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Original Article

Environmental Risks of Insecticides Cholinesterase Inhibitors

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ABSTRACT

This study conducted on 423 inhabitant (372 adults and 51 children) blood samples were collected from patients hosted in the Emergency Hospital of Mansoura University. The clinical diagnosis of such patients was acute insecticides poisoning. The aim of the present study is to study patients with cholinesterase (ChE) inhibitor organophosphorus insecticides intoxication from the laboratory point of view. The plasma samples were analyzed for levels of ChE enzyme and acetyl cholinesterase enzyme activity by spectrophotometer. The pesticides were identified using Gas Chromatography-Electron Captured Detector (GC-ECD). The results of GC-ECD instrument of all patients revealed that parathion (organophosphorous insecticide) poisoning was found in their blood samples. The mode of poisoning was accidentally by inhalation and skin contact. The poisoning cases of children were of mild poisoning. The degrees of poisoning of adults were severe in 138, moderate in 201 and mild in 33 cases. In conclusion, results of the present study revealed that the widely used insecticides in Dakahlyia governorate are the organophosphorous insecticides specifically parathion insecticide.

Key words: Cholinesterase, chromatography, organophosphorous, spectrometry

INTRODUCTION

Pesticides are classified in to four groups; insecticides, herbicides, rodenticides, and fungicides. They are now used intensively in agriculture to control insects (Cooper *et al*., 1994).[1] Special attention has been given to insecticides group. The most widely used types are insecticides as cholinesterase (ChE) inhibitor, mainly organophosphorous compounds and carbamates (WHO 1991).[2] Organophosphorous are the most dangerous class among all synthetic compounds. Chemically, they contain an organophosphorous radical, amino radical ($-NH_2$) and phenyl radical. Parathion (Police el-Nagda)* is the most common one used as powder or liquid. Since, agriculture is one of the major occupations in Egypt, are potentially at high-risk of exposure, especially, those sharing in formulation, mixing and spraying these compounds. Acute poisoning by these compounds affects both human and animals (Sullivan, 1984).[3] Organophosphorous are powerful inhibitors for ChE enzyme (true ChE, which present in human RBCs, nervous tissues and skeletal muscles and pseudo-cholinesterase (PChe), which is present in human plasma and liver). The severity of clinical manifestations in organophosphate insecticide poisoning parallels the degree of inhibition of plasma cholinesterase (PChE) activity.

PChE is the most sensitive to inhibitors (lotti, 1995)[4] and hence that PChE inhibition can be taken only as a marker of exposure (Mason *et al.,* 1993).[5] Carbamates are less toxic than organophosphorous, because, they are rapidly hydrolyzed to methyl carbamic acid and variety of phenol substances which are of low-toxicity. Baygon * is

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the commonest one used for house insects. Environmental exposure of humans to agrichemicals is common and results in both acute and chronic health effects (Weisenburger, 1993).[6]

The aim of the present study is to study patients with ChE inhibitor organophosphorus insecticides intoxication from the laboratory point of view.

MATERIALS AND METHODS

The study was carried out on 423 patients (372 adults and 51 children) with acute poisoning by ChE inhibitor insecticides admitted to poison unit at Emergency Hospital, Mansoura University. Most of these patients were agricultural workers. A control group of 50 healthy subjects were randomly chosen, they were clinically free not exposed to ChE inhibitor insecticides and of the same age group and sex as the patients.

All patients were examined as regards:

1. Biosocial data (age, sex, and circumstance of poisoning),
2. Laboratory investigations (10 ml blood samples were collected on admission from patients and also from control group) for
   a. Plasma levels of pseudo-cholinesterase enzyme and acetyl cholinesterase (AChE) Enzyme activity of blood samples: Levels of PChE and AChE activity was analyzed using UNICO-UV 2100 Spectrophotometer and kits of ChE produced by Boehringer Mannheim GmbH Diagnostics.
   b. Determination of aspartate transaminase (AST) and alanine transaminase (ALT), (Burtis and Ashood, 1994).[7]
   c. Identification method of the pesticides using gas chromatography-electron captured detector (GC-ECD). Extraction of blood samples, (Flanagan *et al.,* 1995):[8]
      - Adjust the pH of each sample to seven by adding solid sodium bicarbonate.
      - Each sample is extracted with 5 ml of methyl tertiary-butyl ether for 5 min using a Rotary mixer.
      - All samples stand for 5 min and then the upper layers were taken and re-extracted with a second 5 ml portion of methyl tertiary-butyl ether.
      - The two extract of each sample were filtered through a Watt Mann filter paper number four in to a clean tube and the extract of all samples were evaporated to dryness under compressed air.
      - All residues of the evaporated extracts were reconstituted by 100 µl chloroform. 1 µl of each extracted sample injected in to GC-ECD (Hewlett Packard 6890 series) under the Following conditions, (Maurer and Weber, 1992):[9]
        - Column Hp-1 (ultra-1) Ion source temperature 200°C,
        - Ionization voltage 70 eV, Initial oven temperature 70°C,
        - Final oven temperature 250°C, Injection port temperature 250°C,
        - Initial time 3.00 min, final time 3.00 min, equilibration time 0.50 min,
        - Rate 8°C/min, carrier gas Helium, flow rate 13.5 ml/min.

Calculations

Actual enzyme activity % = (mean enzyme activity/average control value) × 100.

Statistical analysis

Both statistical analysis and tabulation were carried out. The quantitative data were presented as mean ± standard deviation and the qualitative data were presented as number and percentage.

RESULTS

Age and Sex

Nearly half of the patients (54.84%) were in the age group from 16 years to 25 years and (12.05%) of patients were in age groups <10 and 10-15 years, (24.82%) of patients were in age groups 26-35 years and the other (8.28%) in the age group 36-35 years. Males outnumbered females with a sex ratio of 1.19:1, [Table 1].

Mode of poisoning

Accidental poisoning accounted for more than half of the patients (90.54%) where male significantly outnumbered females (Z = 1.17). Attempted suicide constituted (9.46%) where females significantly outnumbered males (Z = 1.33), [Table 2].

Table 1: Patients with acute poisoning by cholinesterase inhibitor insecticides by age and sex

Parameters	Children		Adults				Total	
			Male		Female			
	No	%	No	%	No	%	No	%
Age in years								
<10	41	80.4	-	-	-	-	41	9.69
10-15	10	19.6	-	-	-	-	10	2.36
16-25	-	-	142	70.3	90	52.94	232	54.85
26-35	-	-	45	22.28	60	35.29	105	24.82
36-45	-	-	15	7.42	20	11.77	35	8.28
Total	51	100	202	100	170	100	423	100

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Table 2: Number and percentage of patients with acute poisoning by insecticides as inhibitors for cholinesterase

Parameters patients (%)	Mode of poisoning		Degree of poisoning		
	Accidental	Attempt to suicide	Mild	Moderate	Severe
Children (*n*=51)	46 (90.2)	5 (9.8)	51 (100)	0 (0.0)	0 (0.0)
Adult males (*n*=202)	187 (92.6)	15 (7.4)	13 (6.41)	97 (48.02)	92 (45.54)
Adult females (*n*=170)	150 (88.2)	20 (11.8)	20 (11.76)	104 (61.18)	46 (27.06)
Total number (*n*=423)	383	40	84	201	138
Percentage of total	(90.54)	(9.46)	(19.9)	(47.5)	(32.6)

Degree of poisoning

Mild, moderate and severe poisoning constituted 19.9%, 47.5%, and 32.6%, respectively as shown in [Table 2].

Type of insecticides

The patients were poisoned by organophosphorous insecticides (parathion). Parathion (diethyl-4-nitrophenylphosphoro-thioate) has molecular weight 291 and chemical formula $C_{10}H_{14}NO_5PS$, [Figure 1].

PChE level and AChE activity

Mean levels of PChE in severe, moderate and mild poisoning were 194.6 ± 42.5, 590.8 ± 194.7 and 1403.5 ± 462.6, respectively. Furthermore, the activities percentages of AChE were 6.87%, 20.85%, and 49.54% in severe, moderate and mild poisoning respectively, [Table 3].

Serum transaminases levels

The mean AST and ALT levels in the poisoned patients were significantly higher than the control group. As regards the degree of poisoning, the mean AST level increased with increasing the severity of poisoning. On the other hand, the mean ALT level was near the control group in mild and moderate poisoning while it was significantly higher in severe poisoning, [Table 3].

DISCUSSIONS

ChE inhibitors from insecticides are irreversible inhibitors, organophosphates or reversible inhibitors, carbamates, (Aaron *et al.*, 1990).[10] Organophosphates insecticides are irreversible ChE inhibitors, causing accumulation of acetylcholine at synapses and over stimulation of nicotinic and muscarinic receptors (Mortensen, 1986).[11] The Environmental Protection Agency (EPA, 1992), reported that over 80% of all hospitalization from pesticide poisoning was due to organophosphate insecticides. The study showed that (54.85%) were in the age group from 16 years to 25 years, (12.05%) of patients was in age groups less than 10 and 16 years, [Table 1]. This percentage of patients could be explained by the emotional immaturity with their inability to carry new responsibilities and facing stresses of life.

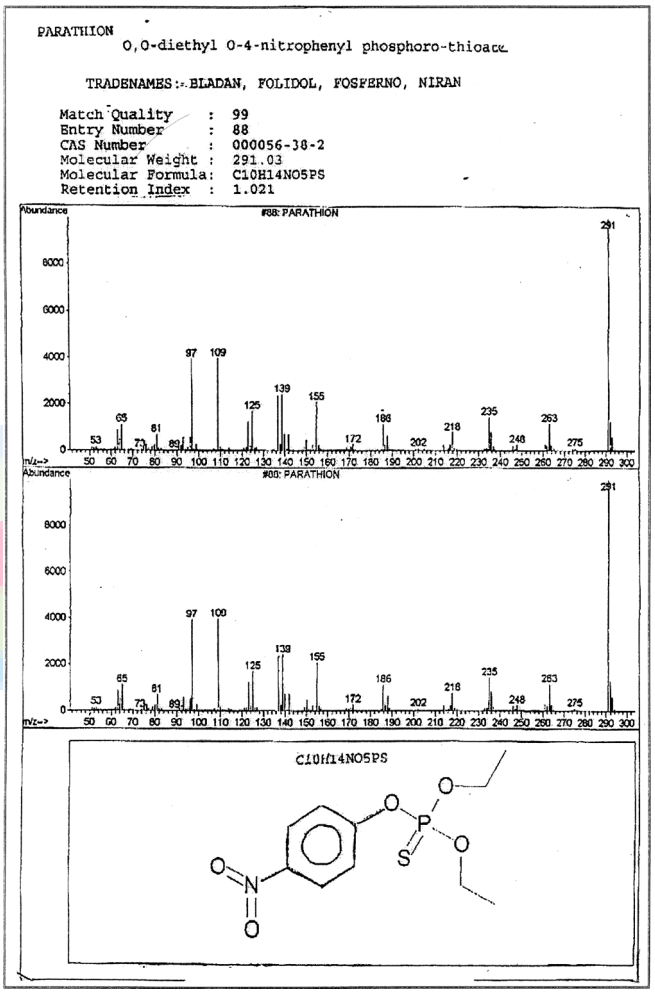


Figure 1: Spectra and structure of parathion

Males outnumbered females with a sex ratio of 1.19:1, this correlates with sex ratio reported by (Marey, 1986).[12] The study also showed that accidental poisoning accounted for (88.18%) and attempted suicide constituted (11.82%). The percentage of attempted suicide by insecticides could be attributed to the low-price, easy availability and wide spread use of insecticides, in addition to, the lack of adequate regulations controlling their sale. Furthermore, the study showed that mild poisoning constituted (19.9%), moderate poisoning constituted (47.5%), while severe poisoning constituted (32.6%), [Table 2]. In Agarwal study (1993),[13] 70.6% of the cases were mild, moderate

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Table 3: patients with acute poisoning by insecticides as ChE activity and mean serum transaminases

Group Parameter	Control 1900-3800 U/l *n*=50	Mild >1000 U/l *n*=84	Moderate 1000-500 U/l *n*=201	Severe< 500 U/l *n*=138
Mean±SD*	2833.2±755.3	1403.5±462.6	590.8±194.7	194.6±42.5
Actual enzyme activity %	100	49.54	20.85	6.87
AST (U/L)	25.8±8.7	30.5±9.8	51.4±9.6	72.6±3.1
ALT (U/L)	23.9±7.3	24.1±9.9	24.3±8.3	51.9±6.7

SD* = Standard deviation (Note; the average control value = 2833.23), AST = Aspartate transaminase, ALT = Alanine transaminase

and severe cases constituted 14.7% each. This could suggest that the pattern of poisoning severity may differ from country to country depending on the quality of life, circumstances of poisoning and amount of insecticide administered. Figure 1 shows that all these cases (423) are of parathion insecticide by GC-ECD analysis. This coincides with (Bey *et al.,* 2001)[14] that recognized that parathion is one of the most widely used organophosphate insecticide owing to its insecticidal activity and physiochemical profile Parathion is the commonest compound used as powder or liquid in both houses and agriculture field. This reflect the wide spread use of organophosphorous insecticides, which attributed to the lack of the governmental regulations, in spite of its danger to the health of both animals and human beings. It is well-known that the most serious effect of organophosphorous insecticides is their ability to induce toxic neuropathy in man and other species (Cavanagh, 1964 and Niesinic *et al*., 1996).[15,16] Regarding to the degree of poisoning according to pseudo-cholinesterase level in adults; there are 138 patients of severe poisoning with PChE level < 500 (U/L), 201 patients of moderate poisoning with PChE level 500-1000 (U/L) and 33 patients of mild poisoning with PChE level > 1000 (U/L). The children poisoning patients (*n*. 51 [12.05% of the total number of patients]) were of mild poisoning. This may be due to the low-exposure incidence, time and early hospital arrival. Mean levels of PChE in mild, moderate and severe poisoning were 1403.5 ± 462.6, 590.8 ± 194.7 and 194.6 ± 42.5, respectively. Furthermore, the activities percents of AChE were 49.54% 20.85% and 6.87%, in mild, moderate and severe poisoning respectively, [Table 3]. Abdel-Magid and Salem (1993)[17] reported a figure of (42.0%), (37.0%) and (6.8%) respectively in their study.

The present study indicated that the mean AST and ALT levels in the poisoned patients were significantly higher than the control group. As regards the degree of poisoning, the mean AST level increased with increasing the severity of poisoning. On the other hand, the mean ALT level was near the control group in mild and moderate poisoning while it was significantly higher in severe poisoning, [Table 3]. Liver damage was mainly attributed to the organic solvents in which the insecticides are usually dissolved (Ellenhorn and Barceloux, 1988).[18]

CONCLUSIONS

Results of our study revealed the widely used insecticides in Dakahlyia governorate are the organophosphorus insecticides specifically parathion insecticide, and the prevalence of insecticides intoxication in adults, which is explained by occupational exposure, lack of knowledge, unsafe attitudes, faulty sprayers, lack of protective equipments, and non-existent first-aid provisions. The lack of information at all levels may be one of the most important causative factors of insecticides intoxications.

Recommendations

- Increasing public awareness about the degree of ChE inhibitor insecticides and their effects on most of the body systems (central nervous system, pulmonary, hepatic, and cardiac).
- Estimation of pseudo-cholinesterase level should be a part of routine investigations for patients with acute poisoning by ChE inhibitor insecticides on admission.
- The use of warning design label on household products which should be issued on national basis and children should be warned and educated not to touch.

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