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Acute Poisoning among Farmers by Chlorpyrifos: Case Report from Gaza Strip

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Abstract

Spraying of organophosphorus insecticides (OPI) using high volume technique may result in poisoning cases among farmers or inhabitants in the spraying zone. This study reported a case among farmers in Gaza Strip, Palestine and discussed the follow up treatments. Results showed sever fasciculation of tongue and all muscle, pinpoint pupils not reacting to light, losing consciousness and disoriented, irritability, followed by diarrhea, vomiting, and severe inhibition of acetyl choline esterase (ACHE). Irritability and low level of ACHE activity were the unique syndromes of this case. Management of the poisoning with Atropine injection was not successful treatment to cure the case. Treatment with Toxogonin as intravenous injection resulted in relieved the irritability of the poisoned case and cure the patient at the end point. During the six weeks of follow up period, the case was severely poisoned in the 1st week, moderate poisoned in the 2nd week and slightly poisoned at the 3rd week. A medical decision to release the patient was made after ACHE activity level reached 4750 U/L, which very close to the normal range. The farmers left the hospital after three weeks of medical treatments and follow up. It is recommended to use Toxogonin in the management of poisoned cases with OPI immediately on arrival of cases with losing consciousness.

Keywords

Organophosphorus Insecticides, Poisoned Case, Toxogonin, Atropine

1. Introduction

Poisoned cases among population may be appeared due to using high volume techniques in windy days or due to misuse of insecticides. In this technique farmers used large fraction of water with motorized sprayers with a capacity of 400 L pesticide solution that discharge 1 L/min. Under wind condition, large fraction of the spray solution became a drift and being transported to long distance far

away from the application point.

So far, poisoning can be divided into four classes such as food poisoning, drug and pesticides poisoning, poisoning of industrial chemicals and poisoning of natural toxins. Pesticides poisoning may occur via indirect ways such as ingestion of contaminated food [1] [2] [3] [4], drinking of contaminated water [5] [6] [7], inhalation of contaminated air [8] and exposure contaminated soil [9] [10] [11] [12].

Organophosphorus insecticides (OPI) are widely used in Gaza [13] [14] [15] for pest control in agricultural lands and in house gardens. Recent study stated that farmers used pesticide container as a domestic tools, which may result in occurrence of poisonous case among farmers. Recently, long term toxicity of pesticides in Gaza Strip has been documented [16] [17] [18].

Furthermore, spraying pesticides in farms have been shown to damage non-target organisms such as cyanobacteria [19] [20] [21] [22] [23], plants [24], fish [25] [26].

Many efforts have been devoted to reduce the toxicity of pesticides among applicators, this included development of clay based and/or organo-clay formulations [27] [28]-[35]. Acute toxicity among population may be appeared due to occupational, accidental exposure to direct insecticide solution and/or suicidal attempt. Here below we presented a case report of acute poisoning of Chlorpyrifos to a young farmer directly expose to pesticides solution.

2. Materials and Method

Mr. Anjelo M, a young farmer, 21 years old, married, has 2 children, was found losing consciousness in the farm. He was brought to the hospital at 10:50 AM with history of sudden loss of consciousness in his farm after spraying chlorpyrifos, using high volume technique at a windy day. Chlorpyrifos, an insecticide, belongs to the chemical group Organophosphorus insecticides (OPI). It is widely used in Gaza for pest control. Its application has been shown to create health problems among farmers [14]. The farmer was received in emergency department at the main hospital, then admitted in the intensive care unit (ICU) for medical treatments. Physical investigations such as blood pressure, temperature, and oxygen saturation were measured immediately at the ICU. Blood samples were contentiously collected from the patient for complete blood chemistry (CBC) and acetyl choline esterase (ACHE) activity determination. Moreover, clinical investigations were conducted during seven days of medical treatments.

Medical treatment

The poisonous case was managed following the procedure described previously [36]. In this protocol, the contaminated cloths of patient were removed and his skin was washed with warm water containing some soups to enhance removal of poison residue from the skin. Then gastric lavage can be undertaken according to the physical status of the case. For the reported case gastric lavage was not applied due to losing consciousness. So far, our case report was dealt very urgent and connected with a monitor that evaluates, oxygen, CO_2, saluta-

tion. Then blood samples were collected for complete blood chemical analysis, and for ACHE activity determination. These steps were repeated every day to monitor the level of ACHE.

The case was given 10 mg atropine in 500 mL normal saline solution (NS) every 6 h intravenous injection (IV), at a late stage double dose of Atropine was given to the patient. Then Diclofen was given as intra muscular injection (IM). After three days stay in the ICU no improvement was seen on the ACHE level, the patient was given 750 mg Toxogonin in 500 mL saline solution every 6 h along with other medical drugs (Table 1) to stop the clinical symptoms such as vomiting, diarrhea. Furthermore, the drugs in Table 1 were given individually without any combination either IM or IV as shown above.

3. Results

It is well known in the literature that OPIs are strong inhibitors for ACHE in human body. Appearance of cholinergic symptoms (Table 2) on the poisoned

Table 1. Medical treatment for the poisoned case during ICU hospitalization.

Type of medical drug	Frequency of treatment						
	1st	2nd	3rd	4th	5th	6th	7th
Diclofen (75 mg); IM	√	√	-	-	-	-	-
Cefazoline (1 g/8 hr); IV	√	√	√	√	√	√	√
Zantac (50 mg/8 hr); IV	√	√	√	√	√	-	-
Pramine (10 mg/12 hr); IV	√	√	√	-	-	-	-
Toxogonin (750 mg/500 mL); IV	-	-	√	√	√	√	√
Atropine (10 mg/500) NS; IV	√	√	√	√	√+	√+	√+

Table 2. Clinical symptoms of the poisoned case.

Symptoms	Values						
	1st	2nd	3rd	4th	5th	6th	7th
Fasciculation of tongue and all muscle	+++	+++	+++	+++	++	++	++
pinpoint pupils not reacting to light	+++	+++	+++	++	+	+	+
Diarrhea	++++	++	+	–	–	–	–
Vomiting	+++	+	–	–	–	–	–
Consciousness and disoriented	+	+	+	+	–	–	–
Irritability of patient	+	+	+	+	–	–	–
Blood Pressure (mm Hg)	**130/70**	**110/58**	**130/80**	**140/100**	**140/80**	**150/97**	**120/70**
pH	7.39	7.39	7.39	7.4	Nd	Nd	Nd
PO_2 (mm Hg)	50	39	50	62	Nd	Nd	Nd
PCO_2 (mm Hg)	38	44	38	37	Nd	Nd	Nd
HCO_3 (mmol/L)	22	26	22	22	Nd	Nd	Nd

Nd = not determined; where +++, ++, +, and – are extreme, high, moderate and disappeared, respectively.

case such as fasciculation of tongue and muscle, pinpoint pupils not reacting to light, diarrhea, vomiting, and losing consciousness and disoriented status of the patient strongly indicate the interaction between the poison (chlorpyrifos) and ACHE the nerves system of the human body. This is also obvious from the low levels of ACHE during the medical treatment (Table 3). In addition, the irritability status of the patient indicated the accumulation of neurotransmitter (Acetyl choline) in the synaptic gaps of the nervous system due to the inhibition status of ACHE. This result is in agreement with [37] who revealed that toxic symptoms are produced by acetylcholine accumulation at cholinergic receptors. Furthermore, the heart rate was in the range of 52 - 102 bit/min during the IUC treatment whereas, blood pressure was in the range of 90/60 - 150/97 mmHg and temperature of the poisoned case was at regular stage (37˚C) during the medical period, indicating that heart was not directly targeted by chlorpyrifos. Some cholinergic symptoms such as vomiting and diarrhea were relieved by Pramine intravenous injection at 10 mg/12 h during the first three days of medical treatment. The irritability status of the patient was gradually relieved after Obidoxime chloride (Toxogonin) intervenes injection at 750 mg.

Moreover, losing consciousness and disoriented disappeared in the 5th day and on during the medical treatment. However, the data in Table 3 clearly shows selected blood indices during the medical treatments. It can be seen that Hemoglobin and WBC can be classified into three cycles as follows: cycle 1 including the first three days (1st - 3rd) 14.7 - 13.5 g/l, cycle 2 including the second three days (4th - 6th) 15.2 - 14 g/l and cycle 3 including the last 2 days (7th and 8th) 14.1 - 12.3 g/l. It is obvious that each cycle started high and ended low. This indicates toxicity of blood. This is also clear from the reduced values of oxygen pressure (PO_2) in the blood which are below the normal range (80 - 100). These data

Table 3. Selected blood indices during the medical period of the case report.

Items measured	Values found							
	1st	2nd	3rd	4th	5th	6th	7th	8th
Hemoglobin (g/dL)	14.7	13.7	13.5	15.2	14.1	14	14.1	12.3
RBC (10^6/μL)	Nd	Nd	4.58	4.92	5.31	5	Nd	Nd
WBC (10^3/mm^3)	25.7	15	7	10.3	9.3	9	12.7	6
HCT (%)	40	40	36.6	41.1	40	42	Nd	Nd
PLT (10^3 mm^3)	259	219	245	228	230	321	211	Nd
creat (mg/dL)	0.7	0.7	0.6	0.8	0.8	1.1	1.8	Nd
Na (mmol/L)	142	149	153	146	149	144	142	Nd
K (mmol/L)	3.5	4.1	4.3	4.3	4.1	4.6	3.5	Nd
Ca (mg/dL)	10	9.97	10	10.72	9.97	10.6	9.4	Nd
ACHE (U/L)	715	626	482	791	650	600	697	892
AST (U/L)	75	80	83	80	85	77	70	60
LDH (U/L)	436	Nd	Nd	Nd	Nd	Nd	Nd	Nd

suggest partial inhibition of cytochrome enzymes that responsible for O_2/CO_2 exchange in the lunge at normal condition. This suggestion is supported by a previous study of El-Nahhal [38] who stated partial inhibition of cytochrome oxidase due to accumulation of CO_2 under closed condition. The appearance of three cycles of hemoglobin and WBCs suggests that poison (chlorpyrifos, **Figure 1**), is slowly metabolized into paraoxon (P= S $\Longrightarrow$ P=O) which reacted with ACHE resulting in accumulation of acetyl choline in the synaptic gap. Moreover, the metabolic process may proceed further to produce less toxic fragments. This is obvious from the high values of Aspartate Amino Transferase (AST) (**Table 3**). AST activity tends to increase up to the 5th day of medical treatment then the value tends to slow a bit down the first measurement but it was still above normal range (10 - 45 u//l). This reduction in AST value suggests that AST activity of the poisoned case was not above range before poisoning. These results indicate the metabolic pressure of the poisoned case. Moreover, Lactic Dehydrogenase (LDH) was above the normal range (105 - 333 u/L) indicating the metabolic activity of the poisoned case. However, LAD is not a specific biomarker of toxicity and/or metabolic activity accordingly further measurements were not made.

This suggestion agreed with our recent published work [39] that revealed elevate level of AST due to long term and short term exposure to insecticides in open field and green houses

Mechanism of poisoning

It is well known in the literature that normal enzymatic reaction in the synaptic gap can be represented by Equations (1) and (2).

$$[\text{Acetylcholinesterase}]+[\text{acetylcholine}]\rightarrow[\text{Acetylatedenzyme}]+[\text{choline base}] \quad (1)$$

$$[\text{Choline base}]+[\text{Acetyle}-\text{CoA}]\rightarrow[\text{Acetylcholine}] \quad (2)$$

These reactions are continuous to transfer the nerve pulse chemically in the synaptic gap. In the poisoned case the enzyme is not available for reaction # 1 accordingly acetylcholine is accumulated in the synaptic gap. This prevents the movement of pulse resulting in cholinergic effects, irritability of patient, and consciousness may occur along with other cholinergic symptoms as seen in **Table 2**. So far the poisoning reaction can be represented by the following equations.

$$[\text{Poison}]+[\text{Organism}]<\frac{K+1}{K-1}>[\text{Toxic effect}]$$

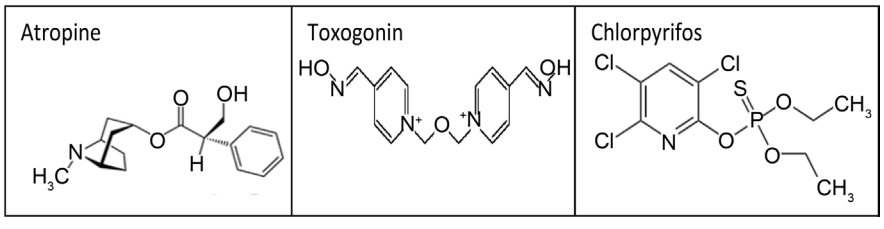


Figure 1. Chemical structure of Atropine, Toxogonin and Chlorpyrifos.

where $K+1$ and $K-1$ represent the irreversible and reversible toxic effect respectively. Moreover, the toxic effect can be acute poisoning and may appear within 0 - 72 h such as this case report. Moreover, chlorpyrifos poisoning may cause sub chronic toxicity and effects appear after 3-up 90 day and chronic effect that may appear within 2 years or more. Moreover, kinetics of toxicity can be denoted as follows: free enzyme (E) reacts with organphosphorus insecticides (OPI) forming phosphorylated enzyme (E-OPI), inhibited enzyme, and/or poisoned case. Toxicity coefficient (K) can be determined according to equilibrium equation (3). So far equation (4) determined the status of toxicity. At higher quantity of bound enzyme or low quantity of free enzyme, the extreme toxicity can be determined by K value.

Mode of poisoning

$$E + OPI \rightarrow E - OPI$$

$$\frac{[E-OPI]}{[E][OPI]} = K$$

$$\frac{[E-OPI]}{[E]} = K[OPI] \tag{3}$$

$$\frac{[\text{Bound Enzyme}]}{[\text{Free Enzyme}]} = k[OPI] \tag{4}$$

Management of toxic case

Management of toxic case was performed by injection of Atropine and Toxogonin. Both molecules are antidotes for OPI but Atropine is not a specific antidote for OPI poisoning and has low ability to cure the poisoned case. However, Toxogonin is more powerful nucleophile than Atropine due to the presence of quaternary ammonium in the chemical structure of the molecules (Figure 1) which enable Toxogonin to react with the anionic site on the enzyme surface followed by reacting with OPI molecules resulting on reactivating the enzyme from the inhibited form according to Equation (5). The mechanism of detoxification based on the ability of Atropine, Toxogonin and/or any antidote to be bound with the OPI and to free the enzyme from the complex (inhibited, phosphorylated). Accordingly, the effective medical treatment for OPI poisoning based on Equation (5) and the value of K3, which based on the affinity of antidote (Atropine, Toxogonin, etc.) to reactivate the enzyme. The high value of K3, indicates the high efficiency of the antidote to reactivate the enzyme and low value indicate low or unsuitability of antidote to reactivate to cure OPI poisoning case.

Mode of treatment (detoxification)

$$E + OPI \rightarrow E - OPI$$

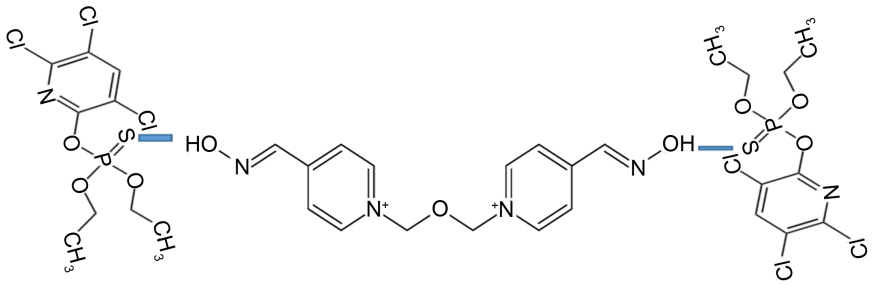
$$E - OPI + Toxogonin \rightarrow [E - OPI - Toxogonin] \rightarrow E + OPI - Toxogonin \tag{5}$$

$$\frac{[E][OPI - Toxogonin]}{[E - OPI][Toxogonin]} = K3$$

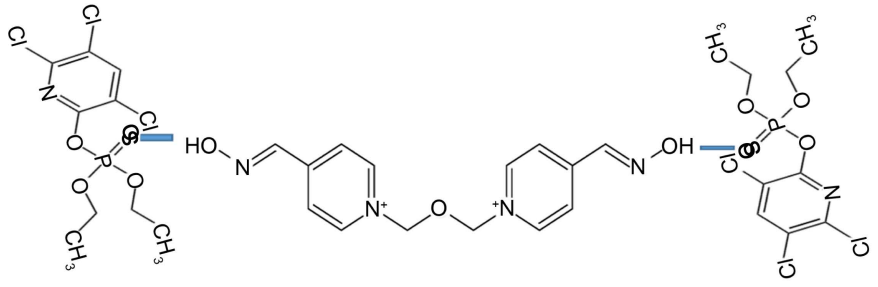
$$\frac{[Free\ Enzyme]}{[Bound\ Enzyme]} = K3[Toxogonin]$$

Mode of detoxification is presented in Figure 2. It can be suggested that two molecules of chlorpyrifos (A, or B, Figure 2) react with one Toxogonin molecules through hydrogen bonding forming a larger size complex than the parent chlorpyrifos. Accordingly the bound chlorpyrifos with Toxogonin will not be able to attack the esteric or anionic site on the enzyme surface. Leaving the enzyme in a free form. Furthermore, the produced complex is a cationic molecule accordingly it is easily be dissolved in the aqueous phase on the blood system and be excreted outside the body. These steps may result in reactivation or protection of ACHE, consequently the cholinergic symptoms be relieved. This explanation agreed with previous reports [40] [41] that found the presence of a cationic molecule in aqueous phase of organic molecules increased the solubility of the organic molecules in water phase due to hydrogen bonding or through hydrophobic hydrophobic interactions between both molecules resulting in a dramatic change in the behavior of organic molecules. Formation of hydrogen bonding between organic and inorganic molecules has be previously demonstrated [42] [43] [44]. This formation may enhance penetration, transport and /or movement of organic molecules through different barriers. Hydrogen bonding is also important in the solubility of inorganic molecules [45] [46]. Formation of hydrogen bonding between poison (chlorpyrifos or its oxon metabolite) and Toxogonin molecules is proposed in Figure 2.

Moreover, recent published work [15] found elevated levels of liver enzymes in farmers having long term exposure to pesticides or exposed to risk factors from pesticides. Further supports to our discussion come from the work of Ahmad *et al.* [47] and Araoud *et al.* [48] who revealed that bendiocarp, carbofuran,



(a)



(b)

Figure 2. Mode of detoxification of chlorpyrifos by Toxogonin. (a) and (b) represent interaction of parent molecule (a) and its oxon metabolite (b) with Toxogonin through hydrogen bonding . Blue dash shows hydrogen bonding.

carbaryl, methomyl and propoxur significantly lowered the AChE activity along with butyrylcholinesterase and paraoxonase activities in OPI treated animals.

4. Conclusion

Application of OPI at high volume technique resulted on acute poisoning at windy days. Cholinergic symptoms were dominant during the first five days of treatment. HB and WBC were changed dramatically during the 1st week of toxicity. Atropine injection was not strong OPI antidote. Acute poisoning (toxicity) was associated with severe reduction on ACHE activity and appearance of clinical symptoms such as pin point pupil's and elevation of AST and LDH. ACHE activity reached the lowest level during the treatment then the level was elevated after several times injection of Toxogonin at 750 mg. Reactivation of ACHE by Toxogonin injection was discussed by two different methods. The patient was released from the hospital after tremendous increase in the activity of ACHE. Contentious application of OPI at high volume at windy days may result in many poisoned case.

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Ethical Statement

This study was not funded by any organization.

Compliance with Ethical Standards.

Conflict of Interest

The author declares that he has no conflict of interest.

The study complies with the international ethics issues. The farmer agrees to participate in this case report without mentioning his personal data.

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