BIOCHEMICAL EFFECTS OF VARIOUS PESTICIDES ON SPRAYERS OF GRAPE GARDENS

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Running Title. Biochemical effects of pesticides.

ABSTRACT

A total of 85 healthy male pesticide sprayers in grape garden exposed to different class of pesticides for 3 to 10 years were compared with 75 controls matched for age with respect to serum cholinesterase, serum total protein, albumin, AST, ALT, hematological parameters such as Hb, Hct, RBC and serum lipid peroxidation. Serum lipid peroxidation was estimated in the form of thiobarbituric acid reactive substances (TBARS) produced. Significant decrease was observed in serum cholinesterase, serum total proteins, albumin and hematological parameters viz. Hb, Hct and RBC. Significant increase in lipid peroxidation, AST, ALT, was observed in exposed group when compared with control. These results suggest that the long term exposure of various pesticides on sprayers of grape garden affect liver, heme biosynthesis and decrease serum cholinesterase.

KEY WORDS

Aspartate aminotransferase, Alanine aminotransferase, Acetyl cholinesterase, hemoglobin, hematocrit, red blood cell, lipid peroxidation.

INTRODUCTION

In developing countries like India with agriculture based economy there is increasing trend of cash crop cultivation. In the horticulture sector the land used for cash crop like grapes is increasing particularly in Maharashtra state. Pesticide exposure represents a major potential health hazard for sprayers in grape garden. Major chemical groups of pesticide used in grape gardens are organophosphate, carbamate, organochlorines, nitro and chlorophenols and pyridyl derivatives (1). There are several factors affecting the levels of exposure during the agricultural application of pesticides (2). It has been observed that maximum exposures occur while mixing and handling of the concentrated solutions. Wind is the single most important factor determining dermal exposure during spraying. Other

factors are equipment used, the duration of exposure, the type of activity and individual protection (3). The biochemical effects produced by certain pesticides can be enzyme induction or enzyme inhibition. The effect of pesticides may be detected by ensuring biochemical changes even before adverse clinical health effects can occur. Organophosphorus and carbamate pesticides are inhibitors of cholinesterase (4, 5). Altered liver enzyme activities have been reported among pesticide workers exposed to organophosphorus pesticide alone or in combination with organochlorine or other pesticides (5, 6). More recently various studies from several parts of the world revealed the toxic effects of pesticides on human beings especially by elucidating free radical mechanism, which can be confirmed by the direct measurement of lipid peroxidation by products such as malondialdehyde(7). There has been no study so far on the biochemical aspects of environmental health with special reference to grape cultivation from developing countries. Therefore we have

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planned to study the biochemical effects of the pesticide pollution on human health.

MATERIALS AND METHODS

Subjects

85 male subjects were taken up for the present study with the age group 20-45 yrs. These subjects were selected on the basis of oral questionnaire from the Tasgaon taluka who have been involved mainly in spraying activities in grape gardens for 3 to more than 10 years. From this group blood samples were collected after 3 months continuous 2 hrs daily spraying, by using petrol pump and HTP spraying instrument, and without any protective equipment like gloves, masks, etc. The control group consisted 75 male volunteers from the same place with same age group, who were not performing spraying activity and did not have any kind of pesticide exposure, and from well socio economic group. Consent was obtained from both sprayers and control group persons. Blood was collected in EDTA bulb and plain tube in the spray field. Serum was separated and analyzed by using standard methods.

METHODS

AChE activity was assayed using kit from Boehringer Mannheim. AST and ALT were estimated by using kits from Accurex Biomedical Pvt. Ltd. Total proteins and albumin were estimated by using kits from Beacon Diagnostics Pvt. Ltd. For lipid peroxidation products, TBA was added to serum sample under acidic condition and absorption of colour that developed after heating was estimated spectrophotometrically at 535 nm and 453 nm and 20% OD of 453 was subtracted from OD of 535 and the values are compared with standard MDA and expressed in nmol per ml of serum (7).

RESULTS AND DISCUSSION

The present study demonstrated a significant decrease in serum cholinesterase ($p < 0.001$) in pesticide spraying workers in grape garden (Table 1). Several studies on volunteers reported that repeated long term exposures of organophosphorus pesticide and carbamate decrease the blood cholinesterase activities without clinical manifestation (8). The normal function of AChE is to terminate neurotransmission due to acetylcholine, liberated at cholinergic nerve ending in response to nervous stimuli. Loss of AChE activity may lead to

a range of effects resulting from excessive nervous stimulation and culminating in respiratory failure and death (9).

Pseudocholinesterase (chE) which is present in blood plasma and nervous tissue, but has no known physiological function is inhibited by organophosphorus compounds in a similar way to AChE, but the specificity of the two enzymes is different, though no toxic effect arises as a result of inhibition of Pseudo-chE, measures of its inhibition can be made for monitoring purposes (10, 11). All these signs and symptoms can occur in different combination and can vary in time of onset, sequence and duration, depending on the dose and route of exposure, mild poisoning might include muscarinic and nicotinic signs only (9, 10).

Skin contact or inhalation are the main hazards for workers. Wind drift during spraying may be a health risk in a number of cases (12). Therefore inhibition of RBC or serum cholinesterase activity is a biomarkers of organophosphorus and carbamate pesticide exposure (13-17). Tolerance to the cholinergic over stimulation may be observed following repeated exposure to cholinesterase inhibiting chemicals. But the cellular mechanisms responsible for the development of tolerance may also lead to the development of other effects i.e. cognitive dysfunction not present at the time of initial exposure (18). Generally the acute cholinergic effects of anticholinesterase compounds are viewed as reversible (14), although longer lasting effects have been reported in animals (19). There is no experimental evidence for lasting or persistent effects of repeated exposure to organophosphate at levels that are not actually toxic (20). In this study, it was observed that the serum total protein and albumin levels were significantly reduced in exposed group as compared to control ($p < 0.001$) (Table 2). But the mean values of serum total protein and albumin in exposed group were on the lower side of normal range. Due to prolonged exposure of OP pesticides, the serum proteins and albumin were significantly reduced in experimental female rats as reported in several studies (21-23).

From the earlier reports and present results, it is tempting to speculate that decreased serum total protein is due to lowered synthesis of albumin in liver and a rise in globulins (mostly α -globulins) is due to prolonged exposures of OP pesticides (24). Serum AST and ALT were increased in the exposed

group as compared to control (Table 1). Several studies were reported that the activities of these enzymes were significantly increased in experimental rats when fed with organophosphorus pesticide and Endrin (17, 22, 25,26).

In this study, the hematological parameters like Hb, Hct, RBC were significantly decreased in exposed group as compared to control (Table 4).

This result is consistent with the result reported in literature. The effect of Hb on humans exposed to organophosphorus pesticides has been observed by several workers. (2, 27-29). The decrease in the Hb alongwith the decrease in the RBC might be due to the effect of pesticides on blood forming organ in rats. Many steps in heme biosynthesis are inhibited by pesticidal residues and this might be a possible physiological reason for the inverse proportion of the result obtained. The poisoning by pesticide residues is the development of anemia due to interference of Hb biosynthesis and shortening of the life span of circulating erythrocytes (29). Hemoglobin, hematocrit and RBC were slightly reduced in mild and high dose males and moderately reduced in high dose female guinea pigs (21, 23, 30).

Serum lipid peroxidation was estimated in the form of thiobarbituric acid reactive substances (TBARS) produced. Significant increase in TBARS was observed in exposed population when compared with control subjects (Table 3). Increased formation of peroxide (TBARS) observed in the sprayer population could be due to the increased peroxidation of membrane. Literature shows that the basic process by which any and related radicals such as superoxide and hydroxy radicals cause membrane

damages in lipid peroxidation (LPO). LPO may be initiated by superoxide hydroxy radicals and also by free radical intermediates. LPO produces disintegration of biomembrane and subcellular organelles. The microsomal membranes rich in PUFA are susceptible to oxidation (31). But in experimental animals, it was reported that the OP, carbamate and Endrin increased lipid peroxidation in both liver and kidney (32).

In conclusion, workers exposed to pesticides spread in grape gardens show toxic response, hence it is compulsory to use protective gear such as gloves, face shields, aprons, shoes. And supplementation of antioxidants viz Vit. C, Vit E in the food of grape garden workers to relieve from toxic effect.

Further research in this regard is in progress.

ABBREVATIONS :

TBARS	—	Thiobarbituric acid reactive substances
OP	—	Organophosphorus
Hct	—	Hematocrit
RBC	—	Red Blood Cell
Hb	—	Hemoglobin
AST	—	Aspartate aminotransferase
ALT	—	Alanine aminotransferase
EDTA	—	Ethylene di-amine tetra acetic acid
AchE	—	Acetyl cholinesterase
LPO	—	Lipid peroxide

Table 1

Values of serum AST, ALT and AchE in exposed and unexposed population. (Mean $\pm$ S.D.)

Sr. No.	Group	No. of cases	AST (U/L)	ALT (U/L)	AchE (U/L)
1.	Control	75	15.25 $\pm$ 6.5	16.53 $\pm$ 5.4	2764 $\pm$ 209
2.	Exposed	85	19.44 $\pm$ 6.3*	21.20 $\pm$ 5.7*	2241 $\pm$ 252*
			p< 0.001	p<0.001	p<0.001

* Values are statistically significant when compared to control. By Student's t-test.

Table 2

Values of serum total protein, albumin, globulin and A:G ratio in exposed and unexposed population. (Mean $\pm$ S.D.)

Sr. No.	Group	Number of cases	Serum total protein (gm %)	Albumin (gm %)	Globulin (gm %)	A : G	p
1.	Control	75	7.03 $\pm$ 0.67	4.80 $\pm$ 0.64	2.2 $\pm$ 0.5	2.3 $\pm$ 0.8	p < 0.001
2.	Exposed	85	6.53 $\pm$ 0.56*	3.90 $\pm$ 0.33*	2.6 $\pm$ 0.6*	1.6 $\pm$ 0.4*	

*Values are statistically significant when compared to control. By Student's t-test.

Table 3

Serum lipid peroxidation levels in exposed and unexposed population. (Mean $\pm$ S.D.)

Sr. No.	Group	No. of cases	Lipid peroxidation (nmols of MDA/ ml.)	p
1.	Control	40	11.40 $\pm$ 3.1	p < 0.001
2.	Exposed	50	14.22 $\pm$ 3.7*	

*Values are statistically significant when compared to control. By Student's t-test.

Table 4

Values of Hb, Hct, RBC and MCHC in exposed and unexposed population. (Mean $\pm$ S.D.)

Sr. No.	Group	No. of cases	Hb (gm%)	Hct (%)	MCHC (gm%)	RBC million/ μ l.
1.	Control	40	13.84 $\pm$ 1.5	42.37 $\pm$ 3.4	32.34 $\pm$ 1.9	5.26 $\pm$ 0.65
2.	Exposed	50	12.47 $\pm$ 1.7*	39.56 $\pm$ 3.6*	33.49 $\pm$ 1.7**	4.56 $\pm$ 0.54*

Values are statistically significant when compared to control. By Student's t-test.

* p < 0.001, ** p < 0.005

REFERENCES

1. Dave S. K. (1998) Occupational health services for Agriculture Workers. *Indian J. Occup. Environ. Med.* 2, 96.
2. Wolfe, H.R., Durham, W.F. and Armstrong, J.F. (1967) Exposure of workers to pesticides. *Arch. Environ. Health.* 14, 622-633.
3. MacCollom, G.B., Currier, W.W. and Baumann, G.L. (1986) Drift comparisons between aerial and ground orchard application. *J. Econ. Entomol.* 79, 459-464.
4. World health organisation (WHO) (1992) Environmental health criteria no.130. Endrin. Geneva, World Health Organisation.
5. World Health Organisation (WHO) (1993) Environmental health criteria no. 145. Methyl Parathion. Geneva, World Health Organisation.
6. World Health Organisation (WHO) (1994) Environmental health criteria no.153. Carbaryl. Geneva, World Health Organisation.
7. Feng N. Ko, George Hsiao and Yueh H. Kuo (1997) Protection of oxidative hemolysis by demethyldiisoeugenol in normal and thalassemia red blood cells *Free Radical Biol. Med.* 22, 217.
8. Aldridge W.N. (1971) The nature of the reaction of OP compounds and Carbamates with esterases. *Bull. World Health Organisation* 44, 25.
9. Costa, L.G. (1988) Interaction of neurotoxicants with neurotransmitters system. *Toxicology* 49, 359-366.
10. World health organisation (WHO) (1986) Environmental health criteria no.63. Organophosphorous insecticides a general introduction, World Health Organisation, Geneva, p-181.
11. Avashia B. H. (1987) Is Carbaryl safe as it's reputation, Letter to the Editor *Am. J. Med.* 83, 1168-1169.
12. Kummer, R. and Van Sittert, N.J. (1986). Field studies on health effects from the application of two OP insecticide formulation by hand-held ULV to cotton. *Toxicol. Lett.* 33, 7-24.
13. ECETOC (1992) Evaluation of the neurotoxic potential of chemicals, Brussels, European Centre for Ecotoxicology and toxicology of chemicals (Monograph No.18).
14. ECETOC (1998) OP pesticides and long term effects on the nervous system. Brussels, European Centre for Ecotoxicology and Toxicology of chemicals. (Technical Report No. 75).
15. Maxwell, D.M., Lenz, D.E., Croff, W.A., Kaminskis, A. and Froehlich H.L. (1987) The effects of blood flow & detoxification on *in vivo* cholinesterase inhibition by soman in rats. *Toxicol. Appl. Pharmacol.* 88, 66-76.
16. Padilla, S., Wilson, V.Z. and Bushnell, P.J. (1994). Studies on the correlation between blood cholinesterase inhibition and tissue inhibition in pesticide treated rats. *Toxicology* 92, 11-25.
17. Meena, K., Gupta, P.K. and Bawa, S.R. (1978) Endrin – induced toxicity in normal and irradiated rats. *Environ. Res.* 16, 373-382.
18. Bushnell, P., Padilla, S., Ward, T., Pope, C. and Oiszyk, V. (1991) Behavioral and neurochemical changes in rats dose repeated with diisopropyl fluorophosphate (DEP). *J. Pharmacol. Exp. Ther.* 256, 741-750.
19. Tendon, P., Padilla, S., Pope, C.N. and Tilson, H.A. (1994) Fenthion produces a persistent decrease in muscarinic receptor function in the adult rat retina. *Toxicol. Appl. Pharmacol.* 125, 271-280.
20. Ray D.E. (1999) Chronic effects of low level exposure to anticholinesterases a mechanistic review. *Toxicol. Lett.* 103, 527-533.
21. Bomhard, E., Loser, E., and Schilde, B. (1981), [E605– Methyl] (Methyl parathion) chronic toxicological studies in rats (2 year feeding trial) Wuppertal – Elberfeld Bayer AG, Institute of toxicology IPCS -145, 155.
22. Kagan, J. (1971) Topical questions about the toxicology of phosphorganic insecticides, *Ernährungsforschung* 14, 503-514 (in German).

23. Schilde, B. and Bomhard, E. (1984) (E 605-methyl (Methyl parathion) supplementary histopathological test further to the 2 year feeding trials on rats Addendum to report No. 9889). Wuppertal-Elberfeld, Bayer A. G. Ins. of toxicology. (unpublished report No. 12559 submitted to WHO by Bayer AG. Leverkusen Germany).
24. Subbotina, S.G., and Belonozhko, G.A. (1968). (The effect of Sevin on the thermoresistance and fractionary protein content of blood serum) Gig. Primen Toxicol. Pestic. Klin. Otrav. 442-444 .
25. Illahi, A., Amin, N., Hashmi, A.S., Nawaz, M. and Naeemur-Rahman (1986) Incidence of endrin residues in Cucumber and it's effect on biological system. J. Pak. Med. Asso. 36, 209-211.
26. Luckens, M.M., and Phelps KI (1969) Serum enzyme patterns in acute poisoning with organochlorine insecticides. J. Pharm. Sci. 58, 569-572.
27. Bhatnagar (1980) Effect of pesticide stress among pesticide factory workers in Delhi. Pub. HIM. 74, 380.
28. Desai I.G. (1986) Biological monitoring of group of green house pesticide sprayers. Toxicol. Lett. 33, 91-101 .
29. Ray (1992) Pollution and Health, Wiley Eastern Ltd., New Delhi (1992).
30. DaLy, I.W.A. (1983) Evalution report for US-EPA of a two year chronic feeding study of methyl parathion in rats. East Millstone, New Jersey, Biodynamics Inc. (Unpublished report, submitted to WHO by Bayer A.G. Leverkusen, Germany.)
31. Freeman B. A. and Capo J. D. (1982) Biology of disease, free radicals and tissue injury. Lab. Invest. 47, 412.
32. Pawar S.S. and Kachole M.S. (1978) Hepatic and renal microsomal electron transport reactions in endrin treated female guinea Pig. Bull. Environ. Contam. Toxicol. 20, 199-205.