at 7.533 and 7.534 min, respectively. The spectrums indicated the same values of retention time as indicated with standard OPA derivatized L-glutamate.

The mechanism by which avermectins produce the pesticide is the release of γ -aminobutyric acid (GABA) and the improvement of its inhibitory action observed that GABA concentrations were elevated in earthworm, (*Allolobophora*

caliginosa) as result of treatment with ivermectin. Yamazaki *et al.* (1989) reported that ivermectin is an agonist for the GABA synapse. In this way, the binding of ivermectin to a neuronal membrane elevate the release of GABA which binds to the

GABA-receptor-chloride channel complex of postsynaptic neuronal membranes causing hyperpolarization of neuronal membrane causing influx of chloride ions, Therefore, it mediates a flaccid paralysis in nematodes and arthropods.

Kass *et al.* (1980, 1984) used *Ascaris lumbricoides* as a model system and reported that avermectins function as a GABA agonist which stimulates GABA release from pre-synaptic inhibitory membranes. Abou-Taleb *et al.* (2009) reported that emamectin benzoate increased the GABA neurotransmitter and glutamic acid concentrations in the field strain of cotton leaf worm to lesser extent than in the laboratory strain. GABA and glutamic acid augmentation are dependant on the emamectin benzoate concentration and the time of exposure. The results of the present study are in agreement with observations of Bedair (2010) and Abdelgalil (2011, 2016, 2018).

The launch of ivermectin in the mid 1980s brought a revolution in the control of animal parasites and was accepted as a potent anthelmintic (Campbell and Benz, 1984). Also, avermectins have wide application as pesticides and antiparasitic drugs for humans and animals (Burg *et al.*, 1979; Ostlind and Long, 1979; Campbell, 1989). The most extensively used compounds of this class are avermectin B1 (abamectin). Abamectin were effectively used for control of the ectoparasite sea lice, the cause of serious problem for the salmon (*Salmo salar*) in aquaculture industry (Westcott *et al.*, 2004). Campbell (1989) stated that the mechanism by which avermectins produce the pesticide and antiparasitic effects in invertebrates and a

neurotoxic effect in vertebrates, is the release of γ -aminobutyric acid (GABA) and the enhancement of its inhibitory action. Yamazaki *et al.* (1989) reported that ivermectin is an agonist for the GABA neurotransmitter.

Toxicants are transported by hemocytes to the hepatopancreas through various routes. The damage of liver, the metabolic center for detoxification of synthetics, is affirmed by changes in the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The activities of such markers show the metabolic changes under the impact of stress. Changes in the degrees of these enzymes in serum are really the most important signal of liver toxicity (Abdelmonem, 2016).

In *Eobania vermiculata* ALT activity significantly increased with an increase in the abamectin dose. Elevation of ALT activity reached 737 and 750% at 0.5 of LD₅₀ after 24 and 72 h, respectively, followed by 516 and 567% activity at 0.2 of LD₅₀ value after 24 and 72 h and 333 and 400% at 0.1 of LD₅₀ after 24 and 72 h, respectively (Fig. 4 A). In *Theba pisana* maximum ALT activity was 478 and 500% at 0.5 of LD₅₀ value after 24 and 72 h, respectively. Abamectin at 0.2 of LD₅₀ concentration provoked 322 and 333% ALT activity in *Theba pisana* after 24 and 72 h exposure (Fig. 4 B).

Increased AST activity (n=5, p <0.05) has been recorded in *Eobania vermiculata* after abamectin at 0.5 of LD₅₀ concentration after 24 (540%) and 72 h (560%) whereas after 0.2 of LD₅₀ abamectin concentration the AST values at 24 and 72 h were 400 and 420% respectively (Fig. 5 A). Abamectin exposure

to *Eobania vermiculata* at 0.1 of LD₅₀ concentration increased AST activity after 24 (340%) and 72 h (360%). AST activity in *Theba pisana* was increased by 500 and 580% after 0.5 LD₅₀ abamectin exposure at 24 and 72 h, respectively (Fig. 5 B). The values of AST activity in *Theba pisana* after 0.2 of LD₅₀ abamectin concentration were 340 and 360% after 24 and 72 h,

(A)

(B)

Fig. 4: Effect of abamectin exposure on the ALT activity in both snails-- (A) *Eobania vermiculata* (BGS) and (B) *Theba pisana* (WGS). Each value represents the mean $\pm$ SD of five animals, Asterisk indicates significant difference from control value ($p < 0.05$; $n = 5$).

(A)

(B)

Fig. 5: Effect of abamectin exposure on the AST activity in both snails-- (A) *Eobania vermiculata* (BGS) and (B) *Theba pisana* (WGS). Each value represents the mean $\pm$ SD of five animals, Asterisk indicates significant difference from control value ($p < 0.05$; $n = 5$).

respectively whereas after 0.1 of LD₅₀ abamectin concentration 180 and 190% after 24 and 72 h, respectively.

Toxicity of abamectin may affect the hepatocytes. Transaminases (amino-transferases) constitute a group of enzymes that catalyze the interconversion of amino acid in α -keto acid by transferring amino group (Moss and Henderson, 1998). Alanine transaminase (ALT) and aspartate transaminase (AST) are present in

molluscan digestive gland (hepatopancreas) and spill out into general flow when hepatic cells are harmed. The transaminases are acceptable marker of tissue sore. This elevation of ALT and AST has long been used as indicators of damage to hepatic and cardiovascular tissues (Wroblewski and La Due, 1956).

Elevation of AST and ALT of gastropods due to exposure to organic pollutants has been reported in past (Henry *et al.*, 1974; Narvia and Rantamaki, 1997). Previous investigation has demonstrated that abamectin causes increased serum aspartate aminotransferase (AST) (Lowenstein *et al.*, 1996; Hsu *et al.*, 2001). Increased AST, a cytosolic enzyme of the hepatocytes, reflects the increase of plasma membrane permeability resulting from the damage of hepatocytes (Plaa and Hewitt, 1982) and is used to detect liver damage (Klaasen and Eaton, 1991). The results of present study are in conformity with the observations of Ibrahim (2001), Badawy (2010) and Abdelgalil (2011, 2016).

Elevation of the enzyme activity may be due to increased synthesis or decreased catabolism of both enzymes, however, the deviation of both enzyme activities from normal range could lead to biochemical disturbances and alterations of liver tissue and cellular function. Also, elevation of these enzymes is related to increased energy demand as organisms attempt to overcome the toxic effects of pollutants (Reddy and Yellamma, 1991). In the ongoing study, increased activities were observed in both enzymes, especially at higher doses of abamectin.

Conclusion

This study indicates that abamectin caused acute toxicity to terrestrial snails in terms of provoking adverse effects on enzymes such as GAD, ALT and AST. Thus abamectin could be alternative to molluscicides such as carbamate and/or other toxic pesticides which cause adverse effects to environment and non-target organisms.

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