

# Assessment of diphenhydramine effects against acute poisoning induced by the organophosphate insecticide dichlorvos in chicks

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**Abstract.** Objective: The study was designed to assess the protective and ameliorative effects of the antihistamine diphenhydramine against a model of acute organophosphate insecticide (dichlorvos) poisoning in 7-14 day-old chicks. Material and Methods: The acute (24 h) oral median lethal doses ( $LD_{50}$ ) of dichlorvos either alone or with concomitant diphenhydramine ( $10 \text{ mg kg}^{-1}$ , i.m.) or atropine ( $2 \text{ mg kg}^{-1}$ , i.m.) were determined in the chicks by the up-and-down method. The protective and ameliorative effects of diphenhydramine on the signs of dichlorvos poisoning were examined and compared with those of atropine. Plasma and whole brain cholinesterase activities of chicks treated with diphenhydramine and/or dichlorvos were measured by an electrometric method. Results: Diphenhydramine at  $10 \text{ mg kg}^{-1}$ , intramuscularly (i.m.) immediately after oral dosing with dichlorvos increased the oral  $LD_{50}$  value of the insecticide from  $6.49$  to  $17.14 \text{ mg kg}^{-1}$ , whereas atropine at  $2 \text{ mg kg}^{-1}$ , i.m. increased it to  $22.55 \text{ mg kg}^{-1}$ . The oral dosing of dichlorvos at  $8 \text{ mg kg}^{-1}$  caused acute signs of cholinergic poisoning in the chicks. The best effective dosage of diphenhydramine was at  $10 \text{ mg kg}^{-1}$ , i.m. immediately after the oral dichlorvos dosing, which significantly increased the latencies to onset of signs of poisoning and significantly prevented the 2 h and 24 h lethality. Diphenhydramine also significantly decreased the occurrence of signs of poisoning and reduced total toxicity score in the chicks. Diphenhydramine, similar to atropine, given at times of -15, 0 and + 5 min relative to the time of dichlorvos dosing also ameliorated dichlorvos-induced poisoning in the chicks to varying extents. Dichlorvos alone at the oral doses of 2 and  $4 \text{ mg kg}^{-1}$  significantly reduced cholinesterase activity in the plasma and whole brain. Diphenhydramine at  $10 \text{ mg kg}^{-1}$ , i.m. immediately after oral dichlorvos dosing at  $4 \text{ mg kg}^{-1}$  significantly but partially ameliorated cholinesterase inhibition caused by the insecticide in the whole brain of the chicks. Conclusion: The results suggest that diphenhydramine has protective and ameliorative effects against a model of acute organophosphate poisoning in chicks.

**Key Words:** antihistamine, organophosphate, insecticide, antidote, cholinesterase.

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## Introduction

Organophosphate (OP) insecticides are commonly used in veterinary practice and public health (Coggon 2002; Jaga & Dharmani 2003; Wilson 2005). They induce poisoning in mammals and avian species by irreversible inhibition of cholinesterase (ChE) activity at the nerve terminals and neuromuscular junctions causing accumulation of acetylcholine. As a result of ChE inhibition, signs of cholinergic toxicity appear which are characterized by muscarinic, nicotinic and central nervous system effects (Osweiler 1996; Kwong 2002; Wilson 2005). The standard antidote for OP poisoning in man and animals is the muscarinic receptor antagonist atropine sulfate (Osweiler 1996; Kwong 2002; Wilson 2005). The antihistamine diphenhydramine has been reported by several studies to counteract OP-induced lethality and poisoning in laboratory animals in a manner similar to atropine (Mohammad *et al* 1989; Faris

& Mohammad 1997; Mohammad *et al* 2002; Bird *et al* 2002; Yürümez *et al* 2007). Earlier reports suggested the possibility of beneficial therapeutic efficacy of diphenhydramine in dogs (Clemmons *et al* 1984) and man (Moody & Terp 1988) suffering from OP poisoning. Thereafter, studies originating from our laboratories have documented and characterized the antidotal and therapeutic effects of diphenhydramine against experimental OP-induced lethality and toxicoses in mice (Mohammad *et al* 1989; Faris & Mohammad 1996a, 1997) and rats (Mohammad *et al* 2002). Later on other investigators further confirmed the action of diphenhydramine against OP-induced lethality in rats (Bird *et al* 2002). Diphenhydramine also attenuates OP induced pancreatic (Yürümez *et al* 2007) and cardiac (Yavuz *et al* 2008) injuries in rats. The protective and antagonistic effects of diphenhydramine against OP poisoning have been attributed to

the antimuscarinic and possibly antinicotinic properties of this antihistamine (Clemmons *et al* 1984; Moody & Terp 1988; Mohammad *et al* 1989; Faris & Mohammad 1997; Mohammad *et al* 2002; Yürümez *et al* 2007). Further, a possible protective interaction between diphenhydramine and OP insecticides on the level of ChE has not been completely ruled out (Mohammad *et al* 2002).

Among the avian species, chicks are used as experimental models of acute OP poisoning (Farage-Elawar & Francis 1988; Malik *et al* 2001; Mohammad & Al-Baggou 2005; Al-Badrany & Mohammad 2007; Mohammad *et al* 2008; Al-Baggou *et al* 2011; Al-Zubaidy *et al* 2011). Signs of cholinergic overstimulation and assessment of ChE inhibition are the accepted standard protocols for monitoring the condition of OP intoxicated chickens (Farage-Elawar & Francis 1988; Vodela & Dalvi 1995; Abdelsalam 1999; Clegg & van Gemert 1999; Al-Badrany & Mohammad 2007; Mohammad *et al* 2008, Al-Zubaidy *et al* 2011). Signs of OP insecticides poisoning in avian species are usually associated with reductions in ChE activities in the plasma, serum and / or the brain (Fairbrother & Bennet 1988; Cairns *et al* 1991; Fossi *et al* 1992; McInnes *et al* 1996; Wilson 1999; Al-Badrany & Mohammad 2007; Mohammad *et al* 2008, Al-Zubaidy *et al* 2011).

Informations on the interaction of diphenhydramine with OP insecticides in the chicken are rather limited. A single case report suggests the usefulness of diphenhydramine in treating a chicken accidentally poisoned with the OP insecticide fenamiphos (Mohammad & Basher 1995). The purpose of the present study was to assess the protective and therapeutic effects of diphenhydramine against a model of acute poisoning with the OP insecticide dichlorvos in young chicks.

## Material and Methods

Day-old Cobb broiler chicks of either sex were purchased from a local hatchery. They were maintained in batches of 25-30 chicks in cages with dimensions of 107 X 64 X 50 cm in a room with constant lighting at a temperature of 32-35°C, which was controlled by electric heaters. The floor litter consisted of wood shavings; water and feed were available ad libitum. The age of the chicks used in the experiments ranged between 7-14 days. The Scientific Committee of the College of Veterinary Medicine at the University of Mosul (Iraq) has approved the present study. All the experiments complied with institutional regulations addressing animal use, and proper attention and care were given to the chicks used in this study.

Diphenhydramine HCl (The State Company for Drug and Medical Appliance-Samara, Iraq) was dissolved in normal saline for intramuscular injection at a volume of 2 mL kg<sup>-1</sup> body weight. A solution of atropine sulfate (1%, Al-Sharq Veterinary Drugs Company, Damascus, Syria) was further diluted with normal saline to obtain the desired dosage (2 mg kg<sup>-1</sup> body weight, i.m.) at a volume of 2 mL kg<sup>-1</sup> body weight. Commercial insecticidal concentrated solution of dichlorvos (50%, Super Nogos, Pacific Agriscience, Australia) was further diluted in distilled water to obtain the desired concentrations of the insecticide for oral dosing by a gavage needle, at a volume of 5 mL kg<sup>-1</sup> body weight. The selections of the doses of diphenhydramine,

atropine and dichlorvos were based on our preliminary experiments in chicks and on the literature (Mohammad *et al* 1989; Mohammad & Basher 1995; Faris & Mohammad 1996a, 1997; Mohammad *et al* 2002).

Effect of diphenhydramine on the LD50 of dichlorvos. The acute (24 h) oral median lethal doses (LD50) of dichlorvos either alone or with concomitant diphenhydramine (10 mg kg<sup>-1</sup>, i.m.) or atropine (2 mg kg<sup>-1</sup>, i.m.) were determined in the chicks by the up-and-down method (Dixon 1980). Diphenhydramine and atropine were injected i.m. immediately after the dichlorvos dosing. The chicks were individually observed for the appearance of signs of cholinergic poisoning for 1 h and then the 24 h lethality was recorded (Al-Badrany & Mohammad 2007; Al-Zubaidy & Mohammad 2007; Mohammad *et al* 2008; Al-Baggou *et al* 2011). The protective ratio for diphenhydramine or atropine was calculated as follows:

$$\text{Protective ratio} = \text{LD50 of dichlorvos with diphenhydramine or atropine} / \text{LD50 of dichlorvos with normal saline}$$

Effect of diphenhydramine on dichlorvos-induced poisoning. Forty chicks were randomly divided into five groups of eight birds each. The chicks were dosed orally with dichlorvos at 8 mg kg<sup>-1</sup> body weight. Immediately after the dichlorvos dosing, the chicks were treated i.m. with the normal saline at 2 mL kg<sup>-1</sup> (the control) or with diphenhydramine at dose rates of 2.5, 5, 10 and 20 mg kg<sup>-1</sup> of body weight. The 2-h and 24-h lethalties were also recorded. The latencies to onset of any sign of poisoning and death were recorded within 2 h. We calculated the total score of the severity of cholinergic poisoning within each treatment group as described previously (Al-Baggou & Mohammad 1999; Al-Zubaidy & Mohammad 2007). For calculating the total toxicity score, the following grades were assigned to the percentage of occurrence of the cholinergic signs of poisoning (salivation, lacrimation, gasping, tremors, frequent defecation, recumbency and convulsion): 1 (1-25%), 2 (26-50%), 3 (51-75%) and 4 (76-100%). For reference, the highest total toxicity score within a treatment group showing all the 7 signs of poisoning mentioned above (100% occurrence) would be 4 × 7 = 28.

In another experiment examining the effects of the time of diphenhydramine application relative to the time of the dichlorvos dosing on the occurrence of the signs of OP poisoning, 56 chicks were randomly divided into 7 groups of 8 birds each. The chicks were dosed orally with dichlorvos at 8 mg kg<sup>-1</sup> body weight. Relative to the time of the dichlorvos dosing, the seven groups of chicks were treated as follows:

- Group 1: Normal saline immediately after the dichlorvos dosing (control group).
- Group 2: Diphenhydramine immediately after the dichlorvos dosing (0 time).
- Group 3: Diphenhydramine 15 min before the dichlorvos dosing.
- Group 4: Diphenhydramine 5 min after the dichlorvos dosing.
- Group 5: Atropine immediately after the dichlorvos dosing (0 time).
- Group 6: Atropine 15 min before the dichlorvos dosing.
- Group 7: Atropine 5 min after the dichlorvos dosing.

**Table 1.** Determination of the 24-h median lethal dose (LD50) of dichlorvos alone or in combination with diphenhydramine or atropine in chicks by the up-and-down method.

Variable	Dichlorvos alone with normal saline treatment	Dichlorvos with diphenhydramine treatment	Dichlorvos with atropine treatment
<b>LD50</b>	6.49 mg kg <sup>-1</sup> , p.o.	17.14 mg kg <sup>-1</sup> , p.o.	22.55 mg kg <sup>-1</sup> , p.o.
<b>Range of the doses used</b>	10-4 = 6 mg kg <sup>-1</sup> , p.o.	19-10 = 9 mg kg <sup>-1</sup> , p.o.	25-10 = 15 mg kg <sup>-1</sup> , p.o.
<b>Initial dose</b>	10 mg kg <sup>-1</sup> , p.o.	10 mg kg <sup>-1</sup> , p.o.	10 mg kg <sup>-1</sup> , p.o.
<b>Last dose</b>	7 mg kg <sup>-1</sup> , p.o.	16 mg kg <sup>-1</sup> , p.o.	22 mg kg <sup>-1</sup> , p.o.
<b>Number of chicks used</b>	6	7	9
<b>Number of chicks died</b>	3	2	3
<b>Increase or decrease in dose</b>	3 mg kg <sup>-1</sup> , p.o.	3 mg kg <sup>-1</sup> , p.o.	3 mg kg <sup>-1</sup> , p.o.
<b>Range of latency to the onset of poisoning</b>	12-2 = 10 min	54-9 = 45 min	16-2 = 14 min
<b>Signs of poisoning</b>	salivation, lacrimation, gasping, frequent defecation, tremors, convulsions, wing drooping and recumbency	salivation, lacrimation, gasping, frequent defecation, tremors and wing drooping	salivation, lacrimation, gasping, frequent defecation, tremors, convulsions, wing drooping and recumbency
<b>Protective ratio</b>		2.64	3.47

Diphenhydramine (10 mg kg<sup>-1</sup>) or atropine (2 mg kg<sup>-1</sup>) was injected intramuscularly immediately after the oral (p.o.) dichlorvos dosing.

After the dichlorvos dosing, the chicks were observed individually for the occurrence of the signs of OP poisoning and the total toxicity score was estimated as mentioned in the previous experiment.

In vivo effect of diphenhydramine on ChE activity in chicks dosed orally with dichlorvos. In this experiment, 48 chicks were randomly divided into 6 groups of 8 birds each. The groups of chicks were treated as follows:

- Group 1: Distilled water at 2 mL kg<sup>-1</sup>, orally, followed immediately with normal saline at 2 mL kg<sup>-1</sup>, i.m. (control group).
- Group 2: Distilled water at 2 mL kg<sup>-1</sup>, orally, followed immediately with diphenhydramine at 10 mg kg<sup>-1</sup>, i.m.
- Group 3: Dichlorvos at 2 mg kg<sup>-1</sup>, orally, followed immediately with normal saline at 2 mL kg<sup>-1</sup>, i.m.
- Group 4: Dichlorvos at 4 mg kg<sup>-1</sup>, orally, followed immediately with normal saline at 2 mL kg<sup>-1</sup>, i.m.
- Group 5: Dichlorvos at 2 mg kg<sup>-1</sup>, orally, followed immediately with diphenhydramine at 10 mg kg<sup>-1</sup>, i.m.
- Group 6: Dichlorvos at 4 mg kg<sup>-1</sup>, orally, followed immediately with diphenhydramine at 10 mg kg<sup>-1</sup>, i.m.

Sixty minutes after the distilled water or dichlorvos dosing, blood samples were obtained from the chicks by jugular vein bleeding into heparinized test tubes (Stevens & Ridgway 1966). Thereafter, the chicks were euthanized by cervical dislocation to obtain the whole brain. Blood samples were centrifuged at 10000 rpm for 15 minutes to obtain the plasma. Plasma and whole brain samples were kept at -20°C pending the determination of ChE activity within one week. The whole brain was homogenized on an ice bath by a glass homogenizer in a pH

8.1 barbital-phosphate buffer solution (1.237 g sodium barbital, 0.163 g potassium dihydrogen phosphate and 35.07 g sodium chloride/L of distilled water) at 3 mL/100 mg wet weight (Mohammad & Al-Baggou 2005; Al-Badrany & Mohammad 2007; Mohammad 2007; Al-Baggou *et al* 2011). We determined ChE activity in the plasma and brain samples by an electrometric method (Alias & Mohammad 2005; Al-Badrany & Mohammad 2007; Mohammad 2007; Al-Baggou *et al* 2011). Briefly, the reaction mixture in a beaker contained 3 mL distilled water, 0.2 mL plasma or whole brain homogenate and 3 mL of pH 8.1 buffer. Initial pH of the mixture (pH1) was measured with a glass electrode using a pH meter (Hanna, Romania), then 0.10 mL of the substrate 7.5% acetylthiocholine iodide was added to the mixture which was incubated at 37 °C for 30 min. At the end of the incubation period, the pH of the reaction mixture (pH2) was measured. The enzyme activity was calculated as follows: ChE activity (ΔpH/30 min.) = (pH1 – pH2) - Δ pH of blank. The blank was without the plasma or brain homogenate sample. The unit of ChE activity was expressed as ΔpH/30 min. The % of ChE inhibition was calculated as follows:

$$\% \text{ ChE inhibition} = \frac{[\text{ChE activity (without dichlorvos)} - \text{ChE activity (with dichlorvos)}]}{\text{ChE activity (without dichlorvos)}} \times 100$$

**Statistics**

The data as multiple means were statistically analyzed by the one way analysis of variance followed by the least significant difference test (Petrie & Watson 1999). The frequency data were subjected to the Fisher’s exact probability test, and the scores of the severity of poisoning were analyzed by the Wilcoxon signed rank test (Runyon 1977; Petrie & Watson 1999). We used the

**Table 2.** Effect of diphenhydramine treatment on dichlorvos-induced (8 mg kg<sup>-1</sup>, orally) poisoning in chicks.

Variables	Diphenhydramine (mg kg <sup>-1</sup> , i.m.)				
	0 (saline control)	2.5	5	10	20
Latency to onset of signs of poisoning (min)	3.00 ± 0.33	2.88 ± 0.95	2.38 ± 0.60	10.50 ± 1.38*ab	5.00 ± 2.47c
Latency to onset of death (min)	11.38 ± 3.02	14.60 ± 3.47	9.20 ± 1.07	-	6.00 ± 2.12
<b>% occurrence of signs of poisoning</b>					
Salivation	100	100	100	50*	37.5*
Lacrimation	100	75	87.5	25*	12.5*
Gasping	100	87.5	100	62.5	50*
Tremor	100	100	100	25*	37.5*
Frequent defecation	100	100	100	37.5*	50*
Convulsions	100	75	50*	12.5*	50*
Recumbency	100	100	75	50*	100
2 h death	100	62.5	62.5	0*	50*
24 h death	100	87.5	87.5	25*	50*
Total toxicity score	28	26	25	12*	15*

Diphenhydramine was injected intramuscularly (i.m.) immediately after the dichlorvos dosing. Latency values are mean + SE. n = 8 chicks/group.

\* Significantly different from the respective control value, p < 0.05.

a Significantly different from the respective 2.5 mg kg<sup>-1</sup> dose group of diphenhydramine, p < 0.05.

b Significantly different from the respective 5 mg kg<sup>-1</sup> dose group of diphenhydramine, p < 0.05.

c Significantly different from the respective 10 mg kg<sup>-1</sup> dose group of diphenhydramine, p < 0.05.

statistical software SPSS v20 to perform the required analyses. The accepted level of statistical significance was at p < 0.05.

## Results

Effect of diphenhydramine on the LD50 of dichlorvos. The acute (24 h) oral LD50 value of dichlorvos alone in the chicks was 6.49 mg kg<sup>-1</sup> (Table 1). The signs of cholinergic poisoning on the chicks appeared within 2-12 minutes and they included salivation, lacrimation, gasping, tremors, frequent defecation, convulsions and recumbency with drooping of the wings before death. Intramuscular injection of diphenhydramine (10 mg kg<sup>-1</sup>) or atropine (2 mg kg<sup>-1</sup>) immediately after the dichlorvos dosing increased the oral LD50 value of dichlorvos to 17.14 and 22.55 mg kg<sup>-1</sup> with protective ratios of 2.64 and 3.47, respectively.

Effect of diphenhydramine on dichlorvos-induced poisoning. Oral dosing of dichlorvos alone at 8 mg kg<sup>-1</sup> produced signs of cholinergic poisoning in the chicks, which included salivation, lacrimation, gasping tremors, convulsions and recumbency and all the chicks died within 1 h (Table 2). Diphenhydramine treatments (2.5-20 mg kg<sup>-1</sup>, i.m.), given immediately after the dichlorvos dosing at 8 mg kg<sup>-1</sup>, decreased the occurrence of toxic manifestations and lethalties in chicks to various extents

(Table 2). Diphenhydramine at 10 mg kg<sup>-1</sup>, i.m. produced the highest protection against the dichlorvos poisoning. It delayed significantly the latency to onset of signs of poisoning, reduced the occurrence of signs of cholinergic poisoning to 12.5 to 50% compared to the control group (100%). Diphenhydramine also prevented dichlorvos-induced 2 and 24 h-lethalties by 100 and 75%, respectively. Correspondingly, diphenhydramine at 10 and 20 mg kg<sup>-1</sup> significantly reduced the total toxicity score by 57 and 46%, respectively in comparison with the control group. Diphenhydramine did not significantly affect the latency to onset of death induced by dichlorvos (Table 2).

Diphenhydramine given at times of -15, 0 and + 5 min relative to the time of dichlorvos dosing also ameliorated dichlorvos induced poisoning in the chicks to varying extents with reductions in the total toxicity score by 11, 43 and 4%, respectively (Table 3). The most effective time of diphenhydramine administration was immediately after the dichlorvos dosing. The least effective time was when the drug was given 5 min after the OP dosing. These effects of diphenhydramine according to the time of administration were closely similar to those of the standard antidote atropine. Atropine also reduced the total toxicity scores by 21, 43 and 7% according to administration times of -15, 0 and + 5 min, respectively (Table 3).



**Table 3.** Signs of organophosphate poisoning in chicks treated with diphenhydramine (10 mg kg<sup>-1</sup>, i.m.) or atropine sulfate (2 mg kg<sup>-1</sup>, i.m.) at different times relative to the time of dichlorvos dosing (8 mg kg<sup>-1</sup>, orally).

Variables	Treatment groups						
	saline control	Diphenhydramine			Atropine sulfate		
		0 time	-15 min	+ 5min	0 time	-15 min	+5 min
Latency to onset of signs of poisoning (min)	1.75 ± 0.25	11.13 ± 3.53*	3.75 ± 0.41 <sup>a</sup>	2.25 ± 0.25 <sup>a</sup>	3.88 ± 0.52 <sup>a</sup>	5.25 ± 0.45 <sup>a</sup>	2.13 ± 0.23 <sup>a</sup>
Latency to onset of death (min)	5.75 ± 0.96	28.50 ± 8.50*	13.50 ± 0.65* <sup>a</sup>	9.60 ± 2.77 <sup>a</sup>	6 <sup>†</sup>	40 <sup>†</sup>	4.00 ± 1.15 <sup>ab</sup>
<b>% occurrence of signs of poisoning</b>							
Salivation	100	62.5	87.5	100	62.5	87.5	100
Lacrimation	87.5	37.5*	75	100	25*	75	100
Gasping	100	62.5	100	100	50*	62.5	100
Tremor	100	37.5*	87.5	100	75	75	100
Frequent defecation	100	37.5*	100	100	50*	100	87.5
Convulsions	100	25*	75	62.5	25*	12.5*	50*
Recumbency	100	62.5	75	100	100	100	100
2 h death	100	25*	50*	62.5	12.5*	12.5*	37.5*
24 h death	100	25*	50*	75	25*	37.5*	62.5
Total toxicity score	28	16*	25	27	16*	22	26

Latency values are mean + SE (except at † since only one chick died within 2 h).

n = 8 chicks/group.

\* Significantly different from the respective control value, p < 0.05.

<sup>a</sup> Significantly different from the respective value of the 0 time-diphenhydramine treatment group, p < 0.05.

<sup>b</sup> Significantly different from the respective value of the -15 min-diphenhydramine treatment group, p < 0.05.

In vivo effect of diphenhydramine on ChE activity in chicks dosed orally with dichlorvos. Oral dosing of the chicks with dichlorvos alone at the dose rates of 2 and 4 mg kg<sup>-1</sup> significantly reduced ChE activities in the plasma (95 and 99%) and whole brain (58 and 69%) compared with the control group (Table 4). Diphenhydramine alone did not significantly affect plasma and whole brain ChE activities in comparison with control values. Diphenhydramine injection at 10 mg kg<sup>-1</sup>, i.m. immediately after the oral dichlorvos dosing (4 mg kg<sup>-1</sup>) partially ameliorated ChE inhibition caused by dichlorvos in the whole brain of the chicks by 46%. However, it did not significantly influence the inhibitory effect of dichlorvos on plasma ChE activity of the chicks (Table 4).

## Discussion

The oral dosing of the OP insecticide dichlorvos in chicks produced a model of acute OP poisoning in a manner similar to other avian studies using the same OP (Al-Baggou *et al* 2011; Mohammad *et al* 2008; Al-Zubaidy *et al* 2011) or different ones (Al-Badrany & Mohammad 2007; Mohammad *et al* 2008; Al-Zubaidy *et al* 2011). Young chicks were found to be suitable for examining acute toxic responses to OP and carbamate insecticides (Farage-Elawar & Francis 1988; Al-Badrany & Mohammad 2007; Mohammad *et al* 2008; Al-Baggou *et al*

2011). The LD50 value of dichlorvos in the chicks is in agreement with the results of another study in chicks (Mohammad *et al* 2008; Al-Zubaidy *et al* 2011). As expected, dichlorvos produced signs of cholinergic overstimulation in chicks. These signs included salivation, lacrimation, gasping, tremors, frequent defecation, convulsions and recumbency with drooping of the wings before death. Similar signs of OP poisoning were reported in chicks intoxicated with the insecticides dichlorvos, chlorpyrifos and diazinon (Al-Badrany & Mohammad 2007; Al-Zubaidy & Mohammad 2007; Mohammad *et al* 2008, Al-Zubaidy *et al* 2011). These effects also correlate with those reported in rodent models of acute OP poisoning using dichlorvos (Faris & Mohammad 1997; Mohammad *et al* 2002; Bird *et al* 2002) and other OP insecticides (Mohammad *et al* 1989; Faris & Mohammad 1996a). The OP insecticides inhibit ChE activity and cause accumulation of acetylcholine at the nerve endings and neuromuscular junctions and they subsequently induce cholinergic poisoning which is manifested as muscarinic (e.g. salivation, lacrimation, respiratory difficulties, defecation), nicotinic (muscle tremors) and central nervous system effects manifested as convulsions (Osweiler 1996; Wilson 1999; Kwong 2002; Wilson 2005).

Diphenhydramine has been shown to be effective in reducing OP acute poisoning and lethality in mice (Mohammad *et al* 1989;

**Table 4.** Effect of diphenhydramine (10 mg kg<sup>-1</sup>, i.m.) on plasma and whole brain cholinesterase activities in the dichlorvos-treated chicks.

Treatment	Plasma		Whole brain	
	DpH/30 min	% inhibition	DpH/30 min	% inhibition
Normal saline (control)	1.01 ± 0.083	-	0.26 ± 0.038	-
Diphenhydramine	1.02 ± 0.111	0	0.23 ± 0.018	12
Dichlorvos 2 mg kg <sup>-1</sup>	0.008 ± 0.004*†	99	0.11 ± 0.008*†	58
Dichlorvos 4 mg kg <sup>-1</sup>	0.046 ± 0.013*†	95	0.08 ± 0.011*†	69
Dichlorvos 2 mg kg <sup>-1</sup> + Diphenhy- dramine	0.15 ± 0.027*†	85	0.12 ± 0.010*†	54
Dichlorvos 4 mg kg <sup>-1</sup> + Diphenhy- dramine	0.16 ± 0.021*†	84	0.20 ± 0.031a	23

Cholinesterase activity values are means + SE, n = 8 chicks/group. Diphenhydramine was injected intramuscularly (i.m.) immediately after the oral dosing of dichlorvos. Cholinesterase activity was determined 60 minutes after the dichlorvos dosing.

\*Significantly different from the respective control value (p < 0.05).

†Significantly different from the respective value of the dichlorvos (2 mg kg<sup>-1</sup>)-treated group (p < 0.05).

a Significantly different from the respective values of the dichlorvos (2 and 4 mg kg<sup>-1</sup>) and dichlorvos (2 mg kg<sup>-1</sup>) + diphenhydramine groups, p < 0.05.

Faris & Mohammad 1996a, 1997) and rats (Mohammad *et al* 2002; Bird *et al* 2002; Yürümez *et al* 2007). Similarly, in the present study, diphenhydramine effectively reduced dichlorvos-induced poisoning and lethality in the chick model of acute OP poisoning. The ameliorative effects of diphenhydramine in the intoxicated chicks were characterized by elevated LD50 value of dichlorvos as well as reduced occurrence of cardinal signs of cholinergic stimulation in a manner similar to the standard antidote atropine sulfate. Similar comparative findings between diphenhydramine and atropine were reported in rats intoxicated with dichlorvos (Mohammad *et al* 2002). Diphenhydramine was also effective in preventing poisoning when given before or immediately after OP insecticides in mice (Mohammad *et al* 1989; Faris & Mohammad 1996a, 1997). Atropine is the standard antidote usually combined with oximes for the treatment of OP poisoning (Osweiler 1996; Bajgar 2004). Such a drug combination has not been attempted with diphenhydramine. However, the effectiveness of diphenhydramine in reducing the cholinergic signs of OP poisoning is most probably attributed to the potent antimuscarinic action of this antihistamine (Mohammad *et al* 1989; Fikes 1990; Al-Baggou' & Mohammad 1999; Faris & Mohammad 1997; Mohammad *et al* 2002). Antinicotinic action of diphenhydramine may also be an added advantage to its potential antidotal effect against OP poisoning (Fernandez *et al* 1975; Clemmons *et al* 1984; Katayama & Tasaka 1985; Moody & Terp 1988; Fikes 1990). The antidotal activity of atropine against OP poisoning, however, is attributed solely to its antimuscarinic properties without any action on the nicotinic effects of OP (Fikes 1990; Osweiler 1996; Kwong 2002; Wilson 2005). In support of the present study, the antihistamine

chlorpheniramine with possess antimuscarinic actions, also ameliorated OP poisoning in chicks (Mousa 2009).

The most effective doses (10 and 20 mg kg<sup>-1</sup>, i.m.) of diphenhydramine in the present study correlate with those (20 and 30 mg kg<sup>-1</sup>, s.c.) reported to be effective in mice intoxicated with OP insecticides (Mohammad *et al* 1989; Faris & Mohammad 1996a, 1997). However, differences according to the routes of administration among various animal species and within the same species should be expected. We did not attempt to use the 30 mg kg<sup>-1</sup> dose of diphenhydramine because the 20 mg kg<sup>-1</sup> dose appeared to be of lesser efficacy compared with the 10 mg kg<sup>-1</sup> dose group. Doses more than 30 mg kg<sup>-1</sup>, i.m. produce tremors in the chicks as the median lethal dose of the drug in chicks is 49.3 mg kg<sup>-1</sup>, i.m. (Mohammad *et al* 2012). However, any difference in response of the chicks to diphenhydramine dosage could be attributed to the route of administration of this antihistamine and the dose of the insecticide used, and there is also the possibility of species difference in response to drugs. Intramuscular route of diphenhydramine injection was chosen in the present study since the subcutaneously administration of drugs in chicks may have the risk of oozing from the site of injection.

The most important diagnostic marker of OP exposure and poisoning is reductions in plasma (or serum) and brain ChE activities in birds (Cairns *et al* 1991; Fossi *et al* 1992; McInnes *et al* 1996; Mohammad & Al-Baggou 2005; Al-Badrany & Mohammad 2007; Mohammad *et al* 2008; Al-Zubaidy *et al* 2011). Determination of ChE activity in the plasma, serum or brain of OP intoxicated chickens is commonly used to monitor

the condition of poisoning and lethality (Farage-Elawar & Francis 1988; Abdelsalam 1999; Clegg & van Gemert 1999; Wilson 1999; Malik *et al* 2001). In the present study, dichlorvos dosing in chicks reduced plasma and whole brain ChE activities. Similarly, dichlorvos and other OP insecticides reduced plasma and whole brain ChE activities in chicks (Al-Badrany & Mohammad 2007; Al-Zubaidy & Mohammad 2007; Mohammad *et al* 2008; Al-Zubaidy *et al* 2011).

In the present study, diphenhydramine (given immediately after the dichlorvos dosing) only partially ameliorated ChE inhibition caused by dichlorvos in the whole brain but not in the plasma of the chicks. Plasma ChE (EC 3.1.1.8) differs from that of the brain (EC 3.1.1.7) in many biological and kinetic aspects including the susceptibility to inhibition by OP insecticides (Wilson 1999, 2005). Dichlorvos inhibits plasma ChE more than that of the brain (WHO 1986). It is also possible that this action of diphenhydramine could be attributed to its weak inhibitory effect on brain ChE activity which might protect the enzyme from further non-reversible ChE inhibitory action of the OP (Fernandez *et al* 1975; Faris & Mohammad 1996b; Mohammad *et al* 1999). Antihistamines (H1-receptor blockers) are known to inhibit erythrocyte ChE (EC 3.1.1.7) in vitro and plasma ChE in vitro and in vivo (Simon & Winter 1970; Fernandez *et al* 1975). Simon & Winter (1970) suggested the possibility that antihistamines may potentiate the toxicity of anticholinesterases. In rats and mice no adverse interactions occurred between diphenhydramine and dichlorvos on the level of brain ChE, and reductions in blood and brain ChE activities occurred regardless of antidotal therapy with diphenhydramine (Faris & Mohammad, 1996b; Mohammad *et al* 2002). We observed this in the present study too.

## Conclusions

In conclusion, the results suggest that diphenhydramine has protective and ameliorative effects similar to atropine against a model of acute OP insecticide poisoning in chicks. The protective effect of diphenhydramine against OP insecticide poisoning in chicks could be attributed to its antimuscarinic properties, and no adverse interaction appeared between diphenhydramine and dichlorvos regarding the state of ChE inhibition in the plasma or the whole brain.

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