TOXICITY OF ABAMECTIN AND FIPRONIL ON SOME BIOCHEMICAL CHANGES IN FEMALE ALBINO RATS

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ABSTRACT

Abamectin and fipronil are relatively new pesticides. LD_{50} values for abamectin and fipronil were 22.29 and 332.02 mg/kg body weight respectively. It was cleared that abamectin fipronil female albino rats. Toxicity of abamectin and fipronil were studied on certain biochemical changes in female albino rats. Results showed that, hemoglobin content, glutathione-S-transferase (GST), acetyl cholinesterase and esterase content were significantly decreased for rats treated with both pesticides in a concentration dependent. While during recovery period (60 days) the results demons trated that biochemical changes no improved for rats when compared to the control groups. Our results, it can be concluded that abamectin and fipronil have toxicity of hemoglobin content, glutathione-S-transferase (GST), acetyl cholinesterase and esterase contents in female albino rats.

Key words: Abamectin, Fipronil, Sub chronic toxicity, Albino rats, Biochemical changes.

INTRODUCTION

Pesticides play an irreplaceable role in integrated pest management due to their efficient, fast, economical, and easily implementation. However, Fipronil and abamectin belong to relatively new pesticides which are broad uses for pest management in several countries. Abamectin and fipronil both bind to the GABA-gated chloride channel. Abamectin activate the chloride channel, causing an inhibitory effect: the activated channel blocks or inhibits normal reactions, which, when excessive, results in the insect's death. Fipronil has the opposite effect on the chloride channel; this insecticide blocks the channel from activating and its normal inhibitory action. Thus fipronil binds to the channel, the nerve is overstimulated, and death eventually occurs. (**Simon 2015).**

The abamectin induced biochemical changes in rats and may cause hazardous effects on human health and other non-target organisms **(Abd-Elhady and Abou-Elghar 2013)**. Commercial form showed signs of liver toxicity, as grounds of increased serum AST activity in rats, significant elevation in serum uric acid and creatinine concentrations were recorded in the rats treated with abamectin **(Eissa and Zidan 2009)**. Abamectin induced

oxidative damage and changes in the activities of antioxidant enzymes in isolated rat hepatocytes **(El-Shenawy 2010) (El-Gendy *et al.* 2015)**.

Fipronil induced lipid peroxidation, oxidative stress, liver, and kidney injury in rats. These pathophysiological changes in liver and kidney tissues could be due to the toxic effect of fipronil that associated with a generation of free radicals **(Mossa *et al.*, 2015).** In same connection, **(Vidau *et al.*, 2011)** showed that fipronil exposure had a profound negative influence on the liver not in kidney injury indices and also on histological changes in liver and kidney tissues liver and kidney tissues. Also, such changes were reversed after an off-dose period of 45 and 60 days. The objective of the present work was to study effects of abamectin and fipronil on biochemical changes in female albino rats

MATERIAL AND METHODS

1. Pesticides used

Two pesticides were used individually in this investigation; the first: Abamectin (formulation, 1.8% EC; Trade name, Vertimec; Manufacturer, Syngenta Agro. Co., Switzerland), the second: Fipronil (formulation, 20% SC; Trade name, Coach; Manufacturer, Zhipang Yungong Chemical Industry Limited, China).

2. Tested animals

The present experiments were carried out on 120 adult female albino rats *Rattus rattus* (90-110 gm) obtained from the farm of general organization of serum and vaccine (Helwan Farm). The animal maintained in the animal house of Plant Protection Department, Faculty of Agriculture, Fayoum University. The females of adult rodents were randomly divided in to five groups per Plastic cage. The cages were kept in air-conditioned room at a temperature 25-27°C and a relative humidity (70 - 80 %) and normal light/dark cycle. The animals were provided with commercial pelleted rodent food and tap water and allowed to acclimate to laboratory condition for a minimum of two week prior to the experiment.

3. Determination of medium lethal dose (LD_{50})

Determination of medium lethal doses LD_{50} of abamectin and fipronil were carried out using 50 female albino rats. Four albino rats per each dose were used. Five serial concentrations were prepared in Distilled water; each rat was given orally adjusted dose of the same active ingredient. In addition to another group of 4 albino rats were used as control for the two insecticides and only Distilled water was used or was given. The dose was administered using a tuberculin syringe with a modified special needle. Rodents' animals were examined during the first four days after administration and mortality was recorded.

4. Sub chronic toxicity tests

Seventy animals for each insecticide were tested and divided in to fourteen groups (5 rats / group). The groups were treated as follows groups (1-2)

were treated with 1/10 LD_{50} pesticide (abamectin or fipronil) for 45 days, groups (3-4) were treated with 1/10 LD_{50} pesticide (abamectin or fipronil) for recovery 15 days, groups (5-6) were treated with 1/50 LD_{50} pesticide (abamectin or fipronil) for 45 days, groups (7-8) were treated with 1/50 LD_{50} pesticide (abamectin or fipronil) for recovery 15 days, , groups (9-10) were treated with 1/100 LD_{50} pesticide (abamectin or fipronil) for 45 days, groups (11-12) were treated with 1/100 LD_{50} pesticide (abamectin or fipronil) for recovery 15 days, group (13-14) used as control. The treated groups with insecticides were in water daily for 45 days given pesticide in water daily for 45days, according to **(Mossa *et al.*, 2015; 2017).** The concentration was adjusted weight dependent. The animals were observed daily for unusual assignments.

5. Biochemical analysis

Hemoglobin was assayed by the colorimetric method described by **Dacie and Lewis. (1984).** The enzyme glutathione –S- transferase (GST) determination was based on the method described by **Scharf *et al.* (1998)**, The acetyl cholinesterase (AChE) activity was determined with the photometric method of **Ellman *et al.* (1961)** using acetylethiocholin iodide (ATC) as a substrate. Determination of esterase activities was carried out using the method described by **Gomori (1953)**. Esterase activity determination was applied in the homogenate of whole body of the adult.

6. Statistical analysis

The LD_{50} values, 95% confidence intervals, and slopes were calculated by Probit analyses. The data obtained from the experiment were analyzed using Multi-way analysis of variance (MANOVA) with means subjected to Duncan multiple range test **(Duncan, 1955)** at 5% probability level.

RESULTS AND DISCUSSION

1. Determinotion of LD_{50} of fipronil and abamectin.

Data presented in Table (1) volues LD_{50}, LD_{90} and slope of abamectin and fipronil against female albino rats when single administered by the acute oral route. The acute oral LD_{50} value for abamectin was 22.29 mg/kg body weight. On other hands, the acute oral LD_{50} value for fipronil was 332.02 mg/kg body weight. It was cleared that abamectin was about 14.90 times more toxic than fipronil to female albino rat under identical conditions.

Table (1). Determinotion of volues LD_{50}, LD_{90} and slope of abamectin and fipronil in female albino rats after 96-hour post treatment.

Pesticide	LD_{50} (mg/kg b. wt.)	95% confidence limits		LD_{90} (mg/kg b. wt.)	Slope ± SE
		Lower	Upper		
Abamectin	22.29	19.04	25.48	100.52	1.96 ± 0.32
Fipronil	332.02	300.89	364.05	891.97	1.99 ± 0.48

2. Effect on hemoglobin in blood serum.

Data in Table (2) reveal the level of hemoglobin in rats exposed to abamectin and fipronil pesticides for different periods. On the other hand a significant decline in hemoglobin content with all concentrations of abamectin and fipronil in treatment period for 45 days groups and recovery groups compared to their control groups. While in treatment period for 45 days, the maximum reduction reached to 17.53 and 20.76% were observed at the highest concentration levels of abamectin and fipronil, respectively as compared with treatment period control.

During recovery period, the results demonstrated that animals did not recover, and hemoglobin levels did not resume to the normal values of the control group. Also, the maximum decrease in hemoglobin level up to 25.86 and 29.53% at the highest concentration levels of abamectin and fipronil, respectively as compared with recovery period control.

These results are in agreement with those obtained by **Eissa and zidan (2009)** who mentioned that this reduction may be attributed to more than one factor i.e. the failure to supply the blood circulation with cells from haemohepatic tissue, since the liver has an important role in the regeneration of erythrocyte and the possible destructive effect on erythrocyte by the toxicants.

Table (2): Level of hemoglobin in serum of female albino rats exposed to daily administered in drinking water of tested pesticides for different periods.

Treatment (mg/kg)	Level of hemoglobin (g/dl)			
	After treatment period (45 days)		After recovery period (60 days)	
	Abamectin	Fipronil	Abamectin	Fipronil
Control	12.38 ± 0.20^{g}*	12.38 ± 0.20^{g}	12.26 ± 0.23^{fg}	12.26 ± 0.23^{fg}
$^{1}/_{100}$ LD_{50}	11.67 ± 0.20^{ef}	11.64 ± 0.23^{ef}	11.55 ± 0.20^{e}	11.49 ± 0.23^{e}
$^{1}/_{50}$ LD_{50}	11.13 ± 0.20^{de}	10.41 ± 0.23^{bc}	10.71 ± 0.20^{cd}	10.43 ± 0.23^{bc}
$^{1}/_{10}$ LD_{50}	10.21 ± 0.20^{bc}	9.81 ± 0.20^{b}	9.09 ± 0.23^{a}	8.64 ± 0.23^{a}

***Means ± S.D followed by the same letter in each column are not significantly different as indicated by the Duncan test (P≤0.05).**

3. Effect on glutathione-S-transferase (GST) in blood serum.

Data in Table (3) indicate the level of glutathione-S-transferase in rats exposed to abamectin and fipronil pesticides. In treatment periods for 45 days, there was a decrease in glutathione-S-transferase content with all concentrations compared to the control group. Also, the maximum decreasing reached to 25.0 % and 35.42% was observed at the highest concentration levels of abamectin and fipronil, respectively as compared with treatment period control. During recovery period (60 days), the results demonstrated that (GST) no improved occurred for rats when compared to the control groups.

These results are in agreement with those obtained **by (Fahim *et al.*, 2016, and Mosssa *et al.*, 2017)** who reveal that abamectin glutathione-S-transferase decrease concentration in female albino rats. Similar results were also

reported in rats by **(El- shafey *et al*., 2011)** who mentioned that this decrease may be attributed to more than one factor i.e. Significant decreases of serum glutathione-S-transferase post abamectin and fipronil treatments in the present study may be due to decreased serum (GST) levels thes are in consistent with the finding of **(El-Shenawy, 2010)** who reported decreased (GST) activities in rat liver following exposure to insecticides abamectin, through a detoxification reaction and/or that (GST). The decrease in (GST) level in the present study may be due to that (GST) involved in detoxification of the abamectin to non-toxic products or by rapidly binding and very slowly turning over the insecticide. (GST) is one of enzyme systems involved in the detoxification of organic phosphorus and carbamte insecticides to non-toxic products or by rapidly binding and very slowly turning over the insecticide. In consistent with the present results, **(El-Demerdash 2007)** reported a significant decrease in (GST) activity after in vivo and in vitro treatment with respectively. Similarly, (GST) inhibition has been documented to occur under other oxidative stress conditions **(Mansour and Mossa 2009)**. Our results demonstrate that GST is a part of adaptive response of rat organ cells to oxidative stress after CPF and/or ABM treatments.

Table (3): Activity of glutathione-S-transferase (GST) in serum of female albino rats exposed to daily administered in drinking water of tested pesticides for different periods.

Treatments (mg/kg)	Activity of glutathione-S-transferase (GST) – mmol/min/ mg			
	After treatment period (45 days)		After recovery period (60 days)	
	Abamectin	Fipronil	Abamectin	Fipronil
Control	$0.48 \pm 0.02^{fg*}$	0.48 ± 0.02^{fg}	0.50 ± 0.03^{g}	0.50 ± 0.03^{g}
$^{1}/_{100}$ LD_{50}	0.46 ± 0.02^{efg}	0.43 ± 0.02^{defg}	0.41 ± 0.03^{cdef}	0.43 ± 0.03^{defg}
$^{1}/_{50}$ LD_{50}	0.41 ± 0.02^{cdef}	0.41 ± 0.02^{cdef}	0.40 ± 0.03^{cde}	0.35 ± 0.03^{bc}
$^{1}/_{10}$ LD_{50}	0.36 ± 0.02^{bcd}	0.31 ± 0.02^{ab}	0.30 ± 0.03^{ab}	0.27 ± 0.03^{a}

***Means followed by the same letter in each column are not significantly different as indicated by the Duncan test (P≤0.05).**

4. Effect on acetyl cholinesterase (AChE) in blood serum.

Data in Table (4) reveal the level of acetyl cholinesterase in rats exposed to abamectin and fipronil pesticides. In treatment periods for 45 days, there was a decrease in acetyl cholinesterase content with all concentrations compared to the control group. Also, the maximum reduction reached to 25.07 and 25.26% was observed at the highest concentration levels of abamectin and fipronil, respectively as compared with treatment period control. During recovery period (60 days), the results demonstrated that acetyl cholinesterase content no improved occurred for rats when compared to the control groups. There was significant decrease in acetyl cholinesterase level up to 22.75 and 26.01 % at the highest concentration levels of abamectin and fipronil, respectively as compared with recovery period control.

These results are in agreement with those obtained by **(Lima *et al*., 2013)** who found that abamectin and fipronil decreased acetyl cholinesterase concentration in female albino rats. Similar results were also reported in rats which mentioned that this decrease may be attributed to more than one factor i.e. by **(Nassar 2014)** Current

results were in agreement with **(Mansour *et al.* 2008)** who reported that abamectin decreased the AChE activity.A plethora of compounds inhibit the AChE activity. AChE is a member of the serine hydrolases family **(Ollis *et al.*, 1992)**. Moreover, serum AChE was reported to be suitable to study the AChE suppression by anti-AChE insecticides and as a measure of brain AChE inhibition by such compounds. Mode of binding of the inhibitors with different structural motives that can bind to the esteratic part of the active site by esterification of serine hydroxyl, or interact with the alpha anionic part of the active site, the aromatic gorge and the peripheral anionic site **(Weiner *et al.*, 2009)**.

Table (4): Activity of acetyl cholinesterase (AChE) in serum of female albino rats exposed to daily administered in drinking water of tested pesticides for different periods.

Treatments (mg/kg)	Activity of acetyl cholinesterase (AChE) –u/min/ mg			
	After treatment period (45 days)		After recovery period (60 days)	
	Abamectin	Fipronil	Abamectin	Fipronil
Control	160.74±4.45^{fg*}	160.74 ± 4.45^{fg}	162.49 ±4.98^{g}	162.49 ±4.98^{g}
$^1/_{100}$ LD_{50}	138.43±4.45^{bcde}	147.46±4.45^{defg}	151.77±4.98^{efg}	146.90±4.98^{def}
$^1/_{50}$ LD_{50}	129.55±4.45^{ab}	130.98 ± 4.45 ^{abc}	144.50±4.98^{cde}	133.47±4.98^{abcd}
$^1/_{10}$ LD_{50}	120.45 ±4.45^{a}	120.14 ± 4.45^{a}	125.53±4.98^{ab}	120.22 ±4.98^{a}

***Means followed by the same letter in each column are not significantly different as indicated by the Duncan test (P≤0.05).**

5. Effect on esterase in blood serum.

Data in Table (5) reveal the level of esterase in rats exposed to abamectin and fipronil pesticides for different periods. In treatment period for 45 days, there was a significant decline in esterase content at all concentrations compared to the control group. Also, the maximum reduction reached to 51.11 and 48.89% was observed at the highest concentration levels of abamectin and fipronil, respectively as compared with treatment period control. During recovery period (60 days), the results demonstrated that esterase no improved occurred of rats when compared to the control groups. Significant decrease in esterase up to 45.24% and 52.38 % by abamectin and fipronil respectively at the highest concentration compared with recovery period control.

Table (5): Activity of esterase in serum of female albino rats exposed to daily administered in drinking water of tested pesticides for different periods.

Treatments (mg/kg)	Activity of esterase (mol/min/ mg)			
	After treatment period (45 days)		After recovery period (60 days)	
	Abamectin	Fipronil	Abamectin	Fipronil
Control	0.45 ± 0.03^{e*}	0.45 ± 0.03^{e}	0.42 ± 0.03^{e}	0.42 ± 0.03^{e}
$^1/_{100}$ LD_{50}	0.36 ± 0.03^{cde}	0.39 ± 0.03^{de}	0.38 ± 0.03^{de}	0.35 ± 0.03^{cde}
$^1/_{50}$ LD_{50}	0.26 ± 0.03^{abc}	0.31 ± 0.03^{bcd}	0.31 ± 0.03^{bcd}	0.31 ± 0.03^{bcd}
$^1/_{10}$ LD_{50}	0.22 ± 0.03^{ab}	0.23 ± 0.03^{ab}	0.23 ± 0.03^{ab}	0.20 ± 0.03^{a}

***Means followed by the same letter in each column are not significantly different as indicated by the Duncan test (P≤0.05).**

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التأثير السام للابامكتين والفيبرونيل علي بعض التغيرات الكيميائية في اناث الفئران البيضاء

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يهدف البحث لدراسة التأثيرالسام للابامكتين والفيبرونيل علي السمية وبعض التغيرات البيوكيميائية في اناث الفئران البيضاء. وقد أظهرت النتائج: ان قيم الجرعة الوسطية المميتة LD_{50} للابامكتين والفبرونيل هي ٢٢.٢٩ و ٣٣٢.٠٢ مجم / كجم علي التوالي موضحا ان الابامكتين اكثر سمية من الفبرونيل بمقدار ١٤.٩٠ مرة لاناث الفئران البيضاء. وقد اوضحت النتائج ايضا حدوث انخفاض في المحتويات من الهيموجلوبين، الجلوتاثيون-اس- ترنسفريز (GST)، انزيم الكولين استريز والاستيراز بشكل ملحوظ عند معاملة الفئران بطريقه تعتمد علي التركيز. وخلال فتره الاستشفاء (٦٠ يوما) لم تتحسن التغيرات البيوكيميائية للفئران المعاملة بالمقارنة مع الكنترول، ومن خلال هذه النتائج يمكن الاستنتاج ان الإبامكتين والفيبرونيل ذات تأثير سام علي محتوي الهيموجلوبين، محتوي الجلوتاثيون-اس- ترنسفريز (GST)، انزيم الكولين استريز والاستيراز في اناث الفئران البيضاء.

الكلمات الدالة: الابامكتين – الفبيرونيل – السمية التحت المزمنة – اناث الفئران البيضاء-التغيرات البيوكيميائية.