# In vitro inhibitory effects of selected anticholinesterase insecticides on human plasma and erythrocyte cholinesterases and their thermal reactivation

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## ABSTRACT

The inhibitory effect of a number of locally used organophosphorus and carbamate insecticides on human plasma and erythrocyte were examined in vitro by using the modified electrometric method for determination of cholinesterase (ChE) activity. The negative logarithum of inhibitor concentration required for ٥٠% inhibition (pIC٥٠) was estimated to range between -١٢.٦ to ٢.٤ and between - ٨.٥ to - ٠.٧٧ for plasma and erythrocyte ChE, respectively. Plasma ChE was more susceptible than erythrocyte ChE to effects of the insecticides. Thermal reactivation technique was applied on plasma and erythrocyte ChE to identify and differentiate between organophosphorus and carbamate insecticides.

Keywords: Cholinesterases, organophosphorus, carbamate, pIC٥٠, thermal reactivation.

## الخلاصة

تم اختبار التاثير المثبط لعدد من المبيدات الحشريه الفسفورية العضوية والكاربامينية المستخدمة محليا على نشاط خميرة الكولين استراز في بلازما الدم وكريات الدم الحمر لدى الانسان في الزجاج با ستخدام الطريقة الكهرومترية المحورة لقياس نشاط خميرة الكولين استراز. كما تم تحديد ال pIC٥٠ اللوغارتم السالب لتركيز المثبط الذي يثبط ٥٠% من الخميرة لكل من المبيدات الحشرية الفسفورية العضوية والكاربامينية. حيث كان ال pIC٥٠ يتراوح بين - ١٢.٦ الى ٢.٤ و -٨.٥ الى -٠.٧٧ لكل من خميرة الكولين استراز في بلازما الدم وكريات الدم الحمر على التوالي. كما تم تطبيق تقنية اعادة التنشط الحراري لخميرة الكولين استراز في بلازما الدم وكريات الدم الحمر للتمييز بين التعرض للمبيدات الحشرية الفسفورية العضوية والكاربيماتية.

Organophosphorus and carbamate insecticides are widely used in veterinary practice and agriculture.[١,٢,٣] They pose major environmental pollution problems and health hazard to man and animals.[٤,٥,٦] The most important toxic action of organophosphorus and carbamate insecticides is inhibition of acetylcholinesterase activity leading to accumulation of acetylcholine at the nerve endings and subsequently causing cholinergic overstimulation characterized by nicotinic, muscarinic and central nervous system effects[١,٧,٨,٩]. Human blood cholinesterase (ChE) are classified as acetylcholinesterase (AChE, mainly found in erythrocytes) and butrylcholinesterase (BChE, mainly found in plasma) according to their substrate specificity as well as sensitivity to various selective inhibitors[١,٥,٧,١٠]. These enzymes differ in their sensitivity to organophosphorus and carbamate insecticides[١١-,١٣] and therefore, the insecticides may show different toxicities. Both in vitro and in vivo tests have been proposed to assess the potential toxicities of organophosphorus and carbamate insecticides like pIC٥٠ (the negative logarithm of molar concentration of compound that inhibit ٥٠% of enzyme activity) and LD٥٠ (the dose of compound that kill ٥٠% of experimental animals).[٧,١٤] In vitro inhibition,
usually expressed as pIC٥٠, is consider as a simple, cheap, relatively non invasive as well as sufficiently correlated with in vivo test ( LD٥٠ ) for determination the sensitivity of human ChE to organophosphorus and carbamate insecticides and for toxicity risk assessment of those chemicals.[٧,١٤,١٥] Although, measurement of ChE activity is considered as a good diagnostic tool for anti-ChE insecticides exposure,[٧,١٢] it cannot differentiate between organophosphate and carbamate exposure.[١٦,١٧].Therefore, as many carbamates are reversible and many organophosphate are irreversible inhibitors, thermal reactivation technique has been successfully applied to identify and differentiate between orgnophosphate and carbamate exposure in different animal species[١٦-,١٨] and this technique has potential application on human plasma and erythrocyte ChE. The aims of this study were to determine the in vitro inhibition of

ChE to evaluate their sensitivity to commercially available organophosphorus and carbamate insecticides and application of thermal reactivation technique on plasma and erythrocyte ChEs to differentiate between inhibition caused by organophosphorus and carbamate insecticides.

## Materials and methods

### Subjects

The subjects included in this study were males and females, age ٢٥± ١٠ years, apparently healthy with no history of exposure to anti-ChE insecticides or drugs. Blood samples were collected in a ٥ ml EDTA-treated test tubes then centrifuged ( Centurion, UK) at ٦٠٠٠ rpm for ١٥ min. The erythrocytes and plasma were separately pooled and kept on ice for ChE assay.

### Electrometric assay of ChE activity

We used a modified electrometric validated in human.[١٨,١٩] For a typical assay condition, the reaction mixture in a ١٠-ml beaker contained ٣ ml distilled water, ٠.٢ ml plasma or erythrocytes and ٣ ml pH ٨.١ barbital-phosphate buffer[٢٠].

The pH of the mixture (pH١) was measured with glass electrode using pH meter (Hanna Instruments, Romania), then ٠.١ml of aqueous solution of acetylthicholine (٧.٥%) was added to the reaction mixture which was incubated at ٣٧ °C in water bath (Shaker bath ٥BS٣٠, UK) for ٢٠ min. At the end of the incubation period, the pH of the reaction mixture (pH٢) waseasured. The enzyme activity was calculated as follows:

ChE activity (ΔpH/٢٠ min.) = (pH١-pH٢)-ΔpH of blank

The blank was without the blood aliquot. The barbital-phosphate buffer solution consist of ١.٢٤g sodium barbital (BDH), ٠.١٦٣g potassium dihydrogen phosphate (Merck, Germany), and ٣٥.٠٧g sodium chloride (BDH) dissolved in one liter distilled water[١٨,٢٠]. The pH of the buffer was adjusted to ٨.١ with ١N HCL.

**In vitro ChE inhibition by organophosphorus and carbamate insecticide**The method of inhibitor-ChE incubation [١٨,٢٠] was used to measure the in vitro inhibition of plasma and erythrocyte ChE activities by organophosphuorus chlorpyrifos (٤٨%, CHEMINOVA, Denemark), triazophos (٤٠%, Shandong Qiaochg Chemical, China), monocroptophos (٤٠٠g/L, Greenriver), and dimetheoate (٤٠٠g/L, Devitayal, India) and carbamate carbaryl (٨٥%, Sociedad Anonima De Agroquimicos, Spain), methomyl (٩٠%, Dobon Donomorz, USA) and propamocarb (٧٢٢g/L, AGRIA SA., Bulgaria). The insecticides were prepared in distill water and individually added in a ٠.١ml to the reaction mixture of the plasma and erythrocytes. The reaction mixtures containing insecticides were incubated at ٣٧°C for ١٠ min. Theraftere, the residual ChE activity in mixture was measured as before.The % of enzyme inhibition was calculated as follows:

% ChE inhibition= [ChE activity (without insecticide)-ChE activity (with insecticide)/ChE activity (without insecticide)]X١٠٠

### Determination of IC٥٠ values

The % inhibition of control activity was plotted against logarithm of inhibitor concentration. The IC٥٠ values were determined by linear regression of the inhibition curve from ٢٠-٨٠% inhibition. When inhibition values was less than ٥٠% the IC٥٠ value was determined by extrapolation[٢١].

**Thermal reactivation of organophosphorus and carbamate inhibited-ChE**In vitro inhibition of plasma and erythrocyte ChE by organophosphorus and carbamate insecticides were done as mentioned above. Before the determination of ChE activity the reaction mixture was incubated in water bath at ٣٧° C for ١٨-٢٤ hours[١٦,١٧]. Theraftere, the ChE was assayed as described before. The % of enzyme reactivation was calculated as follows:

% of ChE reactivation= [ChE activity after reactivation – ChE activity before reactivation /ChE activitry after reactivation

### Statistics

When applicable, the data where subjected to one way analysis of variance followed by the least significant test[٢٢]. Student's-t- test was used for the means of two groups[٢٢]. The level of significance was at P< ٠.٠٥.

## Results

Tables ١-٧ show in vitro inhibition of plasma and eryrthocyte ChEs by organophosphate and carbamate insecticides and their thermal reactivation. The organophosphorus (chlorpyriphos, monocroptophos, dimethoate, and triazophos) and carbamate (carbaryl, methomyl and propamocarb) insecticides in a concentration- dependent manner variably inhibited plasma and erythrocyte ChE activities in vitro (Tables ١-٧).

Table ١ : In vitro plasma and erythrocyte cholinesterase (ChE) inhibition by Chlorpyrifos and their thermal reactivation

| | Plasma | | | | Erythrocyte | | | |
|---|---|---|---|---|---|---|---|---|
| Conc μM | ChE Activity ΔpH/٢٠min | % inhibition | ChE Activity After reactivation ΔpH/٢٠min | % reactivation | ChE Activity ΔpH/٢٠min | % inhibition | ChE Activity After reactivation ΔpH/٢٠min | % reactivation |
| ٠ | ١.١±٠.٠٢ | ٠ | ٠.٧٦ | ٠ | ١.٢١±٠.٠٨ | ٠ | ٠.٨٤ | ٠ |
| ٠.١٢٥ | ٠.٩٥±٠.٠٤ * | ١٣.٦ | | | ١.٠٤±٠.٠٥* | ١٤ | | |
| ٠.٢٥ | ٠.٩١±٠.٠٤* | ١٧.٤ | ٠.٦٣ | ٠ | ١.٢٢±٠.٠١ | ١.١ | ٠.٧٨ | ٠ |
| ٠.٥ | ٠.٧١±٠.٠٣* | ٣٥.٤ | | | ١.١٦±٠.٠٢ | ٤.٤ | | |
| ١ | ٠.٣٨±٠.٠٣* | ٦٥.٣ | ٠.٢٩ | ٠ | ١.١٥±٠.٠٢ | ٤.٧ | ١.١٣ | ٠ |
| ٢ | ٠.٠٩±٠.١ * | ٩٢.٣ | | | ١.١١±٠.٠٢* | ٨.٦ | | |
| ٤ | ٠.٠٦±٠.٠١ * | ٩٤.٧ | ٠.٠٦ | ٠ | ٠.٩٥±٠.٠٢* | ٢١.٤ | ٠.٦٩ | ٠ |

* Significantly different from the respective control (٠ concentration), P<٠.٠٥.
n= ٣-٤/ concentration group.
ChE values are mean±SD

Table ٢ : In vitro plasma and erythrocyte cholinesterase (ChE) inhibition by monocroptophos and their thermal reactivation.

| | Plasma | | | | Erythrocyte | | | |
|---|---|---|---|---|---|---|---|---|
| Conc. μM | ChE Activity ΔpH/٢٠min | % inhibition | ChE Activity After reactivation ΔpH/٢٠min | % reactivation | ChE Activity ΔpH/٢٠min | % inhibition | ChE Activity After reactivation ΔpH/٢٠min | % reactivation |
| ٠ | ١.١٤±٠.٠١ | ٠ | ١.١٤ | ٠ | ١.٠٣±٠.٠٧ | ٠ | ١.١ | ٧ |
| ٠.٠١٥٦ | ٠.٦٨±٠.٠١* | ٤١ | | | | | | |
| ٠.٠٣١٣ | ٠.٤٥±٠.٠١* | ٦٠.٥ | | | | | | |
| ٠.٠٦٢٥ | ٠.٥٤±٠.٠١* | ٥٢.٦ | | | | | | |
| ٠.١٢٥ | ٠.٠٢±٠.٠١ * | ٩٨ | | | ١±٠.٠٤ | | | |
| ٠.٢٥ | ٠.٠٢±٠.٠٦* | ٩٨.٥ | ٠.٠٦[a] | ٢٠٠ | ١.١±٠.٠٥ | ٢.٨ | ٠.٩٦ | ٠ |
| ٠.٥ | ٠.٠٢±٠.٠١* | ٩٨.٢ | | | ١.١±٠.٠١ | ٠ | | |
| ١ | ٠.٢١±٠.١٢* | ٧٦.٩ | ٠.٠٣ | ٠ | ٠.٩٣±٠.٠٣* | ٩.١ | ٠.٦ | ٠ |
| ٢ | ٠.١٢±٠.٠٧* | ٩٢.١ | | | ٠.٨٥±٠.٠٤* | ١٦.٩ | | |
| ٤ | ٠.٠٦±٠.٠١* | ٩٥.٢ | ٠.٠٢ | ٠ | ٠.٨٣±٠.٠٤* | ١٩.٣ | ٠.٢ | ٠ |

* Significantly different from the respective control (٠ concentration), P<٠.٠٥.
[a] Significantly differerent from pre-reactivation values.
n= ٣-٤/ concentration group ChE values are mean±SD

Table ٣ : In vitro plasma and erythrocyte cholinesterase (ChE) inhibition by Triazophos and their thermal reactivation

| | Plasma | | | | Erythrocyte | | | |
|---|---|---|---|---|---|---|---|---|
| Conc. μM | ChE Activity ΔpH/٢٠min | % inhibition | ChE Activity After reactivation ΔpH/٢٠min | % reactivation | ChE Activity ΔpH/٢٠min | % inhibition | ChE Activity After reactivation ΔpH/٢٠min | % reactivation |
| ٠ | ٠.٩٥±٠.٠٧ | ٠ | ٠.٧٣ | ٠ | ١.١±٠.١١ | ٠ | ٠.٧٥ | ٠ |
| ٠.١٢٥ | ٠.٩٢±٠.١٥ | ٣.١٥ | | | ٠.٨±٠.٠٦* | ٢٥.٢ | | |
| ٠.٢٥ | ٠.٨٧±٠.١٥ | ٨.٤ | ٠.٨٩ | ٢.٣ | ٠.٦٧±٠.١٧* | ٣٧.٤ | | |
| ٠.٥ | ٠.٨±٠.٠٢ | ١٠.٥ | | | ٠.٦٨±٠.٠٥* | ٣٦.٤ | | |
| ١ | ٠.٨٣±٠.٠٧ | ١٢.٦ | ٠.٦٧ | ٠ | ٠.٥٨±٠.٠٨* | ٤٦.٧ | ٠.٣ | ٠ |
| ٢ | ٠.٦٧±٠.٠٥* | ٢٩.٥ | ٠.٨٣[a] | ٢٤ | ٠.٥٧±٠.٠٧* | ٤٦.٧ | | |
| ٤ | ٠.٤١±٠.١١* | ٥٦.٨ | ٠.٤٤ | ٧.٣ | ٠.٥٢±٠.١٥* | ٥٢.٣٤ | ٠.٦٨[a] | ٣١ |
| ٨ | ٠.١٨±٠.٠* | ٨١ | | | | | | |

* Significantly different from the respective control (٠ concentration), P<٠.٠٥.

[a] Significantly differerent from pre-reactivation values.
n= ٣-٤/concentration group. ChE values are mean±SD

Table ٤: In vitro plasma and erythrocyte cholinesterase (ChE) inhibition by Dimethoate and their thermal reactivation.

| | Plasma | | | | Erythrocyte | | | |
|---|---|---|---|---|---|---|---|---|
| Conc. μM | ChE Activity ΔpH/٢٠min | % inhibition | ChE Activity After reactivation ΔpH/٢٠min | % reactivation | ChE Activity ΔpH/٢٠min | % inhibition | ChE Activity After reactivation ΔpH/٢٠min | % reactivation |
| ٠ | ١.٢±٠.٠٣ | ٠ | ١.١٩ | ٠.٨٥ | ١.٢٢±٠.٠٥ | ٠ | ١.١١ | ٠ |
| ٠.٢٥ | ١.٣±٠.٠٥ | ٠ | ١.١٩ | ٠ | ١.١٩±٠.٠٢ | ١.٨ | ٠.٧٤ | ٠ |
| ٠.٥ | ١.٣±٠.٠٣ | ٠ | | | ١.١٩±٠.٠٢ | ٢.٢١ | | |
| ١ | ١.١±٠.٠٨ | ٦.٤ | ١ | ٠ | ١.١٣±٠.٠٣ | ١.١٣ | ١.١ | ٠ |
| ٢ | ١.١±٠.٠٣ | ٦.٥ | | | ١.١٦±٠.٠٢ | ١.١٦ | | |
| ٤ | ١.١±٠.٠٥* | ٩.٤ | ٠.٧٣ | ٠ | ١.١٤±٠.٠٢ | ١.٤ | ١ | ٠ |
| ٦ | ٠.٩٨±٠.٠٢* | ١٦.٩٥ | | | ١.١١±٠.٠٣ | ٩.٠٢ | | |
| ١٠ | ٠.٣٧±٠.٠٧* | ٦٨.٦٤ | | | ١.١±٠.٠٢ | ١٣.٥٢ | | |
| ٢٠ | ٠.٣٥±٠.٠٥* | ٧٠.٧٦ | | | ١.٠٨±٠.٠١ | ١١.٨٨ | | |
| ٣٠ | ٠.٣±٠.٠١* | ٧٣.٧٣ | ٠.١٣ | ٠ | ٠.٦٩±٠.٤١* | ٤٣.٨٥ | ٠.٤٢ | ٠ |

* Significantly different from the respective control (٠ concentration), P<٠.٠٥.
n= ٣-٤/concentration group.
ChE values are mean±SD

Table ٥: In vitro plasma and erythrocyte cholinesterase (ChE) inhibition by Carbaryl and their thermal reactivation

| | Plasma | | | | Erythrocyte | | | |
|---|---|---|---|---|---|---|---|---|
| Conc. μM | ChE Activity ΔpH/٢٠min | % inhibition | ChE Activity After reactivation ΔpH/٢٠min | % reactivation | ChE Activity ΔpH/٢٠min | % inhibition | ChE Activity After reactivation ΔpH/٢٠min | % reactivation |
| ٠ | ١.٢±٠.٠٤ | ٠ | ١.١٦ | ٠ | ١.٢±٠.٠١ | ٠ | ١.١٧ | ٠ |
| ٥ | ٠.٨٧±٠.٠٢* | ٣٠.٢٥ | ١.٢٣a | ٤١ | ٠.٧٤±٠.٠٧* | ٤١.٠٣ | ٠.٧٨ | ٥.٤ |
| ١٠ | ٠.٧٢±٠.٠٢* | ٤٢.٨٦ | | | ٠.٦٧±٠.٠١* | ٤٦.١٥ | | |
| ٢٠ | ٠.٦±٠.٠٥* | ٥٢.٩ | ١.١٣a | ٨٨ | ٠. ٦±٠.٠٢* | ٥٢.١٤ | ٠.٦١ | ١.٦ |
| ٤٠ | ٠.٣١±٠.٣١* | ٧٧.٣ | ١.٢٢a | ٢٩٣ | ٠.٤±٠.٠٠٣* | ٧٠.٩٤ | ٠.٤٢ | ٥ |

* Significantly different from the respective control (٠ concentration), P<٠.٠٥.
a Significantly different from pre-reactivation values.
n= ٣-٤/ concentration group.
ChE values are mean±SD

Table ٦ : In vitro plasma and erythrocyte cholinesterase (ChE) inhibition by Methomyl and their thermal reactivation

| | Plasma | | | | Erythrocyte | | | |
|---|---|---|---|---|---|---|---|---|
| Conc. µM | ChE Activity ΔpH/٢٠min | % inhibition | ChE Activity After reactivation ΔpH/٢٠min | % reactivation | ChE Activity ΔpH/٢٠min | % inhibition | ChE Activity After reactivation ΔpH/٢٠min | % reactivation |
| ٠ | ١.٢٧±٠.٠٤ | ٠ | ١ | ٠ | ١.٢٣±٠.٠٣ | ٠ | ٠.٦ | ٠ |
| ١.٢٥ | ٠.٩٣±٠.٠٥* | ٢٧ | | | ٠.٨٢±٠.٠٦* | ٣٣ | | |
| ٢.٥ | ٠.٦٢±٠.٠٣* | ٥١ | ٠.٥١ | ٠ | ٠.٦٧±٠.٠١* | ٤٦ | ٠.٥ | ٠ |
| ٥ | ٠.٤٢±٠.٠٣* | ٦٧ | | | ٠.٥٨±٠.٠٤* | ٥٣ | | |
| ١٠ | ٠.٣١±٠.٠١* | ٧٦ | ٠.٣٣ | ٦.٥ | ٠.٥٢±٠.٠٦* | ٥٨ | ٠.٥ | ٠ |
| ٢٠ | ٠.٢٦±٠.٠١* | ٨٠ | | | ٠.٤٩±٠.٠٣* | ٧٦ | | |
| ٤٠ | ٠.٢١±٠.٠٣* | ٨٣.٢ | ٠.٢٦a | ٢٤ | ٠.٤±٠.٠١* | ٧٣ | ٠.٢٣ | ٠ |

* Significantly different from the respective control (٠ concentration), P<٠.٠٥.
a Significantly differerent from pre-reactivation values.
n= ٣-٤/ concentration group.
ChE values are mean±SD

Table ٧ : In vitro plasma and erythrocyte cholinesterase (ChE) inhibition by Propomocarb and their thermal reactivation

| | Plasma | | | | Erythrocyte | | | |
|---|---|---|---|---|---|---|---|---|
| Conc. µM | ChE Activity ΔpH/٢٠min | % inhibition | ChE Activity After reactivation ΔpH/٢٠min | % reactivation | ChE Activity ΔpH/٢٠min | % inhibition | ChE Activity After reactivation ΔpH/٢٠min | % reactivation |
| ٠ | ١.٠٢±٠.٠٦ | ٠ | | | ١.٠٤±٠.٠٢ | ٠ | | |
| ١٠ | ٠.٩٢±٠.٠٤* | ١١ | | | ٠.٩٦±٠.٠٤* | ٨ | | |
| ٢٠ | ٠.٨٩±٠.٠٢* | ١٤ | | | ١±٠.٠٢ | ٤ | | |
| ٣٠ | ٠.٨٨±٠.٠٢* | ١٤ | | | ١±٠.٠٥ | ٤ | | |
| ٥٠ | ٠.٨٥±٠.٠٣* | ٧٢ | | | ٠.٩٣±٠.٠١* | ١١ | | |
| ٦٠ | ٠.٩١±٠.٠٦* | ١١ | | | ١.٠١±٠.٠٣ | ٣ | | |
| ٨٠ | ٠.٩٧±٠.٠٦ | ٦ | | | ٠.٩٨±٠.٠١ | ٦ | | |
| ١٠٠ | ٠.٨٥±٠.٠٢* | ١٧ | | | ٠.٩١±٠.٠٥* | ١٢ | | |
| ٢٠٠ | ٠.٨٢±٠.٠٧* | ٢١ | | | ٠.٩٦±٠.٠٥* | ٨ | | |

* Significantly different from the respective control (٠ concentration), P<٠.٠٥.
n= ٣-٤/ concentration group. ChE values are mean±SD

Table ٨ shows pIC٥٠ values for the investigated insecticides. Where the pIC٥٠ values ranged from –١٢.٦ to ٢.٤ and from –٨.٥ to –٠.٧٧ for plasma and erythrocyte, respectively.

Table ٨: The pIC٥٠ values of the investigated insecticides

| Insecticides | pIC٥٠ | |
|---|---|---|
| | Plasma | RBC |
| Chlorpyrifos | ٠.٠٥١ | -٨.٥ |
| Monocroptophos | ٢.٤ | -٢.٥٢ |
| Triazophos | -٠.٥٩ | -١.٠٢ |
| Dimethoate | -١.٠٦ | -٣.٤ |
| Carbaryl | -١.١٢ | -١.١ |
| Methomyl | -٠.٦٢ | -٠.٧٧ |
| Propamocarb | -١٢.٦ | -٧.٧٤ |

**Discussion**

Domestic uses of organophosphorus and carbamate insecticides is frequent and widespread in Iraq and presents serious health hazards. Commercially, there are wide variety of organophosphorus and carbamate products, therefore the inhibitory effects of these insecticides is an important issue for toxicity risk assessment. In vitro inhibition (expressed as pIC٥٠) is considered as a simple, cheap, rapid, relatively non-invasive and sufficiently correlated to in vivo studies (LD٥٠) for toxicity risk assessment.[١٤,١٥]

Our research introduces in vitro inhibition of human plasma and erythrocyte ChE by organophosphorate (chlorpyifos,triazophos,monocroptophos, and carbamate (carbaryl, methomyl and propamocarb) insecticides. In vitro inhibition of human plasma and erythrocyte ChE by these insecticides was in agreement with their reported anti-ChE actions[١,٣,١٨,١٩,٢٣]. The present findings suggest that human plasma ChE is more susceptible to the action of organophosphrus and carbamate than erythrocytes ChE as indicated by their pIC٥٠, therefore, plasma ChE activity is more suitable than erythrocyte ChE activity as a biomarker for monitoring exposure to organophosphorus and carbamate insecticide. This finding is in agreement with study conducted by Lotti et al, ١٩٩٥[١٢]. Insecticides used in this study were arranged in an ascending manner from the lower to the higher toxic one in the following order (propamocarb<carbaryl<dimethoate< triazophos<methomyl<chlorpyrifos< monocroptophos) according to their plasma pIC٥٠ and (clorpyrifos <propamocarb<dimethoate<monocroptophos< carbary< triazophos < methomyl) according to their erythrocyte pIC٥٠. The pIC٥٠ is of greatest value for prediction of toxicity when measured for acetylcholinesterase because that is the enzyme related to toxicity.[١٢,١٤]

Our study is considered as a further validation of the modified electrometric method of Mohammad et. al.[٢٠] in human. The present findings suggest the sensitivity of the described electrometric method in detecting human plasma and erythrocyte ChE inhibition by organophosphates and carbamates. Further ChE inhibition should not be excluded from this in vitro system during the ٢٠ min. incubation time. However, the original electrometric method cannot be recommended for the detecting of ChE inhibition induced by carbamate.[١٠,٢٤,٢٥].This findings were in agreement with previous studies in different animal species[٢٦,٢٧,٢٨] and in human.[١٨,١٩]

Thermal reactivation of human plasma and erythrocyte ChE enzymes to differentiate between organophosphate and carbamate pesticides was investigated. When the activity of the initially inhibited sample was equal or greater than control after incubation, carbamate exposure was suspected. When the activity remained below the control, organophosphorus exposure is suspected[١٦,١٧]. The result of the present thermal reactivation study indicates that ٢٤ hour incubation period (at ٣٧ºC) of carbamate-inhibited plasma and erythrocyte ChEs was suitable to cause reactivation of the enzymes, however false negative result may occur with carbamate insecticides due to high level of carbamate which may cause sustained inhibition and prevent reactivation of ChE enzyme after incubation period[١٧]. Sometimes some inhibitors require longer incubation period to be spontaneously reactivated[١٦] which may explain why methomyl – inhibited ChE was not reactivated after the ٢٤ hour incubation period. This finding was in agreement with previous studies on brain acetylcholinesterase in birds[١٦, ١٧] and in mice.[١٨]

**Conclusions**

In vitro ChE inhibition is a useful technique for detecting the potential anti-ChE activity of chemicals like organophosphorus and carbamate insecticides and provide the basis for development of useful and reliable methods to assess possible contamination of ChE- inhibitory pesticides in environmental samples. The result of the present study also indicate the efficiency of the described electrometric method in detecting ChE inhibition by carbamate and extend its value to use the present experimental protocol for in vitro ChE inhibition in preliminary toxicological analysis. Thermal reactivation technique for human blood ChE enzymes is a suitable screening tool for identifying and differentiating field exposure to carbamate and organophosphorus insecticides.

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