# Impact of abamectin and emamectin benzoate against terrestrial snail glutamate decarboxylase (GAD) activity

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## ABSTRACT

We investigated the *in vivo* effects of abamectin, emamectin benzoate and methomyl on - (GAD in *Eobania vermiculata*brown garden snail (BGS) and *Theba pisana* white garden snail (WGS)). GAD activity was evaluated by measuring the formation of gamma aminobutyric acid after derivatization to phenylthiocarbamoyl GABA (PTC-GABA) using HPLC with UV absorbance detection at 245 nm.PTC-GABA and PTC-Glutamic acid were used as standards with retention times of 3.86 and 10.012, respectively. The results revealed thatboth abamectin and emamectin benzoate markedly stimulated GAD activity in both types of the land snails. In contrast, methomyl clearly inhibited GAD activity in a dose-dependent manner. That the activity of GAD enzyme increased by decreasing the dose treatments in both types of snails. Inhibition of GAD activity was more pronounced in BGS than in WGS. Abamectin was more efficacious than emamectin benzoate in stimulating GAD activity, especially in WGS. The stimulatory effects decreased with time, with minimal stimulation observed in BGS at 72 hr with the lowest concentration used (1/10 of $LD_{50}$). Noteworthy, the expression level of GAD in BGS was higher than in WGS, indicating more participation of GABAergic system of *Eobania vermiculata* compared with *Theba pisana* in this respect. These findings may shed light on the mechanism by which abamectin and emamectin benzoate elevate the level of GABA neurotransmission in *E. vermiculata* and *T. pisana* land snails, as they activate the biosynthesis of GABA and inhibit its degradation.

**Keywords**: Abamectin, emamectin benzoate, glutamate decarboxylase, snails

## INTRODUCTION

Terrestrial gastropods are among the most significant threats of sustainable agriculture in many parts of the world (Barker, 2002). From the terrestrial gastropods, land snails are considered as serious economic pests due to its considerable damage of several types of plants. Land snails attack leaves, flowers, roots, buds, and even the trunk of trees causing great damage to the cultivated plants. In Egypt, land snails are known as dangerous pests to field crops, vegetables, orchards and ornamental plants (Kassab and Daoud, 1964; El-Okda, 1979; Ibrahim, 1995; Mohamed, 1995; Abo-Bakr, 1997 and Abdallah *et al*., 1998).Damage caused by snails is due mainly to feeding and to contamination with their bodies, faces or slime, leading

to deterioration of the product quality besides, the financial loss (Lglesias *et al*., 2003). The importance of land snails as pest organisms has drastically increased in the past few decades (Godan, 1983; Gathwaite and Thomas, 1996).

Carbamate and oxime carbamate pesticides have been found to possess high potential use as molluscicides against terrestrial gastropods (Judge, 1969; Hunter and Johnson, 1970; Godan, 1983; Miller *et al*., 1988; El-Okda *et al*., 1989; Radwan and El-Wakil, 1991; Abdallah *et al*., 1992 and 1998; Abo-Bakr 1997; Abdallah *et al.,* 2015; Abdelgalil, 2016). Methomyl is one among the most potent oxime carbamates used especially in bait formulation forms against terrestrial slugs and snails.However, growers and farmers often experience difficulty controlling land gastropods with conventional bait pellets containing molluscicides as in wet conditions the efficacy of these pellets can be very low leading to unsatisfactory control levels (Hata *et al*., 1997; Schuder *et al*., 2003). In addition, poison baits can be toxic to other non-target life farms (Martin, 1993; Purvis, 1996).

Due to the scarce numbers of pesticides with molluscicidal activity, searching about pesticides with molluscicidal activity becomes so urgent. From these pesticide groups which can be used molluscicides is avermectin group because of their acaricidal, gastrointestinal nematicidal activity, also their activity against the sea lice. Avermectins, a group of 16-membered macrocyclic lactones, are fermentation products from *Streptomyces avermitilis*, a naturally occurring soil actinomycete. From the fermentation, eight different avermectins were isolated, which comprise four pairs of homologues. One of these pairs, avermectin B1, that is the mixture of avermectins B1a (>80%) and B1b (<20%), is commercialized under the common name abamectin (Pitterna *et al*., 2009).Abamectin is currently the main avermectin compound used as a miticide/ insecticide in a great variety of crops. Chemical modifications on its original structure with the aim of increasing its insecticidal spectrum resulted in the discovery of emamectin benzoate (4'–deoxy-4'–epi-N-methylamineavermectin B1), one of many 4' – substituted analogs that show an increased potency against lepidoptera larvae (Mrozik, 1994). Emamectin benzoate and avermectins modulate specific glutamate-gated anion channels in synapses and muscle cells (Dunbar *et al*., 1998), thereby increasing the influx of chloride ions. This hyperpolarizes the cell, and prevents depolarization of the neuromuscular endplate beyond the threshold level (Davies and Rodger, 2000). Thus Emamectin benzoate has neurotoxic properties, and it is most effective in arthropods following ingestion (Roberts and Hutson, 1999).

The development of effective alternatives to conventional molluscicides, particularly those that could be used in an integrated control strategy, would reduce plant losses, improve plant quality and offer a sustainable strategy for controlling land snails pests with reduced molluscicide input. The aim of this research is to evaluate *in vivo* effects of abamectin, emamectin benzoate and methomyl against glutamic acid decarboxylase (GAD) of terrestrial snails.

## MATERIALS AND METHODS

### Tested Pesticides

Abamectin **(**5-*O*-demethylavermectin A1a (i) mixture with 5-*O*-demethyl-25-de (1-methylpropyl)-25-(1-methylethyl) avermectin A1a (ii) was supplied by Syngenta.
Emamectin benzoate (4''*R*)-5-*O*-demethyl-4''-deoxy-4'' (methylamino) avermectin A1a + (4''*R*)-5-*O*-demethyl-25-de(1-methylpropyl)-4''-deoxy-4''-(methylamino)-25-(1-methylethyl) avermectin A1a (9:1) was supplied by Syngenta.
Methomyl (methyl N-[[(methylamino) carbonyl]oxy]ethanimidothioate was supplied by E.I. du Pont de Nemours & Co.

### *In vivo* effect of abamectin, emamectin benzoate and methomyl on the glutamic acid decarboxylase (GAD) of *E. vermiculata* and *T. pisana*

*E. vermiculata* and *T. pisana* snails were topically treated with abamectin, emamectin benzoate and methomyl at 1/10 $LD_{50}$, 1/5 $LD_{50}$ and ½ $LD_{50}$ plus untreated snails as a control. Treated snails were collected after 24, 48 and 72 hrs of treatment. Snail shells were removed and the soft tissue was homogenized in 1:10 (w/v) 200 mM potassium phosphate buffer, pH 6.8 using Polytron Kinemetica homogenizer. The homogenate was centrifuged at 5000 rpm for 30 min at 4ºC using IEC-CRU 5000 cooling centrifuge. Supernatant was used as the enzyme source for the determination of enzyme.

### Enzyme assay

Enzyme assay was carried out according to the method of Allen and Griffiths (1984). To 100 µl of enzyme source, 0.5 ml (200 mM potassium phosphate buffer, pH 6.8), 15 µl (50 mM L-Glu) and 55 µl (0.2 mM pyridoxal 5'-phosphate (PLP)) were added. After incubation for 20 min at 37°C, 3 ml absolute ethanol was added at -20°C to terminate the reaction. The suspension was centrifuged at 1500 rpm (10 min, 0°C).GABA and glutamic acid present in the supernatant were derivatized to phenylthiocarbamyl-GABA (PTC-GABA) and PTC-Glutamic acid following the indications of Rossetti and Lombard (1996) as follow GABA and glutamic acid present in the supernatant or in a standard solution of GABA and glutamic acid of convenient concentration was derivatized to phenylthiocarbamyl-GABA (PTC-GABA) and phenylthiocarbamyl-Glu (PTC-Glu) following the indications of Gunawan *et al*. (1990). A 100 µl aliquot of supernatant (or of standard solution of GABA and Glutamic) was dried under vacuum. The residue was dissolved in 20 µl of ethanol-water- triethylamine (2:2:1) and evaporated to dryness under vacuum. A 30 µl volume of ethanol-water-triethylamine-PITC (phenylisothiocyanate) (7:1 : 1 : 1) was added to the residue and allowed to react for 20 min at room temperature to form PTC-GABA and

### 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 during April from El-Maamoura locality, Alexandria, Egypt. These snails were transferred to plastic cups covered with cloth netting and maintained under laboratory conditions of 27 ° C and 65% R.H. The snails were daily fed on lettuce leaves up to the initiation of tests. The snails were allowed to be acclimatized to these conditions for two weeks.

PTC-Glu. Excess reagent was then removed under vacuum.

**HPLC separation and evaluation of PTC-GABA**

The dry residue containing PTC-GABA was dissolved in 100 µl of the mobile phase, consisting of a mixture of 80% solution A (aqueous solution of 8.205 g sodium acetate, 0.5 ml triethylamine, 0.7 ml acetic acid and 5.0 ml acetonitrile in 1000 ml) and 20% solution B [acetonitrile-water (60:40)], adjusted to pH 5.8. Isocratic HPLCseparations were performed on Hewlett Packard apparatus, consisting of a model 32X pump system, a Rheodyne Model 7125 injector, with a 20 µl injection loop and a Model 332 UV detector, in conjunction with a Hewlett-Packard (Avondale, PA, USA) Model 3396 (II series) integrator. A 250 mm. × 4.6 mm. I.D. stainless steel Zorbax SB C18 column was used. The mobile phase for isocratic elution was pumped at 0.6 ml/min, at room temperature; detection was at 254 nm. Standard curves for PTC-GABA and PTC-Glutamic acid were carried out.

**Standard curves of GABA and glutamic acid**

The standard curves for GABA and glutamic acid were potted as shown in Figures 1and 2 were determined by applying the same derivatization procedure to four standard solutions of GABA and glutamic acid (0.125, 0.25, 0.50 and 0.75 mM GABA). The corresponding optical density were recorded as previously mentioned. GABA and glutamic acid were calculated as µg/ mg protein.

Fig. (1): PTC-GABA stander curve

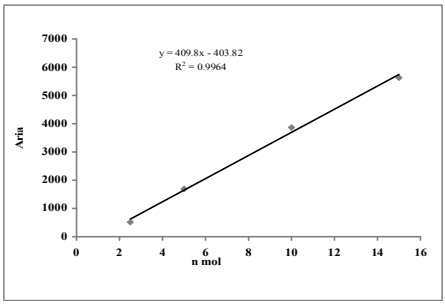
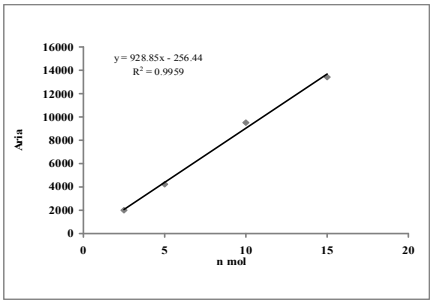



Fig. (2): PTCGlutamic acid stander curve




Selected snails were exposed to 1/10, 1/5 and 1/2 of the $LD_{50}$ values according to Abdallah *et al*., 1998 and Abo-Bakr 1997 of the tested compounds at three different time intervals (24, 48 and 72 hr).

Glutamate decarboxylase (GAD, EC 4.1.2.5) has been shown to play an important role in the regulation of brain excitability through the synthesis of γ-aminobuteric acid (GABA). GABA is major inhibitory neurotransmitter in the central nervous system and is considered a specific marker for GABAergic neurons and their processes (Wu *et al*; 1981, Romanova *et al*; 1994, Rossetti and Lombard 1996). Therefore the evaluation of GAD activity is very important in neurochemical as well as toxicological research (Bedair; 2010). However, in Mollusca, GABA has been shown to elicit

both inhibitory and excitatory actions in the central neurons (Walker, 1986, Yarovsky and Carpenetr, 1978), in *Limax maxima*(Cooke and Gelperin, 1988) and *Helix pomatia* (Hernadi, 1994). In this study, the GAD activity was evaluated by measuring the formed GABA after derivatization to phenylthio carbamoyl GABA (PTC-GABA) using HPLC with UV absorbance detection 245 nm. Figures (3 and 4) show the HPLC of standards PTC-GABA and PTC-Glutamic with retention times 3.86 and 10.012, respectively. PTC-GABA was calculated from the calibration curve using linear least squares regression. The *in vivio* effect of abamectin, emamectin benzoate and methomyl on glutamate decarboxylase activity of both BGS and WGS are recorded in Tables (1 and 2) and Figures (9 to 11). Figures (5, 6, 7 and 8) show the HPLC spectrum of PTC-GABA and PTC-Glutamate separation after 24hr respectively of abamectin treatments of *E. vermiculata*. The spectrums indicated the same values of retention time as indicated with standard PTC-GABA and PTC-Glutamatewith thecorresponding values of abamectin, emamactin benzoate, methomyl and control treatments for both types of used snails, however the spectrums selected were GAD-BGS after treatment with abamectin after 24, 48, and 72 hr, the test of all treatment showed the same retention time. The results revealed that:

1- Same type of response was noticed between both types of the snails BGS and WGS. Methomyl clearly inhibited GAD activity, while abamectin and emamectin benzoate stimulated markedly the GAD activity in both types of the used land snails.
2- The inhibitory effect of methomyl was dose dependent. That the activity of GAD enzyme increased by decreasing the dose treatments in both types of snails. However, the inhibition of GAD activity was more pronounced with BGS than WGS.
3- Natural fermentation products of Streptomyces avermitilis, avermectin B1 (abamectin) and its 4-deoxy-4-epi-methylamine derivative (emamectin benzoate) induced a significant GAD stimulatory effect for both type of snails BGS and WGS as indicated in Tables (1 and 2).
4- Abamectin interaction with GAD activity was higher than emamectin benzoate especially in the case of WGS, while the stimulatory effect on GAD activity was less than BGS.
5- Both compounds abamectin and emamectin benzoate revealed similar degree of GAD stimulation of BGS. The stimulation was dependent, however the stimulatory effect decreased by time, the lowest stimulation obtained for BGS was at 72 hr with the least concentration used 1/10 of LD50.
6- Abamectin and emamectin benzoate caused equal degree of GAD activation with BGS.However abamectin effect was significantly higher in its stimulatory effect on GAD-WGS than GAD-BGS as shown in (Table 2).
7- Specific activity value of GAD-BGS was higher than the value of GAD-WGS indicating more participation of GABAergic system of Eobania vermiculata compared with Theba pisana in this respect.

8- These findings could illustrate how abamectin and emamectin benzoate induce the level of GABA neurotransmitter in E. vermiculata and T. pisana land snails, as they activate the biosynthesis of GABA and inhibit its degradation.

Avermectins cause their pesticidal 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 (Campbell, 1989). Abdel Baky (2004) reported that there was an increase in the concentrations of GABA, when the earthworm, *Allolobophora caliginosa*, was treated by ivermectin. Also, Yamazaki *et al.* (1989) reported that ivermectin is an agonist for the GABA neurotransmitter. Thus, the binding of ivermectin to a neuronal membrane increases the release of GABA. GABA binds to the GABA-receptor-chloride channel complex of postsynaptic neuronal membranes causing an influx of chloride ions. The influx of chloride ions causes hyperpolarization of neuronal membrane. The hyperpolarization of neuronal membrane mediates a flaccid paralysis in arthropods and nematodes. On the other hand Kass *et al*. (1980) and (1984) used *Ascaris lumbricoides* as a model system. Their results demonstrated avermectin's function as a GABA agonist that stimulates GABA release from pre- synaptic inhibitory membranes. In addition, Abou-Taleb *et al*., (2009) reported that, emamectin benzoate increased the GABA neurotransmitter and glutamic acid concentrations in the field strain of cotton leafworm to a lesser extent than in the laboratory strain. The increasing in the GABA and glutamic acid depended on the emamectin benzoate concentration and the time of exposure. The cellular GABA level reflects a dynamic balance between synthesis and catabolism, determined respectively by the relative fluxes through two pyridoxal-5'-phosphate dependent enzymes, glutamate decarboxylase (GAD) and GABA transaminase (GABA-T). Whereas GAD controls the synthesis of GABA from glutamate (Martin and Tobin, 2000), GABA-T catalyzes the first-step in GABA degradation to succinic semialdehyde (SSA) in a transamination reaction with a-ketoglutarate to form glutamate. The results are in agreement with that obtained by Bedair, (2010), that GAD activity was increased upon exposure to emamectin benzoate. The results indicated the stimulatory effect of both abamectn and emamectin benzoate on both tested snails glutamic decarboxylase, that might brought a light shed partially on the mechanism of action of these compounds.

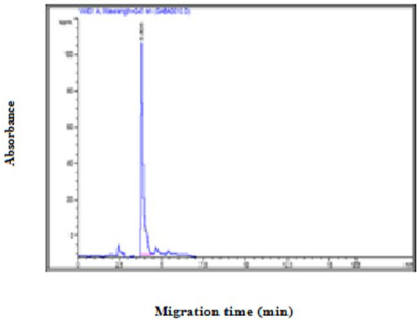



Fig. (3) HPLC chromatogram of PTC-GABA standard

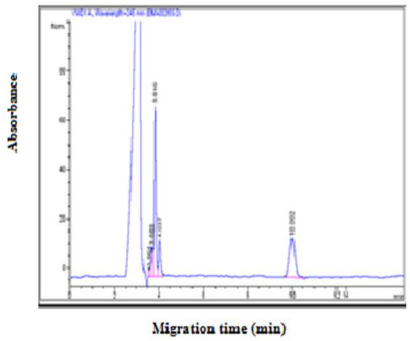



Fig. (6): Spectrum of PTC-GABA and PTC-glutamic acid derivatives HPLC separation due to Emamectin benzoate treatments of *Eobaniavermiculata* after 24 hours.

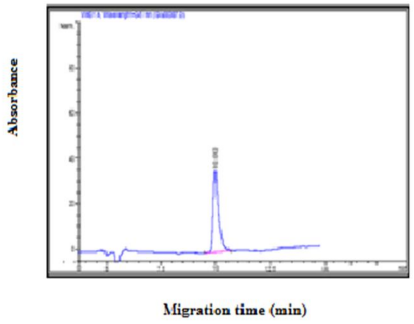



Fig. (4): HPLC chromatogram of PTC-glutamic acid standard

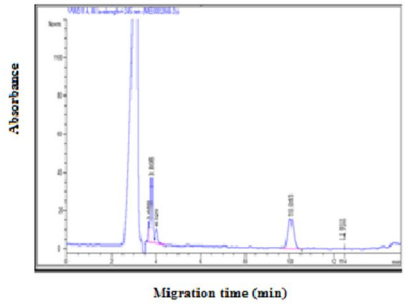



Fig. (7): Spectrum of PTC-GABA and PTC-glutamic acid derivatives HPLC separation due to methomyl treatments of *Eobania vermiculata* after 24 hours.

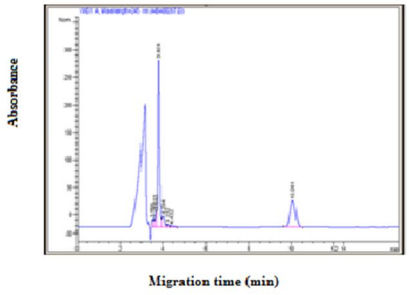



Fig. (5): Spectrum of PTC-GABA and PTC-glutamic acid derivatives HPLC separation due to Abamectin treatments of *Eobaniavermiculata* after 24 hours.

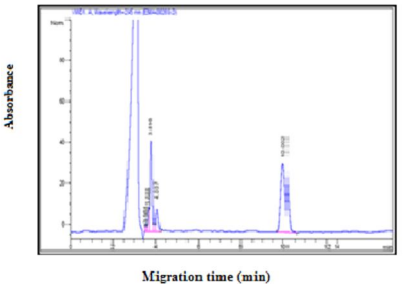



Fig. (8): Spectrum of PTC-GABA and PTC-glutamic acid derivatives HPLC separation due to Control treatments of*Eobania vermiculata* after 24 hours.

Table (1): *in vivo* Effect of abamectin, emamectin benzoate and methomyl on *Eobania vermiculata* (BGS) glutamic acid decarboxylase (GAD) activities

| Dose | | Abamectin | | Emamectin benzoate | | Methomyl | |
|---|---|---|---|---|---|---|---|
| | | S.A (nM glu./mg protein/min) ± SD | Activity % | S.A (nM glu./mg protein/min) ± SD | Activity % | S.A (nM glu./mg protein/min) ± SD | Activity % |
| $LD_{50}$ at 24 hr | 1/10 | 14.804±0.49* | 125.31 | 15.257±0.38* | 129.14 | 10.137±0.99* | 85.80 |
| | 1/5 | 16.356±1.04* | 138.45 | 16.852±0.99* | 142.65 | 8.229±0.92* | 69.65 |
| | 1/2 | 19.243±1.02* | 162.88 | 19.222±0.79* | 162.71 | 7.544±0.74* | 63.86 |
| $LSD_{0.05}$ | | 1.165 | | 1.058 | | 0.911 | |
| $LD_{50}$ at 48 | 1/10 | 17.938±0.67* | 151.84 | 17.157±0.51* | 145.23 | 8.321±0.71* | 70.43 |
| | 1/5 | 20.945±0.94* | 177.30 | 17.964±0.66* | 152.06 | 6.643±0.38* | 56.23 |
| | 1/2 | 22.766±1.10* | 192.71 | 22.173±1.07* | 187.69 | 6.144±0.64* | 52.01 |
| $LSD_{0.05}$ | | 1.206 | | 1.206 | | | |
| $LD_{50}$ at 72 | 1/10 | 14.839±0.38* | 125.61 | 13.836±0.63* | 117.12 | 11.483±0.55 | 97.20 |
| | 1/5 | 14.724±0.72* | 124.64 | 15.463±0.33* | 130.89 | 11.323±0.74 | 95.85 |
| | 1/2 | 16.989±0.49* | 143.81 | 16.829±0.92* | 142.45 | 7.672±0.82* | 64.95 |
| $LSD_{0.05}$ | | 1.188 | | 1.014 | | 0.815 | |

Control specific activity of untreated snail (GAD) is 11.814 ± 0.62( nMglu/mg protein/min) ± SD. Comparisons significant at the 0.05 level are indicated by *

Table (2): *in vivo* Effect of abamectin, emamectin benzoate and methomyl on *Theba pisana* (WGS) glutamic acid decarboxylase (GAD) activities

| Dose | | Abamectin | | Emamectin benzoate | | Methomyl | |
|---|---|---|---|---|---|---|---|
| | | S.A (nM glu./mg protein/min) ± SD | Activity % | S.A (nM glu./mg protein/min) ± SD | Activity % | S.A (nM glu./mg protein/min) ± SD | Activity % |
| $LD_{50}$ at 24 hr | 1/10 | 9.725±0.74* | 158.17 | 7.178±0.22* | 116.73 | 5.768±0.63 | 93.81 |
| | 1/5 | 8.440±0.83* | 137.26 | 6.843±0.18* | 111.28 | 4.457±0.51* | 72.48 |
| | 1/2 | 10.824±0.91* | 176.04 | 9.774±0.42* | 158.95 | 3.864±0.39* | 62.84 |
| $LSD_{0.05}$ | | 0.759 | | | | | |
| $LD_{50}$ at 48 | 1/10 | 7.142±0.43* | 116.15 | 7.470±0.49* | 121.48 | 5.871±0.26 | 95.49 |
| | 1/5 | 12.038±0.88* | 195.79 | 10.328±0.62* | 167.96 | 4.304±0.57* | 70.00 |
| | 1/2 | 11.539±0.86* | 187.66 | 10.636±0.97* | 172.97 | 4.525±0.72* | 73.59 |
| $LSD_{0.05}$ | | 0.991 | | | | | |
| $LD_{50}$ at 72 | 1/10 | 8.863±0.73* | 144.14 | 6.611±0.84 | 107.51 | 5.112±0.33* | 83.14 |
| | 1/5 | 10.479±1.07* | 170.43 | 7.767±0.19* | 126.31 | 5.832±0.56 | 94.84 |
| | 1/2 | 9.901±0.86* | 161.03 | 7.846±0.28* | 127.59 | 4.578±0.28* | 74.45 |
| $LSD_{0.05}$ | | 1.159 | | 0.757 | | 0.984 | |

Control specific activity of untreated snail (GAD) is 6.149±0.78 ( nMglu/mg protein/min) ± SD.

Comparisons significant at the 0.05 level are indicated by *

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## تداخل الابامكتين والامامكتين بنزوات مع نشاط انزيم الجلوتاميك أسيد ديكربوكسيليز في القواقع الارضية


### الملخص العربى

دُرس تأثير تداخل الابامكتين والامامكتين بنزوات ومبيد الميثوميل على نشاط انزيم الجلوتمات ديكربوكسيليز لكل من القوقع البني ايوبانيا فيرميكولاتا والقوقع الابيض تيبا بيسانا. نشاط انزيم الـ GAD تم تقديره عن طريق قياس كمية حمض الجابا امينو بيوتريك بعد تكوين المشتقات مع فينيل ثيو كاربامويل باستخدام جهاز HPLC وقياس الامتصاص عند طول موجة 245 نانوميتر. تم تقدير فينيل ثيو كاربامويل للجابا ولحامض الجلوتاميك القياسيين وكان زمن الاحتباس 3.86 و 10.01 دقيقة على الترتيب وقد اظهرت النتائج ما يلي :-

1- كلا النوعين من القواقع كان لهما نفس الاستجابة بينما كان مبيد الميثوميل مثبطا للانزيم فان الابامكتين والامامكتين بنزوات سببا نشاط الانزيم بدرجة ملحوظة.

2- تثبيط مبيد الميثوميل للانزيم يعتمد على التركيز وعموما فان التثبيط للانزيم في قوقع الحدائق البني اكثر من قوقع الحدائق الابيض

3- تأثير الابامكتين على نشاط الانزيم كان اعلى من الامامكتين بنزوات خاصة في حالة القوقع الابيض.

4- يقل زيادة النشاط بزيادة الوقت حيث ان أقل نشاط تم الحصول عليه بعد 72 ساعة من المعاملة على اقل تركيز 10/1 من قيمة الجرعة المسببة لموت 50%.

5- النشاط النوعي للانزيم في القوقع البني اعلى من النشاط النوعي للانزيم في القوقع الابيض.

6- هذه النتائج تشير الى ان الابامكتين والامامكتين بنزوات لهما تداخل مع الناقل العصبي الجابا لكلا القوقعين الابيض والبني.

7- توضح النتائج قدرة الابامكتين والامامكتين بنزوات على تحفيز الناقل العصبي الجابا وزيادة كميته في كلا القوقعين اما عن طريق تنشيط تخليق الجابا او تثبيط تحطمه