

Comparison between the Effects of Malathion and Deltamethrin to Cholinesterase Activity in Rabbits

Khalil Abdullah Khalil¹ and Kasim Sakran Abass²

¹Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine,
University of Mosul, Mosul, Iraq

²Department of Public Health, College of Veterinary Medicine, University of Kirkuk, Kirkuk, Iraq

All corresponding to: abdullahkhalil863@gmail.com; +9647702360141

Received – January 07, 2017; Revision – March 19, 2017; Accepted 30 March 2017

Abstract

The evaluation of the inhibitory effect of malathion and the deltamethrin on cholinesterase activity in the blood plasma and brain in rabbits was measured by using modified Michel and Ellman methods together in order to confirm the results and to increase the accuracy of measurement have been measuring the cholinesterase activity *in vitro* and *in vivo*. Malathion concentrations ranged from (0.5 – 4 mM) when mixed with the blood plasma and the homogenize of the brain *in vitro* results significant inhibition in the cholinesterase activity and found highest of inhibition was 93% and 94% in the blood plasma and brain respectively, while deltamethrin concentrations ranged from (2.5–20 mM) results significant inhibition in the cholinesterase activity and highest percentage of inhibition was 54% and 48% in the blood plasma and brain respectively, and so when measuring cholinesterase using the Ellman method, but when using the modified Michel method the inhibition percentage when using malathion was 55% and 98% in the blood plasma and brain respectively, and when using deltamethrin ratio was 36% and 70% in the blood plasma and brain respectively. While when malathion administered orally in rabbits, with doses from (150–1200 mg/kg) results significant inhibition in the cholinesterase activity and highest of inhibition when measuring by Ellman method was 84% and 88% in the blood plasma and brain respectively, while deltamethrin when administered orally with doses ranged from (12.5–100 mg/kg) the highest inhibition was 31% and 33% in the blood plasma and brain respectively, whereas when measured by using modified Michel method found highest inhibition when malathion administered orally 75% and 87% in the blood plasma and brain respectively, whereas when deltamethrin administered orally found highest inhibition 32 % and 45% in the blood plasma and brain respectively. From this study shows that malathion more influence on the cholinesterase activity than deltamethrin.

Introduction

There are two types of cholinesterases, A- esterase, which found in the liver and plasma within the high density lipoprotein(HDL), and be highly effective in mammals (Massoulié *et al.*, 1993; Wheelock *et al.*, 2008). The other type is the B-esterase, so which widely distributed in the tissues and the most important enzymes of this type is specific acetylcholinesterase, which are recoverable in the tissues of irritation in cholinergic synapses sites for the analysis of acetylcholine as well as in neuromuscular junctions and musclotendinous (Bahar *et al.*, 2012; Rakonczay, 1986), also called as non-specific acetylcholinesterase, which is in the blood, liver, pancreas, nervous tissue and plasma as well as present in the cholinergic synapses sites and in the end motor plate of nerve fibers with true cholinesterase (Kovarik *et al.*, 2007; Šinko *et al.*, 2007). Determination of cholinesterase activity is very important in the diagnosis and assessment of cases of poisoning pesticide malathion and deltamethrin and help make sure cases of poisoning, especially in the early stages of poisoning, in which the poisoning signs are not clear and can be considered as the percentage decrease of 25-30% in the cholinesterase activity in plasma or red blood cells evidence of exposure to a cholinesterase inhibitors (Adak *et al.*, 2015; Cheng *et al.*, 1998). There are several methods to measure the cholinesterase activity, such as Hestrin colorimetric method (Hestrin, 1949), and this method depends on the remaining cholinesterase interaction reaction after a specified time in the alkaline medium to form acetohydroxamic acid, and the Ellman method (Ellman *et al.*, 1961), which is one of the methods the color depends on the interaction of thiocholine resulting from the decomposition of acetylthiocholine with 5,5'-dithiobis (2-nitrobenzoic acid) component of the color yellow for the new output is 5-thio-2-nitrobenzoate measured color output waves length of 412 nm. The radiometric method (Siakotos *et al.*, 1969), this use of radioactive acetylcholine as a basis of this method relies on selective solubility between acetate and acetylthiocholine in aqueous solutions. There is another method not color depend its Electrometric method (Michel, 1949), the basis of this method is the decomposition of acetylcholine to choline and acetic acid by cholinesterase, acetic acid cause low pH in the reaction mixture (Wills *et al.*, 1972). Cholinesterase spread in several locations in the tissues of rabbits which are found in the liver and the plasma within (HLD) and in tissue negotiable irritation in sites of cholinergic synapses where analyzes of acetylcholine and also found in musclotendinous, neuromuscular junction, neuron body, neuron axis, central nervous system (spinal cord and brain) muscles, blood serum, red blood cells, platelets, T-lymphocytes, pancreas, motor end plate and nerve fibers (Gunduz *et al.*,

2012; Wang *et al.*, 2014). They were chosen malathion organophosphorus insecticide (O,O-dimethyl-S-1,2-bisethoxycarbonylethyl phosphorodithioate) and deltamethrin Pyrethroids insecticide[(S)-cyano-(3-phenoxyphenyl)methyl](1R,3R)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane-1-carboxylate and so for being the extensive pesticide use in the field of agriculture and veterinary medicine and to control the emerging parasites that infect animals and they have important health, environmental and so wide for their use in controlling harmful insects (Dhouib *et al.*, 2015; Pan *et al.*, 2014), and to achieve the objectives of this study was conducted with measuring the inhibition of cholinesterase activity in the plasma and brain *in vitro* and *in vivo* inhibitory by malathion and deltamethrin, furthermore, comparing between the effects of malathion and deltamethrin to cholinesterase activity in rabbits.

Materials and Methods

Animals

In this study we used the local rabbits *Lepus cuniculua domestica* mixed sex and that has been processed from the local markets in Kirkuk, and the ages between 3-4 months, taking into account the weights to be close in a single experiment, and breeding animals for two weeks in a room temperature from 27-35 °C, and the humidity level from 20-25% and has been allocated to give rabbits bush was repopulated in metal cages measuring 80 x 80 x 50 cm dedicated to raising rabbits were supplied from the College of Veterinary Medicine/University of Kirkuk. In this paper, we used of malathion 57% purity supplied by Delta Chemical Industries/Saudi Arabia, and deltamethrin 2.5% Purity supplied by VAPCO Company for Veterinary and Agricultural Industries/ Jordan.

Sample collection

Blood were collected from rabbit ear vein, according to the required experiment, which used anticoagulant heparin and diluted 1:10 with saline solution to rinsing glass test tubes and make it ready for the collection of blood samples. Blood plasma was separated by centrifugation and the device quickly 3000 cycles/min, for a period of 15 min. After the separation of plasma were transferred to glass tubes, clean, dry and testing them. While, the brain was homogenized by open the skull and the brain extract its entirety and placed inside plastic bags, clean, dry and conduct their own tests on it. The homogenized of brain tissue in phosphate buffer solution with a concentration of 3ml/100 mg of tissue weight pH 8.1 by using homogenizer for a period of one min. of Brain tissue and keeping samples in glass test tubes for the purpose of conducting the tests and all samples were preserved in the ices(Abass, 2014a).

Enzyme measurement

Michel method

Placed 3 mL of distilled water in a glass container capacity of 10 ml, then add 0.2 ml of plasma sample or tissue homogenize, add 3 mL of phosphate solution Buffer pH 8.1 mixes, measuring (pH_1) of the mixture by pH-meter, added 0.12 ml of acetylcholine iodide solution 7.5% as a basis, the mixture is transferred to the exact water bath at 37 °C and incubate for 30 min. measuring (pH_2) after taking out the sample directly from the incubator (Abass *et al.*, 2004).

It calculates the amount of change in the value of pH which represents the amount of the difference between pH_1 and 2 in 30 minutes, and this result reflects the activity of cholinesterase used in the sample as follows:

$$\Delta pH/30 \text{ min} = pH_1 - pH_2 - (\Delta pH \text{ of blank}^*).$$

* Blank contain all solutions except plasma or tissue sample

Ellman method

Cholinesterase activity was determined by the Ellman method adapted for use with spectrophotometer and using acetylthiocholine iodide as substrate (1 mM final concentration of acetylthiocholine iodide) for measuring cholinesterase activities. Substrate solutions were prepared and used on the same day and kept on ice during use. Then, enzyme measured by using spectrophotometer for 5 min at 410 nm, at 25°C. In each case the rate of absorbance increase was corrected by subtracting the rate observed for a reagent blank (i.e., without sample). Cholinesterase activities were calculated using an extinction coefficient of 13.6 mM⁻¹ cm⁻¹ for 5-thio-2-nitrobenzoate. All measurements were carried out in triplicate (Abass, 2014b; Dingova *et al.*, 2014).

In vitro inhibition of cholinesterase activity

A. Inhibition by Malathion

The collection of blood and brain samples from twelve rabbit was randomly divided into four groups with each group of four samples of blood plasma and homogenizes brain. Added malathion to mixtures reaction containing blood plasma or sample homogenize brain (0.2 ml) and distilled water (3 ml) and buffer phosphate pH 8.1 (3 ml) to obtain the final concentrations of the following: zero (control), 0.5, 1, 2, 4 mM and after the sample is incubated at a temperature 37 °C for 10 min. to an inhibition process (Abass, 2014b; Pan *et al.*, 2014), and

then measuring the activity of cholinesterase remaining according to the percentage of inhibition, as follows:

% inhibition of cholinesterase activity =

$$\frac{\text{Activity in the control (without malathion)} - \text{activity with malathion}}{\text{Activity in control}} \times 100$$

B. inhibition by Deltamethrin

The collection of blood and brain samples of twelve and a rabbit were randomly divided into four groups containing four samples of blood plasma and homogenize brain. Has been added deltamethrin to mixtures interaction containing blood plasma or sample homogenize brain (0.2 ml) and distilled water (3 ml) and Buffer phosphate PH 8.1 (3 ml) to obtain the final concentrations of the following(0 (control), 2.5, 5, 10, 20 mM. Then the sample is incubated at a temperature 37 ° C for ten minutes to an inhibition process and then measuring the activity yeast choline esterase remaining. According to the percentage of inhibition, as follows:

% inhibition of cholinesterase activity =

$$\frac{\text{Activity in the control (without deltamethrin)} - \text{activity with deltamethrin}}{\text{Activity in control}} \times 100$$

***In vivo* inhibition of cholinesterase activity**

A. Inhibition by Malathion

Used in this test nine rabbits were given a dose of malathion 75, 150, 300, 600, 1200 mg/kg administration orally, and two hours after the administration was taking samples of blood plasma and brain and measuring the cholinesterase activity (Al-Shinnawy et al., 2014).

B. Inhibition by Deltamethrin

Used in this test nine rabbits were given a dose of deltamethrin 12.5 ,25,50,100 mg/kg administration orally and after two hours taking samples of blood plasma and brain and measuring the cholinesterase activity (Yekeen et al., 2016).

Statistical analysis

Results were analyzed statistically using analysis of variance test and the results were subjected to Lest significant difference test (Cornblatt et al., 1989). In the case of two groups were analyzed test results Student`s-t-test (Lenglet et al., 2006).

Results

In vitro inhibition of cholinesterase activity

A. Inhibition by Malathion

Malathion *in vitro* by concentrations 0.5 to 4 mM in the reaction mixture resulted significantly inhibited in cholinesterase activity in plasma and brain and depending on concentrations and has measurements in a Michel method where the highest percentage of inhibition in plasma was 55% and in the brain 98% (Table 1) either when the measurement method Ellman was the highest percentage of inhibition in plasma was 93% and in the brain 94% (Table 2).

Table (1) Cholinesterase activity in the brain and blood plasma *in vitro* malathion inhibition which measuring by using Michel method

Concentration of Malathion (mM)	Plasma cholinesterase activity		Brain cholinesterase activity	
	change in the absorption	% of inhibition	change in the absorption	% of inhibition
0	0.19±0.0044	0	0.17±0.0044	0
0.5	0.15±0.0044*	21	0.075±0.0067*	55
1	0.125±0.0022 ^{A*}	34	0.055±0.0022 ^{A*}	68
2	0.115±0.0067 ^{B*}	39	0.04±0.0044 ^{B*}	97
4	0.085±0.0022 ^{C*}	55	0.03±0.0044 ^{C*}	98

Data in the table represent Mean±SE for samples within the same group (n= 12)

*Value for the control group was significantly different at the ($P < 0.05$)

^Avalue was significantly different from the placebo group at the ($P < 0.05$)

^Bvalue was significantly different from Group B at the ($P < 0.05$)

^Cvalue was significantly different from Group C at the level of the ($P < 0.05$)

Table (2) Cholinesterase activity in the brain and blood plasma *in vitro* Inhibition by malathion which measuring by Ellman method

Concentration of Malathion(mM)	Plasma cholinesterase activity		Brain cholinesterase activity	
	change in the absorption	% of inhibition	change in the absorption	% of inhibition
0	0.60±0.0000	0	0.35 ± 0.0000	0
0.5	0.23±0.0089*	61	0.09±0.0100*	74
1	0.11±0.0034 ^A *	81	0.055±0.0050 ^A *	84
2	0.05±0.0034 ^B *	91	0.035±0.0050 ^B *	90
4	0.04±0.0089 ^C *	93	0.02±0.0000 ^C *	94

Data in the table represent Mean ± SE for samples within the same group (n =12)

*Value for the control group was significantly different at the ($P < 0.05$)

^Avalue was significantly different from the placebo group at the ($P < 0.05$)

^Bvalue was significantly different from Group A at the ($P < 0.05$)

^Cvalue was significantly different from Group B at the level of the ($P < 0.05$)

B. Inhibition by Deltamethrin

Deltamethrin *in vitro* by concentrations 2.5 to 20 mM in the reaction mixture resulted significantly inhibited in cholinesterase activity in plasma and brain and depending on concentrations and has measurements in a Michel method where the highest percentage of inhibition in plasma was 36% and in the brain 70% (Table 3) either way, when measured by Ellman method the highest percentage of inhibition in plasma was 54% and in the brain 48% (Table 4).

Table (3) Cholinesterase activity in the brain and blood plasma *in vitro* Deltamethrin
 Inhibition which measuring by using Michel method

Concentration of Deltamethrin (mM)	Plasma cholinesterase activity		Brain cholinesterase activity	
	Change in the absorption	% of inhibition	Change in the absorption	% of inhibition
0	0.28±0.0089	0	0.235±0.0050	0
2.5	0.225±0.0022*	19	0.21±0.0100*	10
5	0.22±0.0044 ^A *	21	0.18±0.0100 ^A *	23
10	0.205±0.0022 ^B *	28	0.11±0.0200 ^B *	53
20	0.18±0.0089 ^C *	36	0.07±0.0100 ^C *	70

Data in the table represent Mean±SE for samples within the same group (n =12)

*Value for the control group was significantly different at the ($P < 0.05$)

^Avalue was significantly different from the placebo group at the ($P < 0.05$)

^Bvalue was significantly different from Group A at the ($P < 0.05$)

^Cvalue was significantly different from Group B at the level of the ($P < 0.05$)

Table (4) Cholinesterase activity in the brain and blood plasma *in vitro* deltamethrin
 inhibition which measuring by using Ellman method

Concentration of Deltamethrin (mM)	Plasma cholinesterase activity		Brain cholinesterase activity	
	Change in the absorption	% of inhibition	Change in the absorption	% of inhibition
0	0.60±0.0089	0	0.305±0.0050	0
2.5	0.56±0.0044*	7	0.285±0.0150*	7
5	0.52±0.0022 ^A *	13	0.23±0.0001 ^A *	25
10	0.38±0.0089 ^B *	37	0.19±0.0500 ^B *	38
20	0.275±0.0201 ^C *	54	0.16±0.0600 ^C *	48

Data in the table represent Mean±SE for samples within the same group (n = 12)

*Value for the control group was significantly different at the ($P < 0.05$)

^Avalue was significantly different from the placebo group at the ($P < 0.05$)

^Bvalue was significantly different from Group A at the ($P < 0.05$)

^Cvalue was significantly different from Group B at the level of the ($P < 0.05$)

***In vivo* Inhibition of cholinesterase activity**

A. inhibition by Malathion

Administered rabbits by malathion (75, 150, 300, 600, 1200 mg/kg) orally to the emergence of special poisoning signs inhibiting cholinesterase activity and the death of rabbits administered dose 1200 mg/kg after 30 minutes from the time of administration where rabbits malathion treatment showed an increase in the secretion of saliva and ataxia and defecate and shiver in the whole body and narrowing the pupil.

When measuring the cholinesterase activity in the blood plasma and brain in rabbits poisoned by malathion observed a significant decrease in cholinesterase Activity depending on the dose was measurement by Michel method where the highest percentage of inhibition in plasma was 75% and in the brain 87% (Table 5) either when measured by Ellman method the highest percentage of inhibition in plasma was 84% and in the brain 88% (Table 6).

Table (5) Cholinesterase activity in the brain and blood plasma *in vivo* malathion inhibition which measuring by using Michel method

Malathion dose (mg/kg)	Plasma cholinesterase		Brain cholinesterase	
	Change in the absorption	% of inhibition	Change in the absorption	% of inhibition
0	0.315±0.0450	0	0.195±0.0250	0
75	0.235±0.0050*	25	0.165±0.0150*	15
150	0.20±0.0400 ^A *	37	0.145±0.0050 ^A *	27
300	0.12±0.0200 ^B *	62	0.08±0.0001 ^B *	59
600	0.08±0.0002 ^C *	75	0.025±0.0050 ^C *	87

Data in the table represent Mean±SE for samples within the same group (n=9)

*Value for the control group was significantly different at the ($P < 0.05$)

^Avalue was significantly different from the placebo group at the ($P<0.05$)

^Bvalue was significantly different from Group B at the ($P<0.05$)

^Cvalue was significantly different from Group C at the level of the ($P<0.05$)

Table (6) Cholinesterase activity in the brain and blood plasma *in vivo* malathion inhibition which measuring by using Ellman method

Malathion dose (mg/kg)	Plasma cholinesterase		Brain cholinesterase	
	Change in the absorption	% of inhibition	Change in the absorption	% of inhibition
0	0.68±0.0900	0	0.375±0.0150	0
75	0.325±0.0550 *	52	0.205±0.0050 *	45
150	0.275±0.0450 ^{A*}	60	0.15±0.0100 ^{A*}	60
300	0.185±0.0250 ^{B*}	73	0.095±0.0250 ^{B*}	75
600	0.11±0.0100*	84	0.045±0.0150*	88

Data in the table represent Mean±SE for samples within the same group (n=9)

*Value for the control group was significantly different at the ($P<0.05$)

^Avalue was significantly different from the placebo group at the ($P<0.05$)

^Bvalue was significantly different from Group B at the ($P<0.05$)

B. Inhibition by deltamethrin

Rabbits administered by deltamethrin (12.5, 25, 50, 100 mg/kg) orally to the emergence of poisoning markings of inhibiting cholinesterase activity which showed treatment rabbits Deltamethrin increased in salivation and ataxia and defecate and shiver throughout the body and depending on the dose and death of rabbits administered dose 100 mg/kg after 20 minutes from the time of administration. When measuring the cholinesterase activity in the blood plasma and brain in rabbits poisoned by malathion observed a significant decrease in cholinesterase activity depending on the dose measurement by Michel method where the highest percentage of inhibition in plasma was 32% and in the brain 45% (Table 7), while

when measured by Ellman the highest percentage of inhibition in plasma was 31% and in the brain 33% (Table 8).

Table (7) Cholinesterase activity in the brain and blood plasma *in vivo* deltamethrin inhibition which measuring by using Michel method

Deltamethrin dose (mg/kg)	Plasma cholinesterase		Brain cholinesterase	
	Change in the absorption	% of inhibition	Change in the absorption	% of inhibition
0	0.265±0.150	0	0.20±0.0100	0
75	0.23±0.0100*	13	0.15±0.0050*	25
150	0.20±0.0100 ^{A*}	25	0.125±0.0200 ^{A*}	37
300	0.18±0.0200 ^{B*}	32	0.11±0.0200 ^{B*}	45

Data in the table represent Mean±SE for samples within the same group (n=9)

*Value for the control group was significantly different at the ($P<0.05$)

^Avalue was significantly different from the placebo group at the ($P<0.05$)

^Bvalue was significantly different from Group A at the ($P<0.05$)

Table (8) cholinesterase activity in the brain and blood plasma *in vivo* Deltamethrin inhibition which measuring by using Ellman method

Deltamethrin dose (mg/kg)	Plasma cholinesterase		Brain cholinesterase	
	Change in the absorption	% of inhibition	Change in the absorption	% of inhibition
0	0.86±0.1200	0	0.245±0.0250	0
75	0.715±0.0250*	17	0.205±0.0050*	16
150	0.665±0.0850 ^{A*}	23	0.18±0.0300 ^{A*}	27
300	0.59±0.0200 ^{B*}	31	0.165±0.0150 ^{B*}	33

Data in the table represent Mean±SE for samples within the same group (n=9)

*Value for the control group was significantly different at the ($P < 0.05$)

^Avalue was significantly different from the placebo group at the ($P < 0.05$)

^Bvalue was significantly different from Group A at the ($P < 0.05$)

Comparison of the effect of Malathion and Deltamethrin

A. Michel method

Using the method of Michel and when comparing the percentage of cholinesterase inhibitory by malathion and Deltamethrin later added to the reaction mixture found that there is a significant difference clear between them as the inhibition ratio when using malathion higher than Deltamethrin *in vitro* and when using malathion the percentage of inhibition in the plasma and brain was 55% and 98 % respectively, while the inhibition percentage when using Deltamethrin in blood plasma and brain was 36% and 70% respectively. While, when giving malathion and deltamethrin by the dosage orally were inhibition percentage when using malathion in the plasma and brain of 75% and 87%, respectively, while the damping ratio when using deltamethrin in plasma and brain of 32% and 45%, respectively, as shown in (Table 9).

Table (9) Comparison of the effect of malathion and Deltamethrin on the cholinesterase activity in rabbits which measuring by using Michel method

Insecticide type	Inhibition <i>in vitro</i>		Inhibition <i>in vivo</i>	
	Plasma	Brain	Plasma	Brain
Malathion	55 %	98 %	75 %	87 %
Deltamethrin	36 % *	70 % *	32 % *	45 % *

*Value of deltamethrin significantly different at the ($P < 0.05$)

B. Ellman method

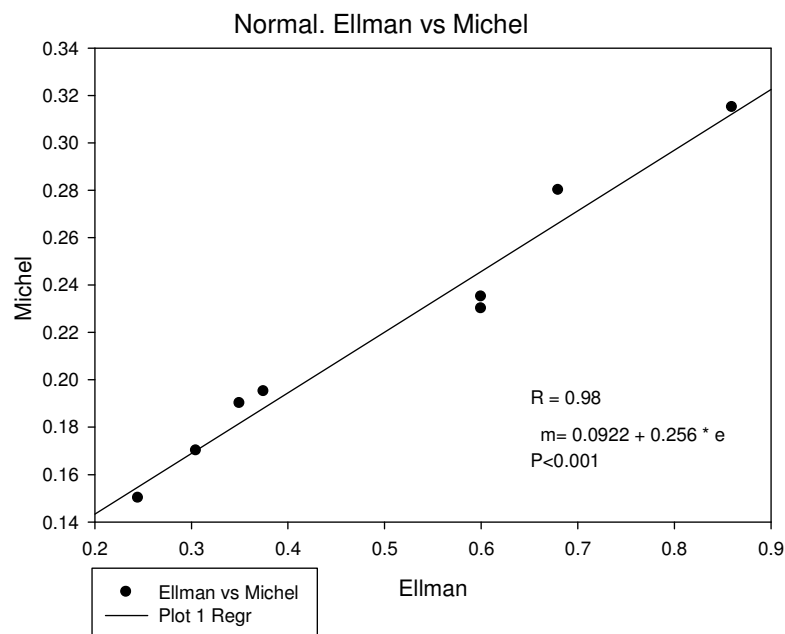
Using the method of Ellman When comparing the percentage of yeast choline esterase and inhibitory by malathion and deltamethrin later added to the reaction mixture found that there is a significant difference clear between them as the damping ratio when using malathion higher than deltamethrin In the glass and when using malathion the percentage of inhibition in plasma and brain 93% and 94 % respectively, while the damping ratio when using deltamethrin in plasma and brain of 54%, 48%, respectively. But when giving malathion and

deltamethrin by the dosage in the mouth were damping ratio when using malathion in plasma and brain 84% and 88%, respectively, while the damping ratio when using deltamethrin in plasma and brain of 31% to 33%, respectively, as shown in (Table 10). Furthermore, there are a linear regression was found between Ellman and Michel method (Figure 1), as well as between brain and plasma cholinesterase (Figure 2).

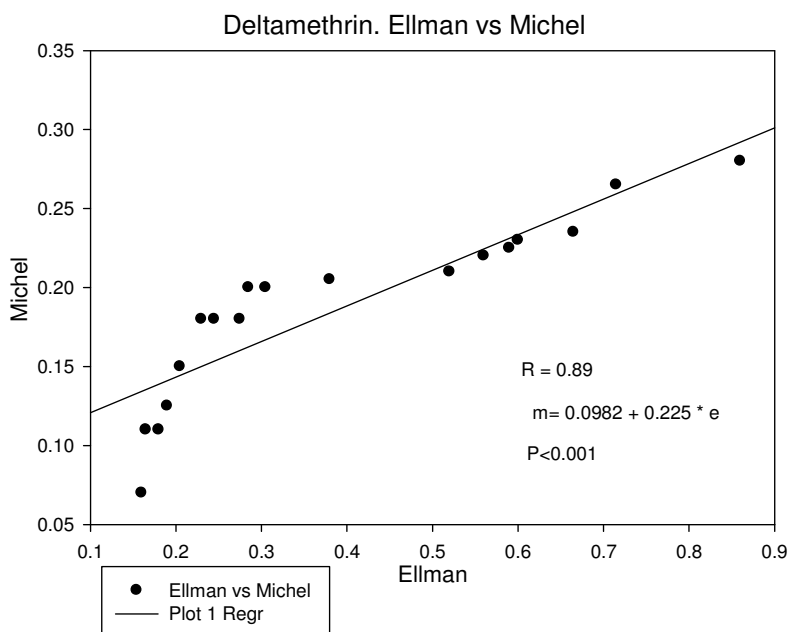
Table (10) Comparison of the effect of malathion and deltamethrin on the cholinesterase activity in rabbits which measuring by using Ellman method

Insecticide type	Inhibition <i>in vitro</i>		Inhibition <i>in vivo</i>	
	Plasma	Brain	Plasma	Brain
Malathion	93 %	94 %	84 %	88 %
Deltamethrin	54 % *	48 % *	31 % *	33 % *

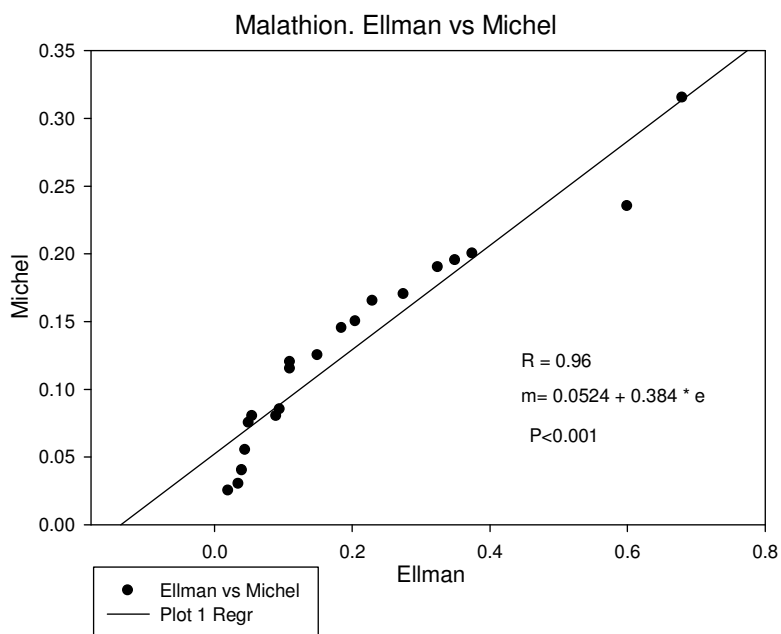
*Value of deltamethrin significantly different at the (P< 0.05)



(a)

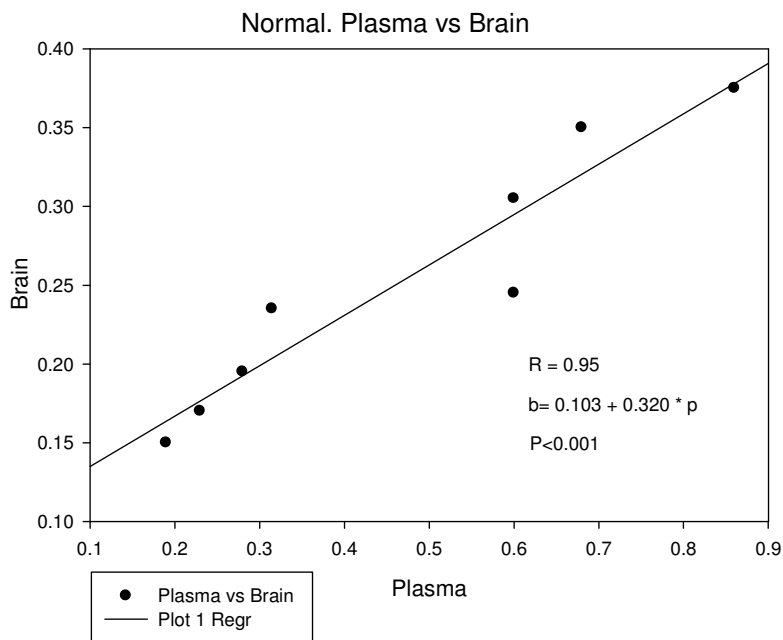


(b)

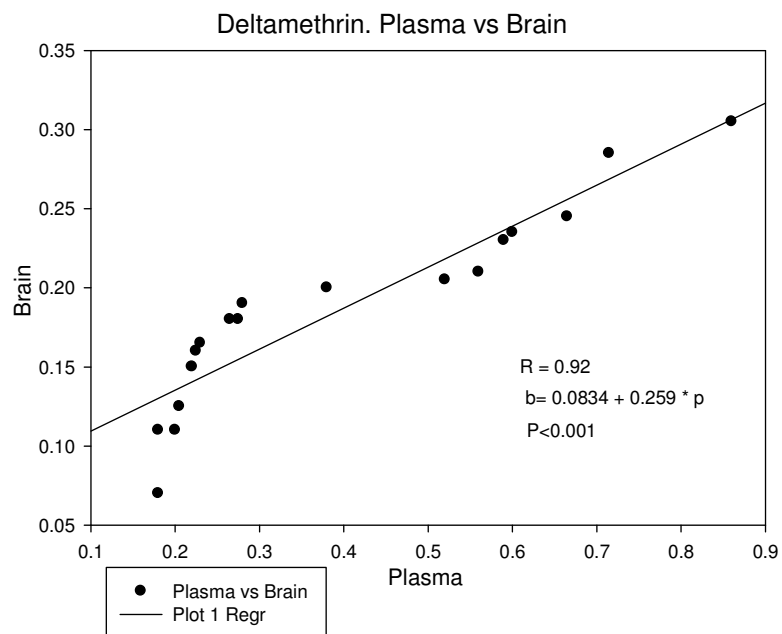


(c)

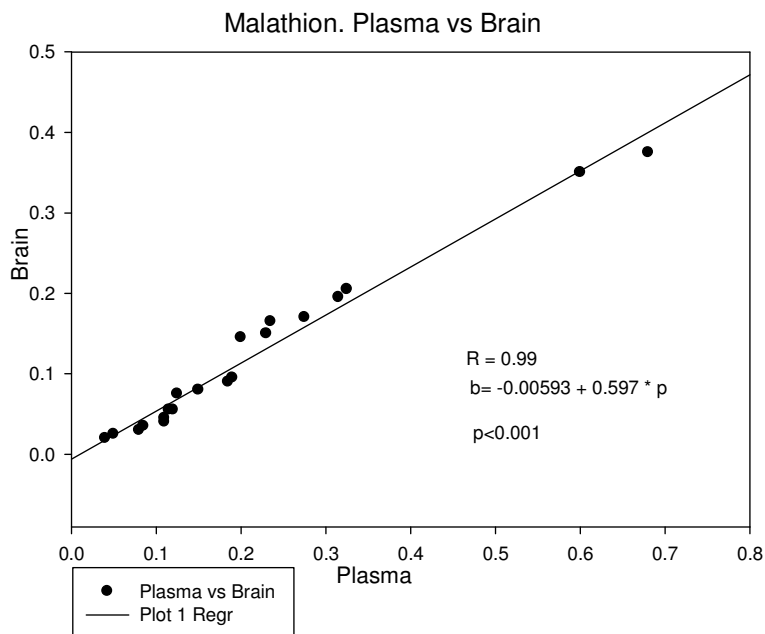
Figure 1: Regression analysis of cholinesterase activity, between Ellman and Michel methods (a), Normal (b), Deltamethrin (c), Malathion.



(a)



(b)



(c)

Figure 2: Regression analysis of cholinesterase activity, between plasma and brain (a), Normal (b), Deltamethrin (c), Malathion.

Discussion

The inhibition in the cholinesterase activity *in vitro* when use a modified Michel method is very clear in the blood plasma and brain samples when incubated with malathion and less than in deltamethrin depending on the concentration, these results are consistent with previous studies (Oropesa *et al.*, 2014), in detecting inhibition resulting in the cholinesterase activity when exposed to cholinesterase inhibitor such as organophosphorus and Carbamate and Pyrethroids. But this method cannot deny the continuation of inhibition during the work of 30 min. so it also was measurement using the method of Ellman to reinforce what has been obtained from the results was a mismatch in the results compatible with what was obtained when using the Michel modified method in the measurement of cholinesterase activity in blood plasma and brain (Askar *et al.*, 2011).

It was necessary to prove the efficiency of the study and the effects obtained in the detection of inhibition cholinesterase activity *in vitro* that held special give malathion and deltamethrin in rabbits experience orally where he found that he could detect inhibition resulting in cholinesterase activity in the blood plasma and brain in rabbits after orally dosage by malathion and deltamethrin (Al-Shinnawy *et al.*, 2014; Yekeen *et al.*, 2016), as well as it has

Michel measurements using the modified method of Ellman and there was a broad consensus in the results between the two methods.

The emergence of private stimulate the nervous system poisoning signs cholinergic act which results from the pool of acetylcholine in the nerve endings to the inability of cholinesterase inhibitors on the analysis of this neurotransmitter supports what has been obtained from the results of the current study (Tse *et al.*, 2013).

The decrease in cholinesterase activity in plasma by 25-30% indicator of exposure to inhibitors of choline esterase, and increase the percentage of inhibition may lead to the emergence of signs of acute poisoning in rabbits that have been dosage (Miranda-Contreras *et al.*, 2013) It was noted after the dosage rabbits malathion that the proportion of inhibition of cholinesterase activity in plasma higher than those in the brain and thus consistent with previous studies which have proved that the phosphorous pesticides inhibit cholinesterase Pseudo than true (Akyildiz *et al.*, 2013)

Additionally when comparing the effect of malathion and deltamethrin on the activity of cholinesterase it found that both insecticides are based inhibition moral of the activity of cholinesterase In the case of the use of the modified Michel method and when comparing the percentage of cholinesterase and inhibitory malathion and deltamethrin later added to the reaction mixture found that there are significant differences clear between them as the inhibition percentage when using malathion higher than deltamethrin *in vitro* and when using malathion the percentage of inhibition in the blood plasma and brain 55% and 98%, respectively, while the inhibition percentage when using deltamethrin in blood plasma and brain 36% and 70%, respectively, and these results were the approval of what has been obtained when using the Ellman method when comparing the percentage of cholinesterase inhibitory by malathion and deltamethrin later added to the reaction mixture found that there is a significant difference clear between them as the inhibition percentage when using malathion higher than deltamethrin in *in vitro* and when using malathion the percentage of inhibition in blood plasma and brain 93% and 94%, respectively, while the inhibition percentage when using deltamethrin in blood plasma and brain 54%, 48%, respectively, as well as there was broad consensus between the results obtained when giving malathion and deltamethrin by the dosage in the mouth were inhibition percentage when using malathion in blood plasma and the brain 75% and 87%, respectively, while the inhibition percentage when using deltamethrin in blood plasma and brain of 32% and 45%, respectively, in Michel modified method. While in the case of using the Ellman method was inhibition percentage

when using malathion in blood plasma and brain 84% and 88%, respectively, while the inhibition percentage when using deltamethrin in blood plasma and brain 31% to 33% respectively. Seen from the experiments conducted in this study that malathion more impact on cholinesterase activity than deltamethrin and that this result was unexpected because the malathion pesticide organophosphorus one of the powerful inhibitors of cholinesterase activity being associated with in Irreversible leading to inhibition cholinesterase once and for all and that this study correspond with what was obtained in previous studies in this area (Wilson, 2003).

Conclusions

This study showed that both of malathion and deltamethrin cause inhibition activity of Cholinesterase in vitro and that this inhibition depends on the concentration, where the greater the concentration of malathion and deltamethrin increased inhibition cholinesterase too well when an inhibition in the body of an organism in vivo by giving malathion and deltamethrin orally cause inhibition of mental activity cholinesterase was malathion more inhibition than deltamethrin and there was a significant difference clear between them and which justifies this difference that malathion from organophosphorus compounds that have a strong influence on the activity of cholinesterase more than deltamethrin from pyrethroids compounds and tastier be less effective because pyrethroids compounds less impact on cholinesterase activity than organophosphorus compounds.

Acknowledgments

The authors are grateful to the College of Veterinary Medicine, University of Kirkuk for allowing the work in laboratory of your collage and supplied the cages and located for animals. In addition, they would like to gratefully thank Dr.Mohammad Al-Mustafa for contributing in statistical advice.

References

Abass KS (2004). Validation of an electrometric method for cholinesterase measurement in the plasma and tissues of the chicken. *Proceedings of the 11th Scientific Congress, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.* pp 241-259.

Abass KS (2014a). Experimental measurements for the effect of dilution procedure in blood esterases as animals biomarker for exposure to OP compounds. *BioMed research international* **2014**. pp 1-13.

Abass KS (2014b). A method for fast assessment of OP/CB exposure in the Japanese quail (*Coturnix coturnix japonica*) using combined esterases enzyme activity as biomarkers. *Enzyme research* **2014**. pp 1-15.

Adak A, Eswarappa R, Nagarajan S, Mukhopadhyay S (2015). Validation of Cholinesterase (Acetyl and Butyryl) Activity Estimation in the Blood and Brain of Wistar Rats.

Akyildiz B, Kondolot M, Kurtoğlu S, Akin L (2013). Organophosphate intoxication presenting as diabetic keto-acidosis. *Annals of Tropical Paediatrics: International Child Health*.

Al-Shinnawy MS, Hassan AR, Ismail DA, Shahin MA (2014). The potential protective and therapeutic effects of aloe vera juice on malathion induced hepatotoxicity in rabbits. *The Egyptian Journal of Hospital Medicine* **55**: 146-159.

Askar KA, Kudi AC, Moody AJ (2011). Comparative analysis of cholinesterase activities in food animals using modified Ellman and Michel assays. *Canadian Journal of Veterinary Research* **75**(4): 261-270.

Bahar FG, Ohura K, Ogihara T, Imai T (2012). Species difference of esterase expression and hydrolase activity in plasma. *Journal of pharmaceutical sciences* **101**(10): 3979-3988.

Cheng DH, Tang XC (1998). Comparative studies of huperzine A, E2020, and tacrine on behavior and cholinesterase activities. *Pharmacology Biochemistry and Behavior* **60**(2): 377-386.

Cornblatt BA, Lenzenweger MF, Erlenmeyer-Kimling L (1989). The continuous performance test, identical pairs version: II. Contrasting attentional profiles in schizophrenic and depressed patients. *Psychiatry research* **29**(1): 65-85.

Dhouib IE-B, Lasram MM, Annabi A, Gharbi N, El-Fazaa S (2015). A comparative study on toxicity induced by carbosulfan and malathion in Wistar rat liver and spleen. *Pesticide biochemistry and physiology* **124**: 21-28.

Dingova D, Leroy J, Check A, Garaj V, Krejci E, Hrabovska A (2014). Optimal detection of cholinesterase activity in biological samples: Modifications to the standard Ellman's assay. *Analytical biochemistry* **462**: 67-75.

Ellman GL, Courtney KD, Andres V, Featherstone RM (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical pharmacology* **7**(2): 88-95.

Gunduz A, Kalkan A, Turedi S, Durmus I, Turkmen S, Ayaz FA, *et al.* (2012). Pseudocholinesterase levels are not decreased in grayanotoxin (mad honey) poisoning in most patients. *The Journal of emergency medicine* **43**(6): 1008-1013.

Hestrin S (1949). The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine, and its analytical application. *Journal of Biological Chemistry* **180**(1): 249-261.

Kovarik Z, Čalić M, Šinko G, Bosak A (2007). Structure-activity approach in the reactivation of tabun-phosphorylated human acetylcholinesterase with bispyridinium para-aldoximes. *Archives of Industrial Hygiene and Toxicology* **58**(2): 201-209.

Lenglet C, Rousson M, Deriche R, Faugeras O (2006). Statistics on the manifold of multivariate normal distributions: Theory and application to diffusion tensor MRI processing. *Journal of Mathematical Imaging and Vision* **25**(3): 423-444.

Massoulié J, Pezzementi L, Bon S, Krejci E, Vallette F-M (1993). Molecular and cellular biology of cholinesterases. *Progress in neurobiology* **41**(1): 31-91.

Michel HO (1949). An electrometric method for the determination of red blood cell and plasma cholinesterase activity. *Journal of laboratory and clinical medicine* **34**: 1564-1568.

Miranda-Contreras L, Gómez-Pérez R, Rojas G, Cruz I, Berrueta L, Salmen S, *et al.* (2013). Occupational exposure to organophosphate and carbamate pesticides affects sperm chromatin integrity and reproductive hormone levels among Venezuelan farm workers. *Journal of occupational health* **55**(3): 195-203.

Oropesa AL, Pérez-López M, Soler F (2014). Characterization of plasma cholinesterase in rabbit and evaluation of the inhibitory potential of diazinon. *Ecotoxicology and environmental safety* **100**: 39-43.

Pan Z, Sai F, Hong ZJ, Hong M, LI JG, ZHANG GW, *et al.* (2014). Application of Gas Chromatography-mass Spectrometry in Analyzing Pharmacokinetics and Distribution of Deltamethrin in Miniature Pig Tissues. *Biomedical and Environmental Sciences* **27**(6): 426-435.

Rakonczay Z (1986). Mammalian brain acetylcholinesterase. *Neurotransmitter Enzymes*: 319-360.

Siakotos A, Filbert M, Hester R (1969). A specific radioisotopic assay for acetylcholinesterase and pseudocholinesterase in brain and plasma. *Biochemical Medicine* **3**(1): 1-12.

Šinko G, Čalić M, Bosak A, Kovarik Z (2007). Limitation of the Ellman method: Cholinesterase activity measurement in the presence of oximes. *Analytical Biochemistry* **370**(2): 223-227.

Tse YC, Sharp CR, Evans T (2013). Mechanical ventilation in a dog with acetylcholinesterase inhibitor toxicosis. *Journal of veterinary emergency and critical care* **23**(4): 442-446.

Wang HP, Liang YJ, Sun YJ, Hou WY, Chen JX, Long DX, *et al.* (2014). Subchronic neurotoxicity of chlorpyrifos, carbaryl, and their combination in rats. *Environmental toxicology* **29**(10): 1193-1200.

Wheelock CE, Phillips BM, Anderson BS, Miller JL, Miller MJ, Hammock BD (2008). Applications of carboxylesterase activity in environmental monitoring and toxicity identification evaluations (TIEs). In: (ed)^(eds). *Reviews of environmental contamination and toxicology*, edn: Springer. p^pp 117-178.

Wills J, Dubois KP (1972). The measurement and significance of changes in the cholinesterase activities of erythrocytes and plasma in man and animals. *CRC Critical Reviews in Toxicology* **1**(2): 153-202.

Wilson JD (2003). *Toxicological profile for malathion*. edn. Agency for Toxic Substances and Disease Registry.

Yekeen TA, Fawole OO, Bakare AA, Emikpe BO (2016). Alteration in haematological, biochemical and reproductive indices of *Rattus norvegicus* treated with lambdacyhalothrin. *Zoology and Ecology* **26**(1): 47-56.