Kufa journal for Veterinary Medical Sciences Vol.(9) No.(2)
Kufa journal for Veterinary Medical Sciences
Kj.vs@uokufa .edu.iq
www.uokufa .edu.iq\journals\index.php\kjvs

The use of the Electrometric Method in Measuring the Activity of Cholinesterase in Local Female Buffalo in north of Iraq

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Received date:20 Sept. 2018 **Accepted:(438)** 14 Nov 2018 **page** (13-22) **Pubished:**31 Dec 2018

Abstract

The aim of present study, was desigen to measurement of cholinesterase (ChE) activity by electrometric method in normal blood of adult Female buffalo, Cholinesterase was used in red blood cells (RBCs)to determine the linear relationship between incubation time and cholinesterase activity. The change in pH of RBCs cells reaction mixture (0.21 to 0.47) agreed with increased incubation time of 10 - 50 minutes and the pH change red blood cells reaction mixture (0.31 to 0.46) was associated with increased incubation temperature of 25-40C. recorded the highest activity of natural ChE the change in pH / 40 C° minutes in the red blood cells (0.46), whole blood (0.42) and least in the blood plasma (0.076). To illustrate the accuracy of the way the coefficient of variation in red blood cells and blood plasma and the whole blood (1.65%, 6.97% and 4.12%), respectively, this was done using 0.1 ml acetylcholine iodide 7.5% as the basis of the combination interaction of the current study. Quinidine sulfate specifically inhibited pseudo ChE in the RBC and whole blood, and it was estimated to be (6.5%,16.7%), where as true ChE activity was (93.5%, 83.3%). Monocrotophos at concentration (0.5, 1 µM) and carbaryl at (5, 10 µM) significantly inhibited RBCs ChE in vitro. These results indicate that the used of modified electrometric method its simple, precise, efficient and have the validity for measurement of ChE activities in adult Female of buffalo.

Keywords: Cholinesterase, buffalo, Electrometric method, Monocrotophos, carbaryl.

إستخدام الطريقة الكهرومترية في قياس نشاط خميرة الكولين أستراز في إناث الجاموس المحلي في شمال العراق

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الخلاصة:

تهدف الدراسة إلى قياس نشاط خميرة الكولين أستراز في دم إناث الجاموس البالغ الغير معامل بالطريقة الكهرومترية. تم تطبيق الطريقة الكهرومترية لقياس النشاط الطبيعي لخميرة الكولين استراز في دم إناث الجاموس البالغة، حيث تم استخدم خميرة الكولين استريز في كريات الدم الحمر لتحديد العلاقة الخطية بين وقت الحضن ونشاط خميرة الكولين استراز, وكان

التغير في الباها لمزيج تفاعل لكريات الدم الحمر من (0.21 إلى 0.47) متوافقا مع زيادة وقت الحضن من 10 -50 دقيقة. ثم استخدمت خميرة الكولين استريز في كريات الدم الحمر لتحديد العلاقة الخطية بين درجة حرارة الحضن C° ونشاط خميرة الكولين استراز, وكان التغير في الباها لمزيج تفاعل لكريات الدم الحمر من (0.31 إلى 0.46) متوافقا مع زيادة درجة حرارة الحضن من 25 – 40°C . حيث سُجل أعلى نشاط طبيعي للخميرة (التغير في الدالة الحامضية/40 دقيقة) في كريات الدم الحمر (0.46)، ثم في عموم الدم (0.42) واقلها في بلازما الدم (0.076) ولبيان دقة الطريقة كان معامل الاختلاف في البلازما 6.97% وفي كريات الدم الحمر 1.65% وفي عموم الدم (4.12%). وتم ذلك باستخدام 0.1مل يوديد الاستيل كولين 7.5% كمادة اساس في مزيج التفاعل للدراسة الحالية . أدى إستخدام كبريتات الكويندين إلى تثبيط نشاط خميرة الكولين استراز الكاذبة في كريات الدم الحمر وعموم الدم أدى ذلك إلى تثبيط نشاط الخميرة الكلي بنسبة 6.5%و16.7% على التوالي ، في حين بلغت نسبة خميرة الكولين استراز الحقيقية 93.5%و83.3% من النشاط الكلي للخميرة في كريات الدم الحمر وعموم الدم على التوالي. سبب إضافة المونوكروتوفوس بتركيز (صفر،0.5 و1مايكرومول/لتر) والكاربارايل بتركيز(صفر، 5 و10مايكرومول/لتر) إلى مزيج التفاعل لكريات الدم الحمر (في الزجاج) تثبيط معنوي في نشاط خميرة الكولين استراز.تشير هذه النتائج إلى أن الطريقة الكهرومترية الحالية تمتاز بالدقة والسهولة والكفاءة لقياس نشاط خميرة الكولين استراز في إناث الجاموس البالغة. لتكون هذه القيم أساساً لمقارنتها مع قيم نشاط خميرة الكولين أستراز للجاموس التي تتعرض للمبيدات الفسفورية العضوية والكاربميتية في البيئة الطبيعية.

الكلمات المفتاحية : الكولين استراز، الجاموس، الطريقة الكهرومترية، المونوكروتوفوس، الكاربارايل.

Introduction:

Compounds like Carbamate and Organophosphorus are uses in veterinary medicine and agriculture (1) to control insects that carrying disease and internal and external parasites (2,3). Over use to Carbamate and Organophosphorus causes environmental pollution of humans and animals (4,5,6,7). And toxicity occurs as a result of misuse or exposure (8), poisoning occurs due to inhibition of Cholinesterase (ChE) activity leading to the accumulation of the Acetylcholine (Ach) at the end of the nerves causing signs to appearance of the Nicotinic, Muscarinic, and CNS-related Poisoning finally lead to death (9,10). Mensuration ChE activity in the blood, helps to detect the toxicity of organophosphate and carbamate pesticides, in the early stages of poisoning. Decrease cholinesterase in 25-30% activity in red blood cells or plasma is evidence to exposure to these compounds (1,4). Many methods appear to measured cholinesterase activity like Ellman method (11), Hystrin method (12), radiometric method (13) and Electrometric method (14). The latter method is inexpensive, simple and does not require complex equipment (only to water bath and pH meters). Michael's original method used only in humans, Therefore, many modifications were made because of the different natural variation in the activity of cholinesterase in tissue and blood between different animal species(15). For that make many modifications on electrometric method (Michael method), by used a different buffer solution of the structure and strength, as well as the volume of the sample involved in the interaction with the use of different temperatures and lap times (15,16). One of the most important modifications is to raise the incubation temperature from 20C° to 37C° and increase blood sample volume in the interaction and reduce the incubation period depends on the type of animal applied this method to sheep (2, 15,17), The electrometric method was used to measure the activity of cholinesterase in cows (18,19). The Electrometric method was not used before on buffalo to measure cholinesterase activity. Local buffalo is exposed to organophosphorus and carbamates because of grazing and swimming in the Water drainage from the city and fields that deal with these pesticides frequently causing the pollution of water on which these animals depend.

Materials and methods

1- **Collection of samples**:

In this study, 31 blood samples of buffalo females ranged from 300-500 kg. Blood samples were collected from the jugular vein and then placed in tubes containing heparin-sodium solution (5000 IU / ml) (B.Braun Melsungen, Germany). And diluted by 1:10 with saline salt solution (20).

Measure by the electrometric method:

After 15 minutes, blood samples were used to measure the normal activity of cholinesterase in the red blood cells and the whole blood. The measured completed in same day via using electrometric method (17), measured pH1 for the sample then use water bath (company elektro. mag, Turkey) controlled at 40 ° C, incubated for 30 minutes, the sample was removed directly from the incubator and measured pH2. The amount of change in the value of the acidic function is calculated by the following equation

(pH2-pH1) -Δ pH of blank = ΔpH / 40min.

The electrometric method was used in preliminary experiments to estimate the incubation time and the appropriate temperature to measure the cholinesterase activity in the red blood cells of the buffalo. cholinesterase activity was measured in blood plasma, whole blood and red blood cells of the buffalo by electrometric method (21). Measure the precision activity of the cholinesterase in red blood cells, plasma, and whole blood in adult buffalo females by an electrometric method, according to mean and standard deviation, as well as Coefficient of variation as follows:

$$\text{Coefficient} = \frac{\text{Sstandard Deviation}}{\text{Mean}} \quad X \quad 100$$

(22)

The percentage of activity of pseudo and true-cholinesterase in red blood cells, whole blood and blood plasma of adult female buffalo was estimated. The samples of blood were divided into two parts. The first part was used to measure the activity of cholinesterase (as mentioned earlier), while the second part of the samples 40 µl of quinidine sulphate (0.1%), (Quinidine sulphate Sigma company, America) for each sample. The samples were then incubated for 10 min at 40 ° C to inhibit pseudo cholinesterase activity (23). quinidine sulfate inhibits pseudo-cholinesterase cholinesterase in red blood cells and whole blood with specific inhibitors (11,24). The total residual activity of cholinesterase was measured in red blood cells and whole blood (True cholinesterase), according to the activity of pseudo- cholinesterase as follows:

Activity of cholinesterase = total cholinesterase – real cholinesterase activity
(Without quinidine) (after adding quinidine)

measured Inhibition of cholinesterase activity in red blood cells of adult female buffalo in vitro by Monocrotophos, 40% of the production of Green River, Italy. (For poisoning) and the carbaryl insecticide 85% powder, Sociedad Anonima De agroquimicos, Spain.

The cholinesterase inhibitor was incubated together with zero concentrations (control), 0.5 and 1 µm/l for monocrotophos treated group and zero concentrations and 5 and 10 µM/ l for the carbaryl group (25) after taking a blood sample (red blood cells) from six adult buffalo females for each experiment incubating the samples at 40 ° C for 10 minutes to inhibit the activity. The percentage was calculated Priority inhibition and as follows:

Percentage of inhibiting activity of cholinesterase activity =

$$\frac{\text{Cholinesterase activity for sample control (without Monocrotophos)- cholinesterase activity with Monocrotophos}}{\text{Cholinesterase activity sample control (without Monocrotophos)}} \times 100$$

Statistical analysis

The results were statistically analyzed using the analysis of variance and then the results were subjected to the Least Significant Difference test. As well as Paired sample T-test (26). The level of moral difference used for all tests at a significant level was less than 0.05 ($P < 0.05$).

Results:

1. Estimation of the incubation period of the interaction sample and its relationship with the activity of the cholinesterase:

An increase in incubation time of 10 to 50 minutes in the measurement of cholinesterase activity in red blood cells sample showed a change in the acid function from 0.21 to 0.47 and a linear increase in cholinesterase activity with increased incubation time by 0.97 correlation coefficient. The incubation time was 40 minutes more suitable for measurement than the rest of the time (Figure 1).

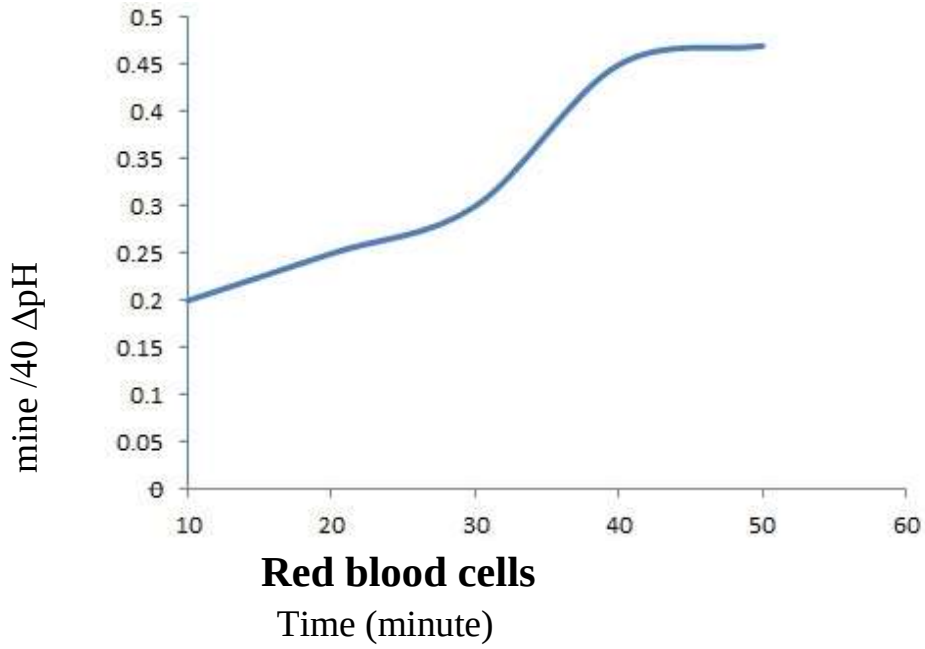

Figure 1: cholinesterase activity in the red blood cells in the adult female buffalo at different times of incubation.

Represents mean value ± standard error of six samples.

2-Determination of the incubation temperature of the reaction sample and its relation to the activity of the cholinesterase:

An increase in incubation temperature from 25°C to 40°C lead to increase in cholinesterase activity in red blood cells showed a change in the acid function from 0.31 to 0.46, with a linear and linear increase of cholinesterase activity with increased temperature of

incubation with a correlation coefficient of 0.96. The temperature of 40 ° C is more suitable for measurement than the rest of the temperature (Figure 2).

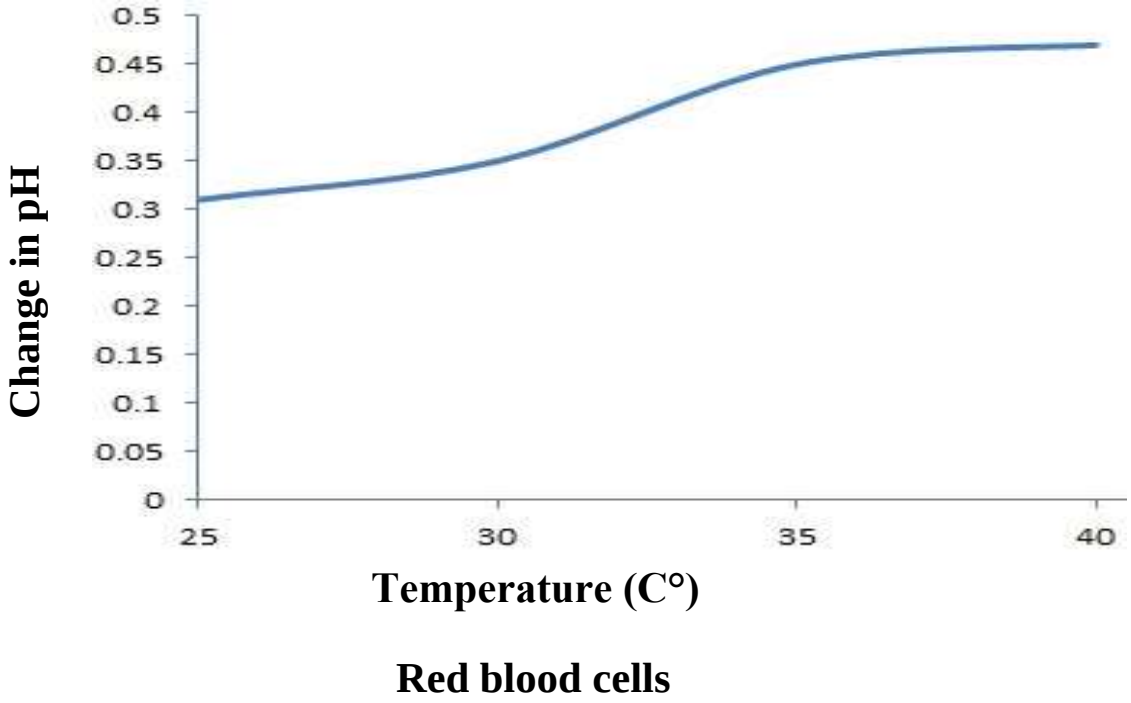

Figure 2: cholinesterase activity in red blood cells in adult buffalo females at different incubation temperatures.

Represents mean value ± standard error of six samples.

3- Measure cholinesterase activity by electrometric method:

Table (1) shows the individual values, mean, standard error, standard deviation, 95% confidence interval and the range for the cholinesterase activity of adult buffalo females. The highest values of cholinesterase activity were in red blood cells (0.46) followed by whole blood (0.42) and blood plasma (0.076).

Table (1)

Measure the normal cholinesterase activity in whole blood, plasma and red blood cells in adult buffalo females

Sample	mine /40 ΔpH		
	whole blood	plasma blood	red blood cells
Mean	0.42	0.076	0.46
standard error	0.0066	0.0020	0.0029
standard deviation	0.0173	0.0053	0.0076
95% confidence interval	0.032	0.099	0.014
range	0.44-0.39=0.05	0.08-0.07=0.01	0.47-0.45=0.02

4- Determination of precision in measuring cholinesterase activity in plasma, red blood cells, whole blood in adult buffalo females by electrometric method:

The coefficient of variation, which indicates the accuracy of the method in measuring cholinesterase activity, was 6.97% plasma, red blood cells 1.65% and whole blood (4.12%) (Table 2).

Table (2)

Determine the accuracy in measuring the cholinesterase activity by electrometric method

Samples	Coefficient of variation	standard deviation	Mean	Number of samples
Red blood cells	%1.65	0.0076	0.46	7
Blood plasma	%6.97	0.0053	0.076	7
Whole blood	%4.12	0.0173	0.42	7

5-Determination of the percentage true and pseudo cholinesterase activity in blood plasma, red blood cells and whole blood in adult buffalo females:

Quinidine sulphate was used to inhibit the activity of pseudo-cholinesterase in red blood cells and whole blood. This inhibited total cholinesterase activity by 6.5% and 16.7%, respectively (Table 3).The percentage of cholinesterase was 93.5% and 83.3% represent total cholinesterase activity in red blood cells and whole blood, respectively (Table 3).

Table (3)

Estimation of the percentage of activity of true and pseudo cholinesterase activity in blood plasma, red blood cells and whole blood adult buffalo females.

Measurements	whole blood		red blood cells	
	Percentages of cholinesterase activity	mine /40 ΔpH ± standard error	Percentages of cholinesterase activity	mine /40 ΔpH ± standard error
Total cholinesterase activity (Without quinidine)	%100	0.007±0.42	%100	0.46 ± 0.0029
True cholinesterase activity *(With quinidine)	83.3%	0.016±0.35	93.5%	0.011±0.43
pseudo cholinesterase activity	16.7%	0.003±0.07	6.5%	0.03 ± 0.004

* Quinidine sulfate was used to inhibit the activity of pseudo-cholinesterase.
Measurements represent the standard error of six samples for each group.

6- Measurement of inhibition of cholinesterase activity in red blood cells in vitro by Monocrotophos and carbaryl:

(A) Inhibition of Monocrotophos:

The addition of Monocrotophos in vitro concentrations (0, 0.5, 1) µM/ l to the reaction mixture for measuring cholinesterase activity in red blood cells resulted in significant inhibition of cholinesterase activity, depending on the concentration of inhibitory material compared to the control group, (Table 4).

Table (4)

Inhibiting cholinesterase activity in red blood cells (adult buffalo females) by Monocrotophos In vitro

Concentration Monocrotophos (µM/ l)	Red blood cells	
	mine /40 ΔpH	Percentage of inhibition
Zero	0.46.±0.003	0
0.5	0.37±*0.013	%19.6
1	0.31± *0.011 A	%32.6

Measurements represent the mean± standard error of six samples for each group.

* The values differ significantly compared to control group values at a probability level less than 0.05.

A* The values differ with group 0.5 µM/ l at a probability level less than 0.05.

(B) Inhibition of carbaryl:

The addition of carbaryl in vitro concentrations (0, 5, 10) µM/ l to the reaction mixture to measure the cholinesterase activity in red blood cells resulted in significant inhibition of cholinesterase activity, depending on the concentration of inhibitory material compared to the control group (Table 5).

Table (5)

Inhibiting cholinesterase activity in red blood cells (adult buffalo females) by carbaryl In vitro

Concentration carbaryl (µM/ l))	Red blood cells	
	mine /40 ΔpH	Percentage of inhibition
Zero	0.003±0.46	Zero
5	*0.008±0.26	%43.5
10	A*0.002±0.23	%50

Measurements represent the mean± standard error of six samples for each group.

* The values differ significantly compared to control group values at a probability level less than 0.05.

A* The values differ with group 5 µM/ l at a probability level less than 0.05

Discussion

The electrometric method currently used in this study is adapted from Michael's original method (14) in measuring the cholinesterase activity in whole blood, plasma and red blood cells, being method, accurate, simple, inexpensive and reliable. (14) can be applied only to human samples (plasma or red blood cells), but they cannot be applied directly to samples Of the different animal species due to the natural variation in the cholinesterase activity in the blood and tissue between species, where cholinesterase not found in poultry in the red blood cells as opposed to mammal (1,27), To measure cholinesterase activity in the blood of field animals such as (cattle, sheep and goats), modification was applied to raise the incubation temperature to 37 ° C and to increase the volume of the reaction sample and reduce the incubation period to between 15-45 min (16). The main current modifications included increasing the sample size of the reaction 0.2 ml and using a uniform plasma sample red blood cells and tissues, a phosphate buffer (ΔpH 8.1) and a reduction incubation period of the sample (between 20 e 40 minutes) (17).

The results showed that the incubation time of 40 minutes gave an appropriate value on the linear relationship to measure cholinesterase activity, which is one of the most suitable times for measuring cholinesterase activity (15,16).

The activity of the cholinesterase was linearly correlated with the increase in the temperature of the samples. The results showed that the temperature of 40C° gave an appropriate value to the linear relationship to measure the cholinesterase activity, which is one of the most suitable times for measuring cholinesterase activity cholinesterase (21) Record the highest normal activity of cholinesterase ΔpH/40 mine In the red blood cells (0.46), whole blood (0.42) and the lowest blood plasma (0.08) (23).

To determine the accuracy of the method, the coefficient of variation in red blood cells, blood plasma and whole blood (1.65%, 6.97% and 4.12%) respectively (21,28).

Quinadine sulfate used to inhibit the activity of pseudo-cholinesterase (29) in red blood cells and the whole blood of adult female buffalo, percentage of pseudo cholinesterase activity was (6.5% and 16.76%) respectively, while the percentage of true cholinesterase in red blood cells and the whole blood of adult female buffalo (93.5% 83.3%).

Monocrotophos addition by concentrations (0.5 and 1 µM / l) to the reaction mixture of red blood cells (in vitro) resulted in significant inhibition of cholinesterase activity (5% and 10 µM / l) to the reaction mixture of red blood in (vitro) resulted in a significant inhibition of cholinesterase activity (43.5% and 50%) respectively (21,23).

This result indicate that the present electrometric method is characterized by accuracy, ease, efficiency, and legitimacy to measure the activity of cholinesterase in the blood of female adult buffaloes, as well as the ability of the method to detect the inhibitory activity in the cholinesterase activity as a result of exposure to organophosphorus compounds and carbamate.

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