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MONITORING BLOOD CHOLINESTERASE ACTIVITY OF FARMWORKERS: IN VITRO INHIBITION BY DIPHENHYDRAMINE AND CARBARYL

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ABSTRACT

Plasma and erythrocyte cholinesterase (ChE) activities of male farm workers exposed to pesticides during their routine work were lower than those of unexposed control subjects by 14 and 4%, respectively. Diphenhydramine and Carbaryl inhibited plasma and erythrocyte ChE activities in vitro in pesticide-exposed and unexposed subjects. The percentages of in vitro ChE inhibition induced by carbaryl in the plasma and erythrocytes of unexposed controls ranged between 47-85% and 19-47%, respectively, whereas they were 35-60% and 3-12% in the pesticide-exposed group, respectively. In vitro pretreatment of plasma and erythrocyte ChE with diphenhydramine (20 μM) significantly reduced the inhibitory effect of carbaryl (10 μM) on them by 18% and 10%, respectively. In conclusion, subjects exposed to pesticides during their routine work in agriculture are at risk of reduced blood ChE activity. Diphenhydramine appeared to partially protect blood ChE in vitro from additional carbaryl-induced enzyme inhibition in both pesticide-exposed and unexposed subjects.

Key words: Antihistamine, biomonitoring, carbamate, cholinesterase, insecticides

INTRODUCTION

Farmers and agricultural workers are at health risk because of possible exposure to pesticides such as organophosphates, carbamates, and pyrethroids (Ecobichon, 2001; Tudi *et al*., 2021). Exposure to pesticides usually occurs dermally, orally, and via respiration during planting, spraying, harvesting, and packing of the agricultural products as well as through other activities related to pesticide application (Damalas & Koutroubas, 2016; Patel e*t al*., 2018). Various organophosphate and carbamate pesticide formulations are widely used, especially in developing countries, in agriculture, public health, and veterinary clinical practice (Ecobichon, 2001; Baynes, 2018; Tudi *et al*., 2021). These products can be misused (Ecobichon, 2001) and even obtained over the counter in some countries such as Iraq (Jongerden *et al*., 2018; Ahmed & Majeed, 2020) without the authoritative control of specialists or the health officers. Such pesticide products are inevitably toxic to animals and workers in agriculture as well as to consumers consuming pesticide-contaminated agricultural products (Ecobichon, 2001; Al-Zubaidy *et al*., 2011; Wilson, 2014; Vale & Lotti, 2015; Baynes, 2018, Boedeker *et al*., 2020). A study conducted by Nagami *et al.* (2017) showed that pesticide-exposed farmers continued to work suffering from watery eyes, coughing, and difficulty in breathing. However, in more severe cases of pesticide poisoning, hospitalization is needed (Zemedie *et al*., 2021).

The most important mechanism of toxic action of organophosphate and carbamate pesticides is irreversible and reversible inhibition of cholinesterase (ChE) activity, respectively, at the nerve terminals leading to cholinergic toxidrome of muscarinic, nicotinic, and central nervous system effects (Wilson, 2014; Vale & Lotti, 2015; Baynes 2018). Therefore, monitoring exposure to ChE inhibiting pesticides is conducted through measurements of the plasma, serum, erythrocyte, whole blood, and to a lesser extent nervous tissue ChE activities in humans, mammals, and birds (except erythrocytes) (Roy *et al*., 2005; Wilson *et al*., 2005; Wilson, 2014; Vale & Lotti, 2015; Cotton *et al*., 2018). This is very important because of the possibility of the injudicious use of pesticides by farmers (Benaboud *et al*., 2021). Studies have been conducted in Iraq for biomonitoring exposure of agricultural workers, farmers, and veterinarians to pesticides, reporting a low level of blood ChE activities but with limited information on the types of pesticides used, their frequency of application,

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and the extent of exposure (Ahmed & Mohammad, 2004; 2007; Al-Haseni & Yahya, 2012; Ahmad, 2013; Othman & Kakey, 2020). A recent review by Boedeker *et al*. (2020) has shown that human unintentional acute poisoning by pesticides whas distributed world-widely, especially among farmers and farm workers. However, no systematic surveys or records are available in Iraq on the acute poisoning of farmworkers hospitalized due to pesticide exposure.

The standard antidotes used in cases of poisoning with organophosphates and carbamates include mainly atropine sulfate which antagonizes the muscarinic effects and diazepam which counteracts convulsions and oximes which are used as enzyme reactivators in organophosphate-inhibited ChE (Wilson, 2014; Vale & Lotti, 2015; Worek *et al*., 2020). In addition, many studies have reported the beneficial antidotal effects of the antihistamine diphenhydramine (a histamine H1-receptor antagonist) against poisoning induced by organophosphate and carbamate insecticides in animals (Fikes, 1990; Al-Baggou' & Mohammad, 1999; Mohammad *et al*., 2002; 2012a; Ojha *et al*., 2014). Diphenhydramine possesses strong antimuscarinic action with potential antinicotinic effects which are not possessed by atropine (Fikes, 1990; Al-Baggou' & Mohammad, 1999; Mohammad *et al*., 2002; 2012a; Ojha *et al*., 2014), and therefore, it can be repurposed for use as an additional antidote against acute and lethal poisoning induced by antiChE pesticides (Fikes, 1990; Al-Baggou' & Mohammad, 1999; Mohammad *et al*., 2012a; Ojha *et al*., 2014). Additional antidotes usually broaden the choice of therapies available for the clinician to deal with pesticides poisoning (World Health Organization, 2008). Few in vivo and in vitro studies on organophosphates and carbamates have suggested that diphenhydramine might interact at the level of the key enzyme, ChE in nervous tissues, to reduce additional ChE inhibition by the insecticides (Faris & Mohammad, 1996; Al-Baggou' & Mohammad, 1999; Mohammad *et al*., 2012a; Ojha *et al*., 2014). To our knowledge, such an interactive study with diphenhydramine and antiChE insecticides has not been conducted on human blood ChE which is the favorite biomonitoring tool for exposure to antiChE pesticides (Wilson *et al*., 2005; Wilson, 2014; Vale & Lotti, 2015; Cotton *et al*., 2018).

The purpose of the present study was to examine blood ChE activity in farmers and agriculture workers (farmworkers) who might have been exposed to pesticides and then to examine the effects of in vitro exposure of plasma and erythrocyte ChE to diphenhydramine and /or carbaryl which is a carbamate insecticide (Wilson, 2014; Baynes, 2018). Carbaryl is available in the local market as an -the-counter insecticide. Diphenhydramine was found to be of therapeutic benefits when used in a chick model of acute and lethal antiChE poisoning in vivo as well as in vitro when blood and brain ChE inhibitions were assessed (Mohammad *et al*., 2012a; Mohammed & Mohammad, 2022).

MATERIALS AND METHODS

Study subjects

After interviewing more than 100 subjects, the study included 15 male farmworkers (age 33-53 years) from Duhok, Iraq who volunteered to participate in the study. They were farmers and agriculture workers who testified that they were exposed to anti-ChE pesticides during their routine work for about 5-25 years. However, they were not hospitali for any reason related to pesticide spraying/handling during their work. The types and amounts of the pesticides the subject used, and the frequency of use could not be verified, but they havformulated products intended to combat pests in agriculture. Suitable 15 male non-farm workers (age 38-55 years) without a history of exposure to pesticides during their routine work were also recruited for the study as a control group. All the participant volunteers were not suffering from chronic disease conditions that might affect their work. The number of the subjects in each group was within the range of sample size estimation for clinical research set by Benchmarksixsigma.com (https://www.benchmarksixsigma.com/calculators/sample-size-calculator-for-2-sample-t-test/), considering the confidence level 95%, power of the test 80%, exposure level difference 20% between the unexposed and exposed groups (e.g. 1.2 vs 0.96, SD 0.23). We informed all the participants about the purpose of the study, the procedure of blood sampling, and the expected outcome of the study, and written consent was obtained from each one of them.

Laboratory investigations

Venous blood samples were obtained from all participants into heparinized test tubes. Plasma was separated from erythrocytes by centrifugation at 3000 rpm for 15 min. All samples were stored at –20 °C to be analyzed within one week. We used the electrometric method as described earlier for the measurement of plasma and erythrocyte ChE activities (Ahmed & Mohammad, 2004; Mohammad, 2007; Mohammad *et al*., 2007). In a 10 mL glass beaker, the reaction mixture was constituted by adding together 3 mL distilled water, 0.2 mL plasma or erythrocytes, and 3 mL of barbital-phosphate buffer (1.237 g sodium barbital, 0.163 g potassium dihydrogen phosphate, and 35.07 g sodium chloride/L of distilled water, pH 8.1). The initial pH of the mixture was measured with the glass electrode of a pH meter (Camlab Co., Cambridge, U.K.) before the addition of 0.1 mL acetylcholine iodide (7.1%) as a substrate. Thereafter, the reaction mixture was incubated at 37ºC in a water bath for a single period of 20 min. At the end of the

20-min incubation period, we measured the pH of the reaction mixture again. The activity of ChE in the plasma or erythrocytes was calculated as follows:

ChE activity (Δ pH/20min) = (pH1 – pH2) – Δ pH of blank (no blood sample in the reaction); e.g. (8.1–6.8)–0.1= 1.2 Δ pH/20min.

Plasma and erythrocyte ChE activities of pesticide-unexposed participants (healthy controls) were regarded as reference values for calculation of the percentage of enzyme inhibition in subjects exposed to pesticides (Mohammad *et al*., 2007), as follows:

% ChE inhibition= [ChE activity of unexposed-ChE activity of exposed / ChE activity of unexposed] × 100

For the in vitro ChE inhibition (Table 1), blood samples were used from randomly chosen five pesticide-exposed male participants and from another five unexposed ones, with 5 replicates per each in vitro treatment (Mohammad *et al*., 1997; Padilla *et al*., 2004; Mohammad *et al*., 2006; 2007; Verdín-Betancourt *et al*., 2019). The method of 10-min incubation of the inhibitor with plasma or erythrocyte ChE was used to determine in vitro enzyme inhibition (Mohammad *et al*., 1997; Ahmed & Mohammad, 2004; Mohammad, 2007; Mohammad *et al*., 2007; 2014) with either carbaryl insecticide powder (85%, Ferasin 85wp, Fertil, Turkey) or diphenhydramine HCl (The State Company for Drug and Medical Appliance, Samara, Iraq), or with both. At first, aqueous solutions of carbaryl or diphenhydramine were separately and freshly prepared so that the final concentrations of carbaryl in the reaction mixtures were 5, 10, and 20 μM and those of diphenhydramine were 10, 20, and 50 μM. Base-line control reaction mixtures contained only the vehicles with no drugs added. The volume of the added inhibitor or vehicle solution to the reaction mixture was 0.1 mL. The in vitro concentrations of carbaryl and diphenhydramine were adopted in the present study depending on previous reports (Mohammad *et al*., 2006; Mohammed & Mohammad, 2022) as well as on our preliminary experiments. The reaction mixtures of inhibitor-enzyme sources were subjected to an initial incubation at 37 ºC for 10 min to facilitate ChE inhibition. Thereafter, the residual ChE activity in the mixture was measured electrometrically as described above.

In another experiment, blood samples from five male subjects unexposed to pesticides were used for the combined in vitro ChE inhibition by either carbaryl (10 μM), diphenhydramine (20 μM), or both. For the combined effect, diphenhydramine was added to the enzymatic reaction mixture 10 min before the carbaryl addition. The choice of these two concentrations of carbaryl and diphenhydramine depended on the results of the previous in vitro experiment we conducted as mentioned above.

The percentage of ChE inhibition in the plasma or erythrocytes was estimated by the following equation:

% ChE inhibition = [ChE activity (base-line control) – ChE activity (with carbaryl or diphenhydramine, or both) / ChE activity (base-line control)] × 100; e.g. [1.2–0.60/ 1.2] × 100 = 50% (Mohammad *et al*., 2006; 2007).

Table 1. The outline of experiments for in vitro inhibition of plasma and erythrocyte cholinesterase (ChE) activities by diphenhydramine and/or carbaryl in pesticide-exposed male farmworkers and unexposed human volunteers.

Blood samples from subjects	
Pesticide-unexposed (control), *n*=5	Pesticide-exposed, *n*=5
In vitro inhibition of plasma and erythrocyte ChE activities by diphenhydramine and carbaryl (Mohammad *et al*., 2007; 2014)	
Diphenhydramine (0, 10, 20, 50 μM) *n*=5/each concentration	Carbaryl (0, 5, 10, 20 μM) *n*=5/each concentration
3 mL distilled water + 0.2 mL blood sample + 3 mL barbital-phosphate buffer + 0.1 mL of inhibitor (diphenhydramine or carbaryl) Incubate at 37 ºC for 10 min Measure ChE activity electrometrically (Mohammad, 2007, Mohammad *et al*., 2007)	
% ChE inhibition = [ChE activity (base-line control) – ChE activity (diphenhydramine and/or carbaryl) / ChE activity (base-line control)] × 100 (Mohammad *et al*., 2007; 2014)	
Pesticide-unexposed, *n*=5	
In vitro inhibition of plasma and erythrocyte ChE activities by diphenhydramine and/or carbaryl	
Blood samples were subjected to ChE inhibition by: Carbaryl (10 μM), Diphenhydramine (20 μM), or both (diphenhydramine, 10 min later carbaryl) *n*=5/each ChE inhibition	

Statistics

In 2021, we used the statistics software package SPSS-IBM to analyze the data of multiple means (± SE of the mean) by one-way analysis of variance followed by the least significant difference test (Petrie & Watson, 2013). Student's t-test was used to compare the means of two groups when applicable (Petrie & Watson 2013). The level of statistical significance was at $p<0.05$.

RESULTS

Measurements of plasma and erythrocyte ChE activities in pesticide-exposed farmworkers indicated that the plasma ChE activity was significantly lower than that of the unexposed controls by 14%, whereas the erythrocyte ChE activity was non-significantly below the control value by only 4% (Figure 1). Diphenhydramine significantly and in a concentration-dependent manner inhibited plasma and erythrocyte ChE activities in vitro in both pesticide-exposed and unexposed subjects (Table 2). The percentages of ChE inhibition in the plasma and erythrocytes of unexposed controls ranged between 27-74% and 7-18%, respectively, whereas they were 11-53% and 8-19% in the pesticide-exposed group, respectively (Table 2). In both groups, diphenhydramine reduced plasma ChE activity in vitro more than that of erythrocyte ChE (Table 2). It also appeared that the plasma ChE of the control subjects was susceptible to in vitro diphenhydramine inhibition more than that of the exposed ones (Table 2).

Carbaryl significantly and in a concentration-dependent manner inhibited plasma and erythrocyte ChE activities in vitro in both pesticide-exposed and unexposed subjects (Table 3). The percentages of ChE inhibition in the plasma and erythrocytes of unexposed controls ranged between 47-85% and 19-47%, respectively, whereas they were 35-60% and 3-12% in the pesticide-exposed group, respectively (Table 3). It appeared that plasma and erythrocyte ChE activities of the control subjects were susceptible to in vitro carbaryl inhibition more than those of the exposed ones (Table 3). In vitro pretreatment of plasma and erythrocyte ChE of healthy subjects with diphenhydramine (20 μM) significantly reduced the inhibitory effect of carbaryl (10 μM) on these two enzymes by 18 (43 vs 25%) and 10% (23 vs13%), respectively (Figure 2).

DISCUSSION

Many studies have used measurements of blood (plasma, serum, erythrocyte, or whole blood) ChE activities to monitor and assess exposure to products of pesticides containing carbamates or organophosphates, especially among farmworkers (Jaga & Dharmani, 2003; Wilson *et al*., 2005; Damalas & Koutroubas, 2016; Cotton *et al*., 2018; Patel *et al*., 2018; Benaboud *et al*., 2021). True ChE (acetylcholinesterase) is mainly found in the nervous tissues and erythrocytes, whereas pseudo-ChE (butyryl ChE) is found in the liver and the plasma (serum) (Wilson *et al*., 2005; Wilson, 2014; Vale & Lotti, 2015). Depression of blood ChE activity by 20-30% from baseline values usually suggests exposure to antiChE pesticides (Jaga & Dharmani, 2003; Wilson *et al*., 2005; Wilson, 2014). To confirm the diagnosis of antiChE poisoning, especially in acute cases, the reduction in enzyme activity should be >50%, and immediate healthcare is recommended (Jaga & Dharmani, 2003; Wilson *et al*., 2005; Lionetto *et al*., 2013). In the present study, 14 and 4% reductions were found in plasma and erythrocyte ChE activities, respectively in pesticide-exposed subjects in comparison to unexposed ones. This finding suggests a marginal plasma ChE inhibition, which is difficult to assess or interpret in the absence of pre-exposure ChE activities of the subjects (Jaga & Dharmani, 2003; Wilson *et al*., 2005; Wilson, 2014; Vale & Lotti, 2015). Contributing factors to such a marginal low ChE inhibition we encountered in our subjects possibly include but are not limited to the fact that we could not confirm the types of pesticides used and the exposure frequency and duration in the participants. It is also possible that the exposure of the participants to pesticides was gradual and limited to small levels (doses) since the exposed subjects did not suffer from exposure-related overt ill effects usually seen in acute antiChE poisoning (Cocker *et al*., 2002; Wilson *et al*., 2005; Lionetto *et al*., 2013; Wilson, 2014; Vale & Lotti, 2015). In this context, it should be stressed that antiChE pesticides possess differential ChE inhibitory actions on the plasma and erythrocytes (Roy *et al*., 2005; Wilson *et al*., 2005; Lionetto *et al*., 2013; Wilson, 2014; Cotton *et al*., 2018; Hongsibsong *et al*., 2018; Assis *et al*., 2018). However, inhibition of plasma ChE activity strongly correlates with the intensity and duration of exposure to pesticides (He, 1999; Lionetto *et al*., 2013). Even low-level pesticide exposure can result in reduced plasma ChE activity in routine clinical examination (Kapka-Skrzypczak *et al*., 2011). Variability of the relationship between erythrocyte and brain ChE inhibitions and plasma carbaryl levels was also reported (Moser *et al*., 2013).

A study conducted in Mosul (Iraq) reported 21-30% reductions in plasma and erythrocyte ChE activities in agriculture workers and veterinarians (Ahmed & Mohammad, 2007). However, in another study conducted in Erbil (Iraq), plasma ChE in agriculture workers and veterinarians exposed to pesticides for up to 19 years was below control levels by only 11 and 10%, respectively (Al-Haseni & Yahya, 2012). In a related study in Kirkuk (Iraq), whole blood ChE activity of agriculture workers after six years of exposure was 22% below control values

(Ahmad, 2013). Therefore, based on these results cited above and ours in the present study, a comprehensive approach is needed to monitor blood ChE activities in the local agriculture workforce and farmworkers to assess their health conditions repeatedly and more comprehensively.

In vitro, ChE inhibition by organophosphate and carbamate insecticides is a versatile tool to assess the potential toxicity of such pesticides and the results usually correlate well and are supportive of in vivo poisoning studies, especially in laboratory animals (Mohammad *et al*., 1997; Ahmed & Mohammad, 2004; Padilla *et al*., 2004; Mohammad *et al*., 2006; 2014). Carbaryl, in the present study, expectedly inhibited both plasma and erythrocyte ChE activities in vitro. This is in agreement with similar in vitro ChE inhibitory effects of carbaryl using humans (Mohammad *et al*., 2007; Ahmad, 2013) or animals (Mohammad *et al*., 2006; Mohammed & Mohammad, 2022) blood samples. Carbaryl is a commonly used insecticide in agriculture, public health as well as in veterinary medicine (Wilson, 2014; Baynes, 2018) with considerable inhibitory, though reversible, action on blood ChE activities. And we utilized this ChE inhibitory action of carbaryl in the present study to examine its interaction with the H1 antihistamine diphenhydramine. Diphenhydramine was found to alleviate organophosphate and carbamate poisoning in a manner comparable to the standard antidote atropine sulfate (Fikes, 1990; Al-Baggou' & Mohammad, 1999; Mohammad *et al*., 2002; 2012a; Ojha *et al*., 2014). Additional possible beneficial effects of diphenhydramine include its antinicotinic effects (Fikes, 1990; Al-Baggou' & Mohammad, 1999; Mohammad *et al*., 2012a) and possibly weak ChE inhibitory action (Faris & Mohammad, 1996; Al-Baggou' & Mohammad, 1999; Mohammad *et al*., 2012b; Mohammed & Mohammad, 2022). In vitro and in vivo studies in laboratory animals suggest that diphenhydramine could partially protect the ChE from additional inhibition caused by either organophosphates or carbamates (Faris & Mohammad, 1996; Mohammad *et al*., 2012a; Mohammed & Mohammad, 2022). The present study is the first of its type using the in vitro ChE inhibitory challenges for possible interaction between this antihistamine and carbaryl. These results, though in vitro, suggest that diphenhydramine might act as a ChE inhibitor (probably weak) to prevent further ChE inhibition induced by carbaryl. In this context, diphenhydramine in vivo ameliorated carbaryl-induced acute and lethal toxicity in the chick model of antiChE poisoning (Mohammed & Mohammad, 2022). Furthermore, our results are by those of the in vitro interactions, resulting in reduced ChE inhibition, between diphenhydramine and carbaryl, using plasma and brain ChE of chicks (Mohammed & Mohammad, 2022). It is well known that weak ChE inhibitors prevent acute organophosphate poisoning (Al-Zubaidy & Mohammad, 2007; Lorke & Petroianu, 2019). This could be an added benefit of diphenhydramine to its antimuscarinic action against antiChE poisoning induced by pesticides (Fikes, 1990; Ojha *et al*., 2014). Still, clinical trials are needed to affirm the antidotal properties of diphenhydramine as most of its effects are observed when given experimentally in laboratory animals before intoxications with organophosphates or carbamates (Al-Baggou' & Mohammad, 1999; Mohammad *et al*., 2002; Mohammad *et al*. 2012a; Ojha *et al*., 2014; Mohammed & Mohammad, 2022).

CONCLUSION

Farmworkers exposed to pesticides during their routine work in agriculture are at risk of reduced blood ChE activity; therefore, routine biomonitoring of blood ChE activities is needed to detect any earlier risk due to exposure to antiChE pesticides. Diphenhydramine appeared to partially protect both plasma and erythrocyte ChE from additional

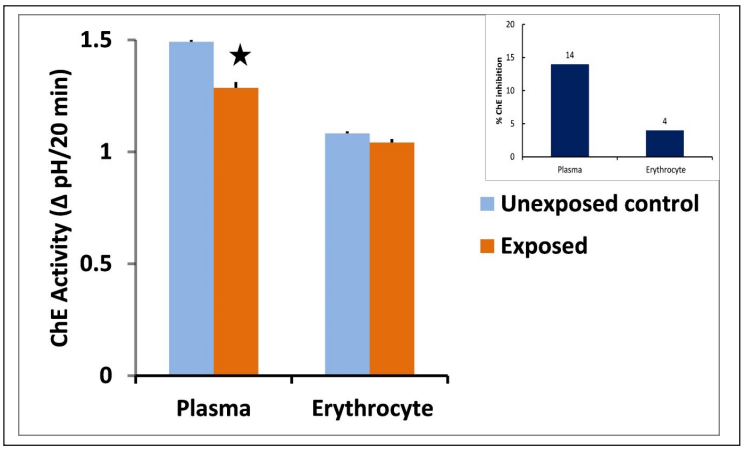

Fig. 1. The plasma and erythrocyte cholinesterase (ChE) activities in pesticide-exposed male farmworkers and unexposed human volunteers. The insert indicates the % inhibitions of the plasma and erythrocyte ChE activities of pesticide-exposed farmworkers. * Significantly different from the respective unexposed group, $p<0.05$. Values of ChE activities are mean ± SE, $n=15$/group.

Table 2. In vitro inhibition of plasma and erythrocyte cholinesterase (ChE) activities (Δ pH/20 min) by diphenhydramine in pesticide-exposed male farmworkers and unexposed human volunteers.

Diphenhydramine (μM)	Pesticide-unexposed (control)			
	Plasma ChE	% inhibition	Erythrocyte ChE	% inhibition
0 (control)	1.148 ± 0.053	0	0.830 ± 0.031	0
10	0.844 ± 0.039*	27	0.773 ± 0.017	7
20	0.690 ± 0.095*, a	40	0.718 ± 0.034*, a	14
50	0.300 ± 0.033*, a,b	74	0.693 ± 0.044*, a,b	18
	Pesticide-exposed			
0 (control)	1.288 ± 0.029	0	0.833 ± 0.014	0
10	1.142 ± 0.050*	11	0.764 ± 0.018*	8
20	0.960 ± 0.038*, a	26	0.724 ± 0.013*	13
50	0.612 ± 0.020*, a,b	53	0.675 ± 0.010*, a,b	19

All samples were incubated at 37 ºC for 10 min to facilitate ChE inhibition; Enzyme activity values are mean ± SE, *n*=five subjects/group for each ChE assay.
*Significantly different from the respective control group, $p<0.05$.
[a]Significantly different from the respective 10 μM concentration group, $p<0.05$.
[b]Significantly different from the respective 20 μM concentration group, $p<0.05$.

Table 3. In vitro inhibition of plasma and erythrocyte cholinesterase (ChE) activities (Δ pH/20 min) by carbaryl in pesticide-exposed male farmworkers and unexposed human volunteers.

Carbaryl (μM)	Pesticide-unexposed (control)			
	Plasma ChE	% inhibition	Erythrocyte ChE	% inhibition
0 (control)	1.235 ± 0.043	0	1.097 ± 0.029	0
5	0.650 ± 0.070*	47	0.887 ± 0.025*	19
10	0.438 ± 0.067*, a	65	0.813 ± 0.023*	26
20	0.192 ± 0.071*, a,b	85	0.582 ± 0.019*, a,b	47
	Pesticide-exposed			
0 (control)	1.263 ± 0.023	0	1.182 ± 0.011	0
5	0.827 ± 0.042*	35	1.142 ± 0.021	3
10	0.640 ± 0.037*, a	49	1.110 ± 0.012*	6
20	0.512 ± 0.028*, a,b	60	1.043 ± 0.016*, a,b	12

All samples were incubated at 37 ºC for 10 min to facilitate ChE inhibition; Enzyme activity values are mean ± SE, *n*=five subjects/group for each ChE assay.
*Significantly different from the respective control group, $p<0.05$.
[a]Significantly different from the respective 5 μM concentration group, $p<0.05$.
[b]Significantly different from the respective 10 μM concentration group, $p<0.05$.

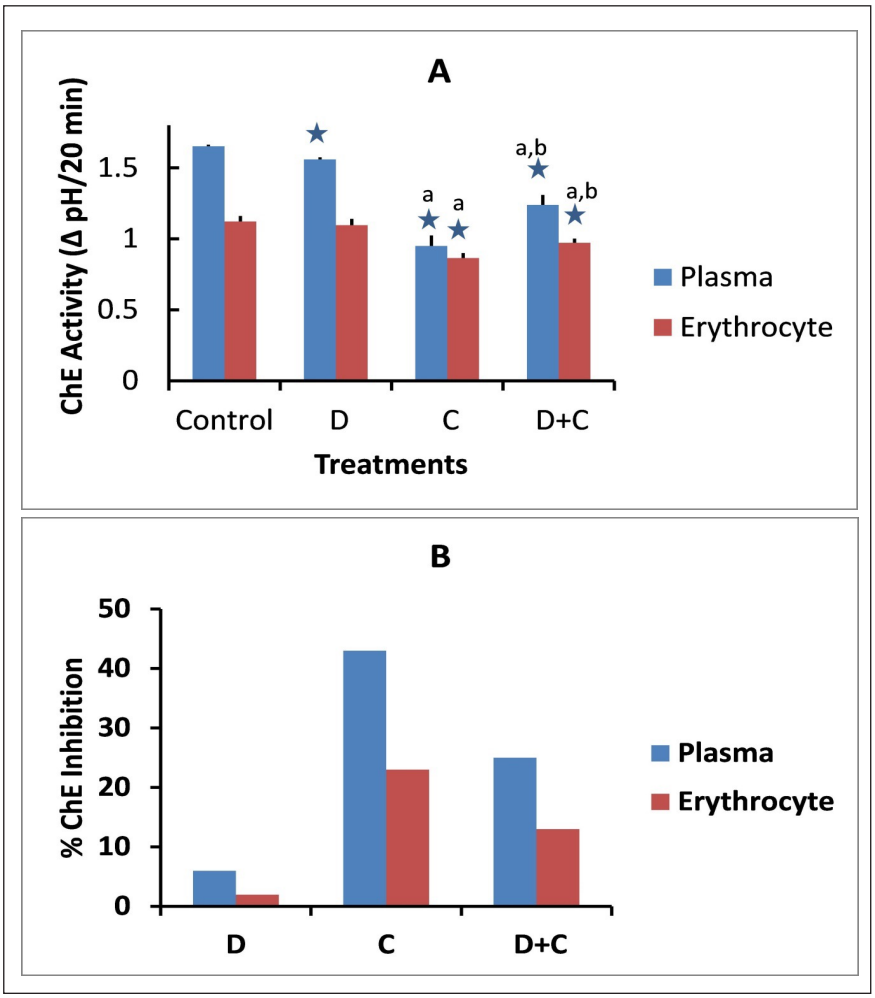

Fig. 2. (A). In vitro effect of diphenhydramine (D, 20 µM) pretreatment on carbaryl (C, 10 µM)-induced inhibition of cholinesterase (ChE) activities in the plasma and erythrocytes of male subjects not exposed to pesticides. (B). Percentages of in vitro ChE inhibition by diphenhydramine and/or carbaryl. Diphenhydramine was added to the mixture 10 min before the carbaryl addition. All samples were incubated at 37 ºC for 10 min to facilitate ChE inhibition. The values of ChE activities are mean ± SE, *n*=five subjects/group for each ChE assay.*Significantly different from the respective control (distilled water) group, $p<0.05$. [a]Significantly different from the respective diphenhydramine group, $p<0.05$. [b]Significantly different from the respective carbaryl group, $p<0.05$.

inhibition caused by carbaryl, an effect that might contribute to its antidotal action against poisoning induced by antiChE pesticides.

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ETHICAL STATEMENT

Ethical approvals were obtained in writing from the Committee of Post Graduate Studies in the College of Medicine, University of Duhok (Iraq) to conduct the study as a part of the MSc research work of the first author, as well as from the Local Research Ethics Committee at the Duhok Health Directorate, Duhok, Iraq.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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