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Largactil As an Antidote to Organophosphorus Pesticide Poisoning in Local Doves

Ashraf Saddiek Alias [1*]

[1*] Department of Environmental Health, College of Environmental Sciences, University of Mosul, Iraq. E-mail: ashraf.saddik@uomosul.edu.iq

Abstract

Many organophosphorus compounds are toxic compounds that are harmful to humans, animals, and the surrounding environment. To treat the toxicity of these compounds, researchers use another compound as an antidote for cholinergic toxicity, that is, Largactil. Largactil is a kind of phenothiazine that contains the active ingredient chlorpromazine hydrochloride, which is used as an anticholinergic, antihistamine, and analgesic. This study aims to use Largactil as an alternative to atropine in the treatment of poisoning of collared doves exposed to organophosphorus insecticide (dichlorvos). This study is based on a sample of thirty birds (collared doves) divided into three groups of ten birds each. The control group (Group 1) received a dose of dichlorvos, followed by 2 ml/kg of saline solution administered intraperitoneally. Group 2 and Group 3 are treatment groups that were given atropine and Largactil, respectively and separately, in addition to dichlorvos. The oral median lethal doses (LD50) of dichlorvos alone or with atropine and Largactil in doves were determined by the up-and-down method for acute toxicity testing. The results showed that the oral LD50 of dichlorvos with intraperitoneal atropine increased by 30.845 mg/kg, with a protective ratio of 1.47 (Group 2), while an increase in the oral LD50 for Largactil ranged from 21.0 mg/kg to 55.7 mg/kg, with a protective ratio of 2.65 (Group 3). Largactil significantly reduced the signs of toxicity and decreased the overall toxicity score of the birds more than atropine. These results indicate that Largactil has better protective and mitigating effects than atropine in the treatment of acute organophosphorus pesticide poisoning in birds.

Keywords:

Largact, anti-poisoning, organophosphorus, insecticide, atropine

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**Corresponding Author: Ashraf Saddiek Alias, E-mail: ashraf.saddik@uomosul.edu.iq*

Introduction

Organophosphates (OPs) are chemical compounds containing phosphoric acid and alcohol and are produced by esterification. These compounds are the main component of a certain group of insecticides and are toxic to humans, animals, and birds because they act as inhibitors of the enzyme acetylcholinesterase (AChE) at nerve endings or neuromuscular junctions. This enzyme blocks the transmission of acetylcholine-mediated synaptic signals in the brain, autonomic nervous system, and neuromuscular junctions (Mohammad et al., 2007). The hydrolysis of acetylcholine is prevented by the binding of OPs to and phosphorylation of nucleophilic serine at the catalytic site of the enzyme. As a result, signs of cholinergic toxicity, affecting the central nervous system, appear. Hyperstimulation of nicotinic cholinergic receptors can lead to immediate death of insects, animals, or humans. Exposure to OPs causes varying degrees of toxicity to humans, animals, plants, and insects and a range of potentially lethal hazards (Aroniadou-Anderjaska et al., 2023; Adeyinka et al., 2023; Rusyniak & Nanagas, 2004; Naser & Mohammad, 2011; Ogutcu et al., 2008). Dichlorvos, also known as 2,2-dichlorophenyldimethylphosphate, is an organophosphate widely used as an insecticide to control some agricultural or household pests and protect crops from damage. It affects aphids, spider mites, caterpillars, thrips, whiteflies, and mushroom flies (Peng et al., 2024; Salem et al., 2023). However, exposure to such pesticides through inhalation, drinking, or eating has many negative and harmful effects and can cause damage to the kidneys, liver, heart, or other organs (Jaga & Dharmani, 2003; Salem et al., 2015; Worek et al., 2005; Ashraf, 2019).

Organophosphates are widely used in Iraq as a pesticide for human health, agriculture, and veterinary medicine. The injudicious use of OP can lead to toxicity and environmental pollution (Sethi & Jain, 2024; Ranjan & Jindal, 2022). The absorption of these organic compounds through the skin is considered to be the highest percentage of toxicity to which humans or animals can be exposed. These compounds are broken down via hydrolysis in the liver and decompose at different rates, depending on their structure. Some OPs are characterized by rapid decomposition, while others are slower, leading to their solubility and storage in body fat, which can delay the subsequent manifestation of their toxicity. Delayed toxicity occurs with organic compounds such as dichloropentane and dimethyl thione, which are converted from thion compounds to oxon compounds by oxygen or light when these compounds are present in the environment (Caner et al., 2025; Farajizadeh & NematBakhsh, 2015) However, when OPs are stored in the body, these are broken down and converted into more water-soluble forms by the action of microsomal liver enzymes (Wilson, 2005; Alias & Mohammad, 2005).

Symptoms of cholinergic toxicity caused by AChE inhibition are characterized by effects on the muscarinic and nicotinic receptors of the parasympathetic and central nervous systems, respectively. In addition, some clinical signs of poisoning include muscle spasms, weakness, mental status impairment, bladder incontinence, vomiting, and diarrhea. The respiratory system is also affected, with symptoms including shortness of breath, bronchial rhinorrhea, bradycardia, and bronchospasm, which can lead to death (Syed et al., 2015). Atropine sulfate is one of the antidotes used to treat OP poisoning. It is a commonly used antidote for OP addiction and is antimuscarinic in humans and animals (Mustafa & Al-Baggou, 2020; Sinha et al., 2023).

Largactil is a compound containing the active ingredient chlorpromazine hydrochloride. It belongs to a class of medications known as phenothiazines (Thevenon et al., 2022). Largactil can be used temporarily or permanently to treat various ailments (Yürümez et al., 2007). It is also used to treat OP poisoning and a variety of problems, including behavioral disorders and severe depression. Additionally, it is effective in treating extreme pain, nausea, vomiting, and uncontrollable hiccups. Moreover, it is used as an alternative treatment

to atropine sulphate in OP poisoning due to its potent anticholinergic, antihistam inic, and analgesic effect, as explained in some studies (Juza et al., 2023, Sinha et al., 2023, Dixon, 1980). This study chose local doves as experimental models for acute OP poisoning because they are close to the human environment (Pushpavalli et al., 2024). Due to the lack of information on the interaction of Largactil with OP insecticides, this study aims to evaluate the preventive and therapeutic effects of Largactil in a model of acute dichlorvos poisoning in local doves.

Materials and Methods

Materials

The various compounds used in the current work are summarized in Table 1.

Table 1. The chemical compounds used in the study

Sr. No.	Name of compounds	Percentage, company name, and country
1	Dichlorvos	50%, Super Nogos, Pacific Agriscience, Australia
2	Largactil	0.5%, Eczacıbaşı, Turkey
3	Atropine sulfate	1%, Veterinary and Agricultural Products Manufacturing Co VAPCO, Jordan

Doves Characterization

The current study was conducted on 30 local doves (collared doves) representing both male and female individuals. The subjects weigh between 250 g and 350 g and are housed under laboratory conditions in a sizing cage with dimensions (100×90×60) cm, where they have free access to food and water.

Procedures

A commercially available emulsified solution with a 50% dichlorvos concentration was mixed with distilled water and administered orally at a dose of 3 ml/kg bird. Largactil was added in normal saline to prepare a solution of 10 mg/kg bird, with a dose of 5 ml/kg bird injected intraperitoneally. Atropine sulfate was also mixed with normal saline to obtain a solution of 2 mg/kg bird and injected intraperitoneally into the bird at a dose of 2 ml/kg.

The selection of doses of Largactil, atropine, and dichlorvos was based on our primary study in birds and the information from other researchers (Bajgar, 2004). The acute (24 hour) oral LD50 of dichlorvos alone or in combination with Largactil (10 mg/kg, intraperitoneally [i.p.]) or atropine (2 mg/kg, i.p.) was determined directly in birds using high-dose and low-dose methods (Al-Badrany & Mohammad, 2007). Largactil was immediately administered i.p. after oral administration of atropine and dichlorvos. The birds were individually observed for signs of cholinergic toxicity (Al-Badrany & Mohammad, 2007, Petrie & Watson, 1999).

The protective ratio of Largactil or atropine was calculated as follows:

$$protective\ ratio = \frac{Dosage\ amount\ (with\ treatment)}{Dosage\ amount\ (without\ treatment)}$$

Method

Thirty birds (collared dove) were divided into three groups of ten birds each. The birds were treated orally with dichlorvos at a dose of 21 mg/kg body weight. Immediately after oral administration of dichlorvos, the birds in Group 1, which represented the control group, was administered an i.p. dose of 2 ml/kg bird normal saline solution. Atropine was administered to the second group of birds (Group 2) with a dose of 2 mg/kg body weight. Group 3 was given Largactil at a dose of 10 mg/kg bird. The onset of any signs of poisoning or death was recorded at 3 p.m. After dichlorvos was administered, the birds were observed individually for signs of OP toxicity and the toxicity scores were compared. To calculate the total toxicity score, each symptom of cholinergic toxicity were assigned a number from 1 to 10 (salivation–1, lacrimation–2, running–3, breathing–4, wing change–5, frequent urination–6, ataxia–7, tremor–8, confusion–9, and death–10).

Statistical analysis

Data were statistically analyzed using one-way ANOVA, followed by the least significant difference test (Mohammad et al., 2008). Toxicity severity scores were analyzed using the Wilcoxon Signed-Rank test, and frequency data were analyzed using the Fisher test (Runyon, 1977, Jain et al., 2023). The statistical software SPSS v20 was used to perform the necessary analyses. The statistical level of this value was $p < 0.05$.

Results and discussion

Oral LD50 of dichlorvos determined using the up-and-down method

After 24 hours of saline water in local doves, the oral LD50 for dichlorvos was 21 mg/kg body weight. It took approximately 0.15–0.40 minutes for the birds to show signs of cholinergic poisoning. The signs include vomiting, salivation, dyspnea, lacrimation, feather erection, ataxia, defecation, tremors, convulsions, and death; see Table 2.

Table 2. Lethal dose of dichlorvos and signs of poisoning in doves.

Measurements	Result
Dichlorvos LD50	21 mg/kg orally
Dose range	25 - 20 = 5 mg/kg
First dose	35 mg/kg
Last dose	20 mg/kg
Up-and-down dose	5 mg/kg
Onset of signs of toxicity	0.15–0.40 minutes
Signs of toxicity	Salivation, dyspnea, vomiting, lacrimation, feather erection, ataxia, defecation, tremors, convulsions, death

Table 2 shows that after injecting the LD50 of dichlorvos into the birds (Group 1), some characteristic signs of toxicity manifested, including convulsions, tremors, other symptoms that disrupt the functions of the central nervous system and respiratory system, and, in cases of severe poisoning, death. This is due to the inhibition of the enzyme acetylcholinesterase, which leads to cholinergic poisoning. These results as well as the symptoms observed in the birds are consistent with various studies, such as that of Jain et al. (Eddleston et al., 2008, Mohammad et al., 2012).

24-hour average oral LD50 of dichlorvos with atropine in doves determined using the up-and-down method

The oral LD50 of dichlorvos with atropine (2 mg/kg, i.p.) revealed an increase to 30.845 mg/kg, with a protective ratio of 1.47. Different signs of cholinergic toxicity were observed in the birds at 1.5–4.0 minutes, such as salivation, lacrimation, dyspnea, feather erection, defecation, ataxia, tremors, and convulsions; see Table 3.

Table 3. Lethal dose of atropine + dichlorvos LD50 and signs and symptoms of toxicity in doves.

Measurements	Result
Atropine + dichlorvos LD50	3**0.845** mg/kg orally
Dose range	35 - 25 = 10 mg/kg
First dose	**3**5 mg/kg
Last dose	30 mg/kg
Up-and-down dose	5 mg/kg
Onset of signs of toxicity	1.5–4.0 minutes
Signs of toxicity	Salivation, dyspnea, vomiting, lacrimation, feather erection, ataxia, defecation, tremors

When observing the signs and symptoms (Table 3), we found that the second group of birds injected with atropine (Group 2) had some alleviated symptoms, such as nervous convulsions, that occurred in Group 1, which was not injected with atropine (Table 2). This is consistent with the study by Eddleston et al.(Alias, 2009), which showed that taking an appropriate dose of atropine has a positive effect on overcoming the toxicity of organophosphorus compounds.

24-hour average oral LD50 of dichlorvos with Largactil in doves determined using the up-and-down method

The oral LD50 values of dichlorvos with Largactil were as high as 55.7 mg/kg, and the protection factor was as high as 2.65 (Group 3).

Various symptoms of cholinergic poisoning in the birds appeared within 4–8 minutes and included salivation, vomiting, shortness of breath, and lacrimation; see Table 4.

Table 4. Lethal dose of Largactil + dichlorvos LD50 and signs of toxicity in doves.

Measurements	Result
Largactil + dichlorvos LD50	55.7 mg/kg orally
Dose range	60 - 35 = 25 mg/kg
First dose	35 mg/kg
Last dose	60 mg/kg
Up-and-down dose	5 mg/kg
Onset of signs of toxicity	4–8 minutes
Signs of toxicity	Salivation, dyspnea, vomiting, lacrimation

Comparison of results between the first, second, and third groups

Table 5. and Figure 1 show a comparison of the symptoms of OP toxicity in the birds after the administration of dichlorvos (25 mg/kg, oral) followed by treatment with atropine sulfate (2 mg/kg, i.p, Group 2) or Largactil (10 mg/kg, i.p, Group 3).

Table 5. Different clinical indicators of cholinergic toxicity in birds for a single dose of 25 mg/kg of dichlorvos in the three groups.

Variables	Dichlorvos + normal saline (Group 1)	Atropine at 0 time (Group 2)	Largactil at 0 time (Group 3)
Time to onset of signs of poisoning (min)	0.30 ± 0.020	4.59 ± 0.111	0.94 ± 0.114
Salivation	100	100	100
Lacrimation	100	100	70
Vomiting	100	70	30*
Gasping	100	70	100
Feather erection	100	70	0*!
Frequent defecation	100	30*	20*
Ataxia	100	80	0*!
Tremor	100	70	0*!
Convulsions	100	0*	0*
3-h death	100	0*	0*
Total toxicity score	40	23.6*	12.8*!
Reduced total toxicity score	-	41%	68%

Time values are in mean + SE. N = 10 birds/group.

* The value is morally different from the control value, $p < 0.05$.

Significantly different from the value of 0 times atropine treatment, $p < 0.05$.

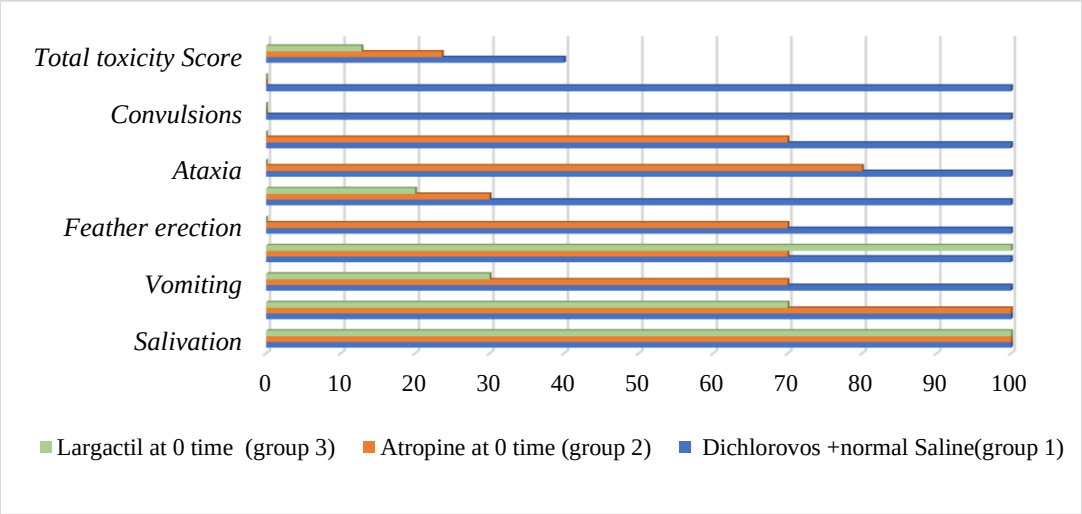

Figure 1. Different clinical indicators of cholinergic toxicity in birds for a single dose of 25 mg/kg of dichlorvos in the three groups

Treatment with Largactil (10 mg/kg, i.p., Group 3) and atropine (2 mg/kg, i.p., Group 2) after administration of 25 mg/kg of dichlorvos alleviated the signs of toxicity that caused the death of the birds. Compared to the control group (Group 1), the toxicity levels were significantly reduced by 68% and 41% in the groups treated with a dose of Largactil (Group 3) and atropine (Group 2), respectively. We found that the use of both Largactil and atropine had a positive effect on strongly inhibiting death caused by dichlorvos. The results we obtained showed the signs of toxicity in the birds due to the ingestion of a dose of dichlorvos (LD50 for dichlorvos) and confirmed the results of earlier studies such as Mohammad et al. [30] and Alias

(Robb et al., 2023). Ingestion of dichlorvos caused symptoms of parasympathetic hyperactivity in birds, which included salivation, tearing, shortness of breath, feather cleaning, bathing, staggering, tremors, convulsions, and death. These symptoms support the results of previous studies on birds exposed to pesticides such as dichlorvos, diazinon, and chlorpyrifos. In addition, we found that in birds poisoned with dichlorvos, the third group (Group 3), which was treated with Largactil, had a more positive outcome compared to the group with atropine sulfate (Group 2), as seen with the increase in the LD50 value of dichlorvos and a decrease in clinical symptoms after treatment (Ali et al., 2020). Largactil reduced the parasympathetic symptoms of OP poisoning through its potent antimuscarinic, anticholinergic, antihistaminic, and analgesic properties (Sinha et al., 2023). The efficacy of Largactil as an antidote was successfully observed at a dose of 10 mg/kg i.p. In this study, the intraperitoneal injection method of Largactil was chosen because subcutaneous injection of the drug into the bird may result in leakage of the drug from the injection site.

Conclusions

Organophosphorus pesticides are pesticides that inhibit acetylcholinesterase, causing cholinergic toxicity and other symptoms. This study aimed to find a drug that can help reduce and alleviate the symptoms of toxicity resulting from exposure to these pesticides. Therefore, the focus was on the use of Largactil as an alternative to atropine in the treatment of dichlorvos poisoning, due to its protective effectiveness as an anticholinergic, antihistamine, and analgesic. In this study, Largactil significantly reduced the indicators of cholinergic toxicity and total toxicity to birds resulting from acute OP poisoning in local dove models. This drug can contribute to the conservation of biodiversity and directly or indirectly prevent its extermination.

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Conflict of Interest

The authors declare that they have no competing interests.

Author Contributions

All authors' contributions are equal for the preparation of research in the manuscript

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