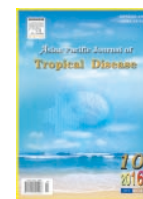




Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Entomological research

doi: 10.1016/S2222-1808(16)61138-6

©2016 by the Asian Pacific Journal of Tropical Disease. All rights reserved.

Activities of cholinesterase enzyme among diazinon and sevin insecticides sprayers in the western part of Iran

Ali Jalilian¹, Hasan Bakhshi², Sahar Bazrafkan³, Farzad Shayeghi⁴, Masoumeh Pirmohammadi¹, Nutifafa Godwin Gidiglo¹, Mansoureh Shayeghi^{1*}¹Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran²Malaria and Vector Research Group, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran³International Campus, Tehran University of Medical Sciences, Tehran, Iran⁴Medical Science Branch, Islamic Azad University, Tehran, Iran

ARTICLE INFO

Article history:

Received 15 Aug 2016

Received in revised form 23 Aug, 2nd

revised form 25 Aug 2016

Accepted 28 Aug 2016

Available online 1 Sep 2016

Keywords:

Diazinon

Sevin

Cholinesterase enzyme

Ilam

Iran

ABSTRACT

Objective: To measure the activities of cholinesterase enzyme among farmers who used the selected insecticides for the purpose of preventing the growth of agricultural pests on their farms.

Methods: A total of 21 people used diazinon to spray their agricultural lands and 13 people also used sevin to spray theirs in western part of Iran. Lovi Bond method was used for the measurement of cholinesterase activity.

Results: Results revealed that the enzyme level before spraying with diazinon was 100.0% among 3 workers and 87.5% in 18 of them. This level decreased to 75.0% among 13 workers and 67.5% in 5 workers. The number of workers that had headache, pale, dizziness with headache, nausea, diarrhea with cramps and stomachache were 5, 9, 5, 3, 4 and 7 respectively. These symptoms decreased after 72 h. Out of 13 workers who sprayed with sevin, the enzyme level before spraying was normal (100.0%) among 5 workers and 87.5% in 8 workers. After spraying, the enzyme level was 87.5% in 5 workers, 75.0% in 5 workers and 67.5% in 3 workers.

Conclusions: These workers were in danger of chemical poisoning. Measurement of pre-cholinesterase and post-cholinesterase exposures is recommended in order to compare the values after pesticide application.

1. Introduction

Among the numerous pesticides, organophosphorus (OPs) agents and carbamates are widely used all over the world[1,2]. Previous investigations have revealed that these toxic compounds can be absorbed especially through skin surfaces, mucous membranes, gastrointestinal and the respiratory tracts[1,2].

These toxic compounds produce their toxic effects by the acetylcholinesterase (AChE) inhibition and the subsequent accumulation of synaptic acetylcholine in peripheral and central nervous systems[3]. The widespread use of OPs as pesticides and the frequent misuse of OP nerve agents in military conflicts or terrorist attacks emphasize the high clinical relevance of OP usage to poisoning[3,4].

For many years, phosphorous and carbamate insecticides have been used for the control of agricultural pests and vector-borne diseases in Iran. Poisoning of spray workers and residents by these insecticides may occur through their exposure to such chemicals during the handling of chemical tools, dish washing, drinking of contaminated water and eating of polluted foodstuffs[5-7]. These afore mentioned compounds have physical and chemical characteristics that may cause human

*Corresponding author: Mansoureh Shayeghi, Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, P.O. Box 6446-14155, Tehran, Iran.

Tel: +98(21) 42933169

Fax: +98(21) 88951393

E-mail: mansorehshayeghi@yahoo.com

The study protocol was performed according to the Helsinki Declaration and approved by the Research Ethics Committee of Tehran University of Medical Sciences. Informed written consent was obtained from Research Ethics Committee of Tehran University of Medical Sciences.

The journal implements double-blind peer review practiced by specially invited international editorial board members.

poisoning and lead to an acute or a chronic disease such as mild or severe disorders in the cholinesterase enzyme functions and consequently block the activities of neurons by disrupting cholinesterase enzyme functions with different degrees of toxicity in birds, mammals and some other creatures.

It is important to recognize and treat poison-related conditions. According to current investigations, inhibition of AChE is a very important toxic action of OP compounds; so, it is essential to follow the AChE activity in order to quantify the degree of inhibition and to assess possible reactivation; also, an easy accessible source for AChE in humans is the red blood cell-AChE. Enzymatic activities in fluids of the body are utilized as diagnostic markers for the recognition of physiological conditions and diseases. So, the measurement of cholinesterase enzyme function in spray workers especially carbamates and phosphorus compounds is a routine assessment which is recommended to be measured. It should be mentioned that some of these workers work in some seasons and there is no control regarding the conduct of their activities.

2. Materials and methods

The study was carried out in Chardaval and Darreh Shahr districts, located in Ilam Province, west of Iran during 2013. This province shares border with Iraq. The use of OPs (diazinon) and carbamate (sevin) insecticides is common in this province since 2013. Totally, 21 people used diazinon to spray their agricultural lands located in Chardaval and 13 people used sevin to spray theirs in Darreh Shahr. In this study, the activity of cholinesterase enzyme in spray workers was measured using the Lovi Bond method, considering the fact that a minimum cost was involved in its usage, which was practicable under any condition and a small volume of blood was required[8]. Solutions like bromo thymol blue and acetylcholine perchlorate as substrate, distilled water without CO₂, tools for compressing Lovi Bond and timer were prepared. For confirming the validity of materials, 0.5 mL bromo thymol blue and 0.01 mL blood of a testifier who had no contact with any insecticides and 0.5 mL substrate solution which

Table 1

The changes of enzyme level in spray workers by the use of Lovi Bond method before and after spraying diazinon and sevin insecticides in Chardaval and Darreh Shahr districts, Ilam Province.

Insecticide	Time	Experimental groups	No. of selected people	No. of examined people	The enzyme level (%)	Comments
Diazinon	Before spraying	Treatment	21	3	100.0	Natural
				18	87.5	
	After spraying	Treatment	21	3	87.5	The risk of toxicity
				13	75.0	
Sevin	Before spraying	Treatment	13	5	67.5	Natural
				8	87.5	
	After spraying	Treatment	13	5	87.5	The risk of toxicity
				5	75.0	
				3	67.5	
Control	No expose	Control	6	6	87.5	Natural

was obtained from Lovi Bond machine were prepared. Another sample was obtained from testifier's blood and water without CO₂ was placed in the left part of the machine, then disks were circled in front of the light until the color of filter was similar to that of the solution[6,8]. In order to observe more than 12.5% of changes, the test was repeated.

The blood samples of the spray workers from the studied area were taken before and after the work. Before the spray workers started work, blood samples were taken. An experimental pipe was allocated to each of the spray workers on a condition that the workers washed their hands with water and soap. In each pipe, 0.5 mL and 0.01 mL blood samples from the finger-tips of each spray worker as well as that of the testifier's pipe were taken and added to bromo thymol blue solution; then, 0.5 mL of substrate was poured into the pipes and the time was noted. As soon as 1 min passed, testifiers' samples were added and placed in the right part of the Lovi Bond compressor machine[6,8]. Color of solution altered in the control pipe revealed the occurrence of the experiment and also spray workers' blood color which were altered after 1 min revealed the occurring of the experiment, hence the levels of cholinesterase enzyme activity[6,8]. Finally, significant differences between variables were analyzed by One-way ANOVA using SPSS program.

3. Results

Activity level among spray workers by the use of the Lovi Bond method in blood samples of workers of agricultural farms was evaluated. A total of 21 spray workers used diazinon to spray their agricultural lands located in Chardaval and 13 spray workers also used sevin to spray their agricultural lands at Darreh Shahr. The enzymatic range varied between 87.5%–100.0% before spraying with diazinon in Chardaval. This range of enzymes was variable between 67.5%–87.5% after spraying. The people were at risk of chemical poisoning after spraying. The number of workers that had headache, pale, dizziness with headache, nausea, diarrhea with cramps and stomachache were 5, 9, 5, 3, 4 and 7 respectively. These symptoms decreased after 72 h. Out

of 13 workers who sprayed with sevin in Darreh Shahr, the enzyme level before spraying was normal (100%) among 5 workers and 87.5% in 8 workers. Obviously, it ranged between 87.5%–100.0%. After spraying, the enzyme level ranged between 67.5%–87.5%. The enzyme level was 87.5% in 5 workers, 75% in 5 workers and 67.5% in 3 workers. These workers were in danger of chemical poisoning (Table 1). Due to the similarity in climatic conditions, this investigation was similar to that of Shayeghi *et al.*[6,7].

3.1. Ethical statement

The study protocol was performed according to the Helsinki Declaration and approved by the Research Ethics Committee of Tehran University of Medical Sciences. Informed written consent was obtained from Research Ethics Committee of Tehran University of Medical Sciences.

4. Discussion

Despite their harmful effects, the use of chemical pesticides has increased in recent years and farmers widely use pesticides to increase the yield of their agricultural products due to the resistance of pests to so many pesticides. Many people are at risk of exposure to these pesticides. In the OP compounds, insecticides like diazinon and carbamates compounds like sevin are mostly used as insecticides in agriculture and hygiene pest control[5-7]. OP compounds are examples of pesticides that have so many pharmacological effects[5-7]. It should be mentioned that some of these workers work in just some seasons and there is no control regarding their cholinesterase activities. These poisoning effects on cholinesterase enzymes especially AChE[3,4]. These toxic compounds can be absorbed through the skin, mucous membranes, gastrointestinal and respiratory tracts and produce their toxic effects by the inhibition of AChE and the subsequent accumulation of synaptic acetylcholine in peripheral and central nervous systems[3,4]. Inhibition of AChE leads to the appearance of poisoning effects up to 50% and between 80%–90% of poisoning effects can lead to death in mammals. The workers encounter pesticides repeatedly for long periods and this can lead to chronic poisoning which may show no clinical symptoms. In this study, 21 people used diazinon to spray their agricultural lands located in Chardaval and 13 of them also used sevin to spray their agricultural lands in Darreh Shahr. Lovi Bond method was used to measure the activities of cholinesterase enzymes. Results revealed that the enzyme level before the spraying with diazinon was 100% among 3 workers and 87.5% in 18 workers. This level decreased to 75% among 13 workers and 67.5% in 5 workers. The number of workers that had headache, pale, dizziness with headache, nausea, diarrhea with cramps and stomachache were 5, 9, 5, 3, 4 and 7 respectively. These symptoms decreased after 72 h. Out of 13 workers who sprayed with sevin in Darreh Shahr, the enzyme level

before spraying was normal (100%) in 5 workers and 87.5% in 8 workers. After spraying, the enzyme level was 87.5% in 5 workers, 75% in 5 workers and 67.5% in 3 workers. These workers were in danger of chemical poisoning. In another similar investigation, the effects of malathion were studied in Tonekabon[7]. Recently, another investigation was carried out on the activity of cholinesterase enzyme among spray workers in Tehran, the capital of Iran in 2009[6].

Due to similar climatic conditions, this investigation is similar to those that were carried out in 2001 and 2009[6,7]. Finally, measurement of pre-cholinesterase and post-cholinesterase exposures is recommended in order to compare the values after pesticide application.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors wish to render their sincerest gratitude to the Research Assistance of Tehran University of Medical Sciences in Iran for the financial support.

References

- [1] Pakravan N, Shokrzadeh M, Bari MA, Shadboostan A. Measurement of cholinesterase enzyme activity before and after exposure to organophosphate pesticides in farmers of a suburb region of Mazandaran, a northern province of Iran. *Hum Exp Toxicol* 2016; **35**: 297-301.
- [2] Vale A, Lotti M. Organophosphorus and carbamate insecticide poisoning. *Handb Clin Neurol* 2015; **131**: 149-68.
- [3] Čolović MB, Krstić DZ, Lazarević-Pašti TD, Bondžić AM, Vasić VM. Acetylcholinesterase inhibitors: pharmacology and toxicology. *Curr Neuropharmacol* 2013; **11**(3): 315-35.
- [4] Pohanka M. Acetylcholinesterase inhibitors: a patent review (2008-present). *Expert Opin Ther Pat* 2012; **22**(8): 871-86.
- [5] Khan DA, Ahad K, Ansari WM, Khan H. Pesticide exposure and endocrine dysfunction in the cotton crop agricultural workers of southern Punjab, Pakistan. *Asia Pac J Public Health* 2013; **25**: 181-91.
- [6] Shayeghi M, Nasirian H, Nourjah N, Baniardelan M, Shayeghi F, Aboulhassani M. Cholinesterase activity among spray workers in Iran. *Pak J Biol Sci* 2009; **12**(9): 696-701.
- [7] Shayeghi M, Shayeghi S. Effect of malathion insecticides on function of cholinesterase enzyme among the agriculture sprayers. *Armaghan Danesh* 2003; **7**(28): 31-6.
- [8] Ellman GL, Courtney KD, Andres V, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 1961; **7**: 88-95.