Biochemical Indices of Sprayers Exposed to Organophosphorous Pesticides in the Local Area NUZVID

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Abstract:
Chronic and acute toxicity of OP pesticides exposure in human being habituated with smoking and also non – smoking resulted not only hepatocellular damage, lipid peroxidation and due to oxidative stress. Significantly effected in the biochemical profiles of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), malonylaldehyde (MDA), when a comparison is made to controls. Increased levels were observed in the above enzymes and a decrease in glutathione (GSH). These people are the labour engaged in pesticide spraying and also owner farmers as sprayers. The effects are significant and serve as a measure of biochemical parameters as indices.

Key words: *acute exposure group, chronic exposure group, op pesticide, non smokers and smokers sprayers.*

INTRODUCTION

Pesticides are ubiquitous, capable of killing all forms of life. They are some of the deadliest; poisons produced by man, hence present a health hazards in long term exposure even at low levels. Organophosphates (OP) pesticides are commonly used worldwide in agricultural and in pest control. The worldwide use of different groups of pesticides leads to global cross-contamination and unintentional exposure of humans. The majority of people are continually exposed to low OP concentrations, and long-term exposures link to changes in biochemical profile of some enzymes (Brown et al., 1990; Waddell et al., 2001).

REVIEW OF LITERATURE

The route of entry is transdermal, conjunctival, gastrointestinal, genitourinary mucosal membranes. After absorption, they get accumulated in liver, kidney, adipose tissue etc. These compounds are lipophilic, so that they can cross the Blood Brain Barrier (Vale, 1998).The toxicity of organophosphorous compounds is mediated by generation of free radicals which may alter the liver metabolism and will be evidenced by changes in the levels of liver enzymes, and lipid peroxidation. The primary indicators for liver function are **Aspartate Amino Transferase (**AST), **Alanine Aminotransferase (**ALT), and **Alkaline Phosphatase** (ALP). Lipid peroxidation (LPO) is an indicator of oxidative stress in cells. OP poisoning may induce oxidative stress leading to generation of free radicals and are thus formed increases oxidative destruction of lipids (lipid peroxidation), is a destructive self processed chain of reaction, releasing malonylaldehyde (MDA) as the end product, (Cheeseman (1993). The glutathione (GSH) belonging to the second line of defense enzyme systems as an antioxidant in living cell detoxifies to protect the cells from the reaction oxygen species ROS such as free radicals and peroxides.

MATERIALS AND METHODS

The present study was carried out to determine the impact, management and outcomes of pesticide poisoning and the key dysfunction following OP exposure is throughout the body and the biochemical enzymes analysed are: AST, ALT, ALP, MDA and GSH. The agricultural workers in the present study are in the age group between 25–45 years having various exposure periods which may range between minimum 1 to a maximum of more than 10 years. According to the duration of exposure to organophosphorous pesticides, the agricultural workers i.e., the study group is divided into two groups on the basis of their exposure as Group I the workers having acute exposure (<1 year) and Group II will include having prolonged chronic exposure (>10 years). The study group is also divided into three groups according to the type of organophosphorous pesticide (OP) sprayed. The pesticide applicators spraying only methylated OP pesticides are Group I, spraying only ethylated OP pesticides, Group II and both methylated and ethylated OP pesticide applicators spraying as Group III. Blood samples were collected from sprayers among the above three groups and controls were maintained. The blood was immediately centrifuged at 3000 rpm for 15 minute and the plasma was also separated. The cells were washed with normal saline and RBC's were subjected to lysis.

The activity of alkaline phosphatase was determined by the method described by Reitman and Frankel (1957). AST effects the conversion of alpha, keto-glutarate and aspartate to glutamate and oxaloacetate respectively, by the transfer of amino group. Thus the Oxaloacetate formed is coupled with 2, 4-dinitrophenylhydrazine to produce a coloured

complex; the absorbance in alkaline solution is measured at 505 nm. The activity of alkaline phosphatase was determined by the method described by Reitman and Frankel (1957). ALT effects the conversion of alpha- ketoglutarate and alanine to pyruvate. Pyruvate formed is coupled with 2, 4-dinitrophenylhydrazine to produce a coloured complex. The absorbance in alkaline solution is measured at 505 nm. The activity of alkaline phosphatase was determined by the method described by Mod.Kind and King's (1954) with some modifications by Varley (1975). 4- Nitro phenyl phosphate is hydrolysed by alkaline Phosphatase at pH 10.3 at 37^0C and 4- Nitro phenol is liberated. Alkali is added to stop the enzyme activity at the end of the timed incubation period and the increase in absorbance is due to the 4- Nitro phenol released is measured at 410 nm. Malondialdehyde (MDA) was estimated in the blood by method Stocks et al (1971). 0.5 mL of blood in phosphate buffer (pH 7.4, 100 mM) was incubated for 30 min at 37°C and centrifuged. To the supernatant (3 mL) 1 mL 1% TBA was added and kept in boiling water bath for 15 min. Contents were cooled in ice water and centrifuged for 15 min at 2500 rpm. The absorbance was taken at 532 nm and converted to equivalent of MDA (nmol/mL blood) using molar extinction coefficient of $1.56x10^5$ mol L^{-1} cm^{-1}. The level of GSH was estimated in the blood by Tietz method (1969). 0.5 mL of blood was mixed with 1.5 mL of water, 2 mL of 10% TCA and centrifuged at 2000 rpm for 15 min. To the supernatant (1 mL), 4 mL 0.1 M phosphate buffer (pH 7.4) and 0.1 mL of 0.4% DTNB in phosphate buffer was added and the color was read at 412 nm.

RESULTS

The mean values of various biochemical enzymes in the controls and Sprayers were presented in table 1 and also depicted as figure 1 AST, ALT, ALP, MDA increased significantly in the exposed group as compared to the level measured in the control group 69.31±20.10 > 24.52±12.11; 29.69±12.20 >21.40±12.61; 18.61±4.21 > 9.67±3.01; 42.2±6.81> 17.93±2.61. While the GSH levels were decreased significantly in the exposed group as compared to the level measured in the control group shown in table 1and as figure 1. The mean values of the enzymes of biochemical profile in relation to smoking status as smokers and non smokers among exposed group and control group were presented in table 2 and also figure 2. Significantly increased levels of AST, ALT, ALP, MDA observed among the Smokers of exposed group than Smokers of control group were77.29±18.10 > 27.21±11.10; 58.61±13.11 > 24.11±10.67; 21.67±3.71 >13.15±4.01; 44.67±3.17 > 21.29±2.83 in contrast to their non-smoking counterparts. While the GSH levels were decreased significantly in the smokers of exposed group as compared to the level measured in the smokers of control group shown in table 2 and as figure 2.The mean values of AST, ALT, ALP and MDA were significantly increased in the workers belonging to the chronic exposure group as compared to acute exposure group in table 3 and also figure 3 were 63.72±20.21 > 55.25±18.15; 59.51±14.20 > 47.15±13.57; 24.21±4.30 > 18.13±5.10; 47.95±2.83 > 31.67±3.07. While the GSH levels were decreased significantly in the chronic exposed group as compared to the level measured in the acute exposed group shown in table 3 and as figure 3.The mean values of the enzymes measured in the three sub-sets of the exposed group were presented in table 4 and figure 4. The workers involved in spraying the mixtures of methylated and ethylated OP pesticides (group–III) showed significantly elevated values of AST, ALT, ALP, MDA in comparison to the values obtained in (methylated) group–I and (ethylated) group –II were 67.21±16.51>61.57±18.35>52.93±16.21; 47.61±18.21>26.11±17.21<31.57±18.11; 29.67±3.11>17.88±4.01<21.27±3.71; 47.61±3.02 > 37.95±3.11 >34.39±3.40. GSH levels were decreased significantly in comparison to the levels of group III < group II < group I shown in table 4 and as figure 4.

Table-1 Biochemical profile in Controls and Pesticide Sprayers

Liver Functions	Controls (n=50)	Sprayers (n=125)
AST	24.52±12.11	69.31±20.10
ALT	21.40±12.61	29.69±12.20
ALP	9.67±3.01	18.61±4.21
MDA	17.93±2.61	42.2±6.81
GSH	38.73±3.45	23.1±3.44

Mean ± SD, n=50, n=125; p<0.001. Standard Deviation is indicated as (±);
Values are statistically significant when compared between two exposure groups

Figure- 1 Biochemical profile in Controls and Pesticide Sprayers

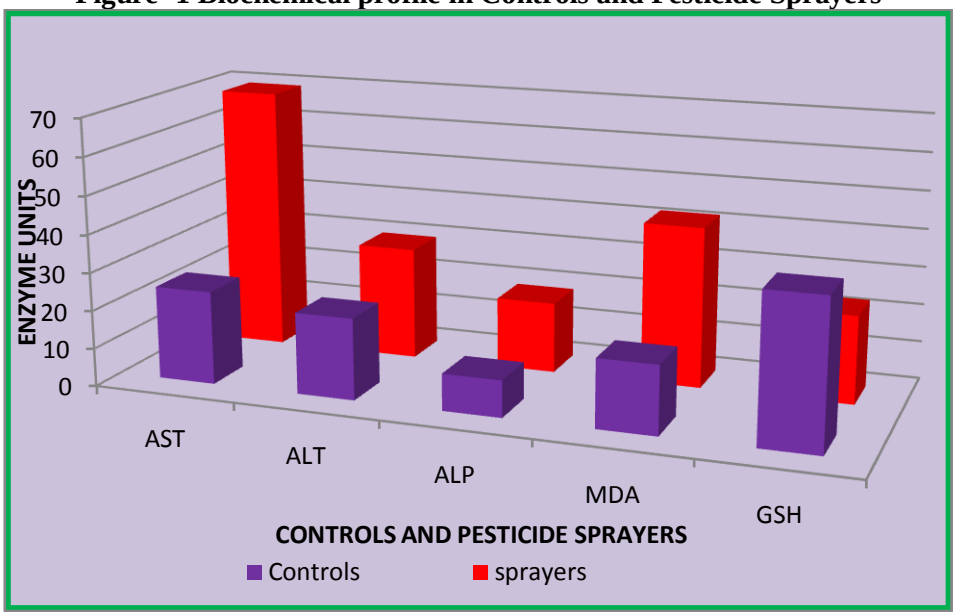


Table-2 Biochemical profile in relation to smoking status

	Controls		Sprayers	
	Non-smokers	Smokers	Non-Smokers	Smokers
AST	18.65±10.23	27.21±11.10	61.57±14.20	77.29±18.10
ALT	16.89±12.11	24.11±10.67	48.65±16.10	58.61±13.11
ALP	7.71±4.11	13.15±4.01	16.21±4.31	21.67±3.71
MDA	16.88±1.95	21.29±2.83	35.77±4.12**	44.67±3.17**
GSH	37.69±1.85	31.73±2.61	26.85±1.71	24.35±2.40

Mean ± SD, n=50, n=125; **p<0.001. Standard Deviation is indicated as (±);
Values are statistically significant when compared between the two exposure groups.

Figure-2 Biochemical profile in relation to smoking status

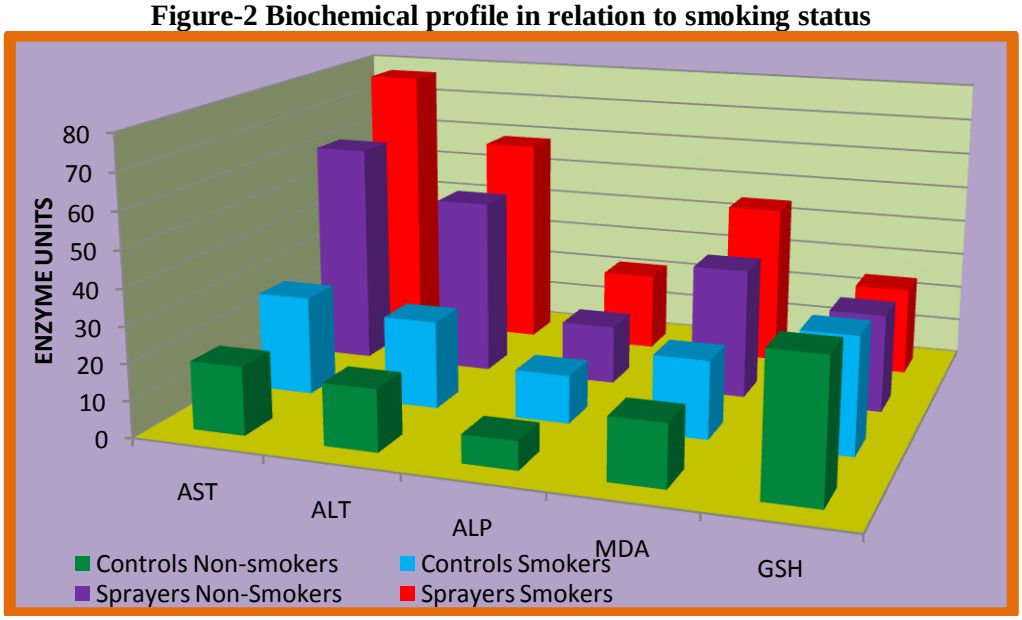


Table-3 Biochemical profile in relation to duration of exposure to pesticides

	Acute Exposure (< 1 Year) (n=75)	Chronic Exposure (> 10 Years) (n=50)
AST	55.25±18.15	63.72±20.21
ALT	47.15±13.57	59.51±14.20
ALP	18.13±5.10	24.21±4.30
MDA	31.67±3.07	47.95±2.83*
GSH	27.71±1.39	21.50±1.40

Mean ± SD, n=50, n=125; *p<0.005; Standard Deviation is indicated as (±);
Values are statistically significant when compared between the two exposure groups.

Figure-3 Biochemical profile in relation to duration of exposure to pesticides

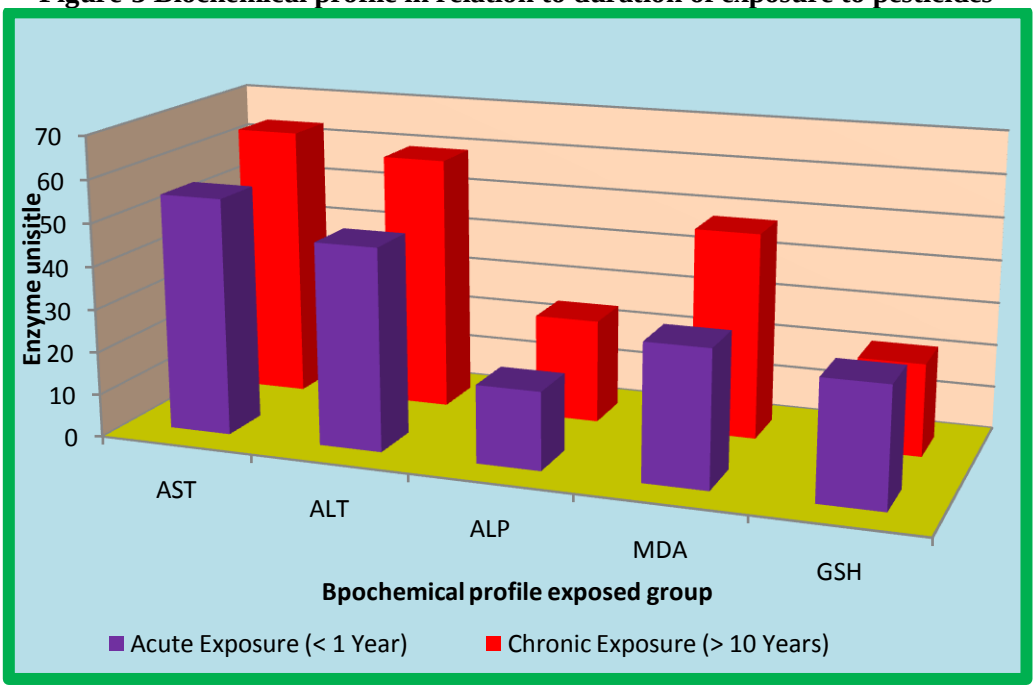
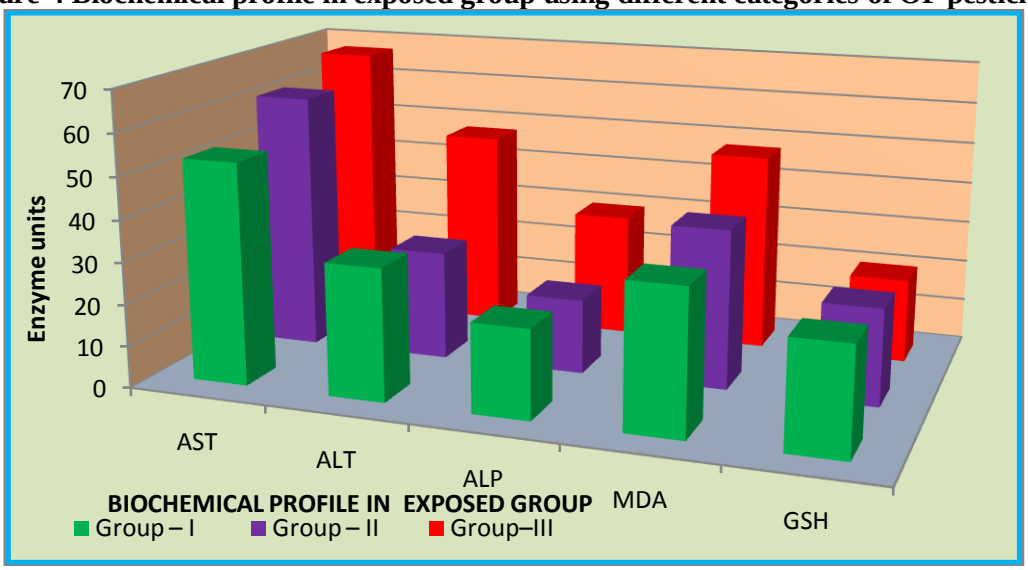


Table-4 Biochemical profile in exposed group using different categories of OP pesticides

	Group – I (n=39)	Group – II (n=41)	Group–III (n=45)
AST	52.93±16.21	61.57±18.35	67.21±16.51*
ALT	31.57±18.11	26.11±17.21	47.61±18.21*
ALP	21.27±3.71	17.88±4.01	29.67±3.11*
MDA	34.39±3.40	37.95±3.11	47.61±3.02*
GSH	25.65±1.37	23.11±1.21	20.17±1.11

Values are mean ± SD; *p<0.05; Standard Deviation is indicated as (±);
Values are statistically significant when compared between the two exposure groups.

Figure-4 Biochemical profile in exposed group using different categories of OP pesticides



DISCUSSION

OP pesticide poisoning is primarily a problem of developing countries like India. In the present study biochemical parameters studied in pesticide sprayers and in controls included are Aspartate Amino Transferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Malondialdehyde (MDA) levels were significantly elevated in Table 1, 2, 3, 4 and as Figure 1, 2, 3, 4. Glutathione (GSH) showed decrement was studied in Table 1, 2, 3, 4 and as Figure 1, 2, 3, 4. Significantly elevated levels observed in the exposed workers when compared to the controls. Both the smokers and non- smokers in the exposed group showed increased levels of liver enzymes as liver is the main organ involved in detoxification process and hence alters the metabolism of the liver. Higher values of transaminases are seen in the present study are due to the chronic exposure group which indicates that longer time period of pesticide exposure and in the mixed OP pesticide exposed individuals than in single type of either methylated or ethylated type of pesticides. ALT is a more specific indicator of liver inflammation, and may be elevated also in diseases affecting other organs, such as myocardial infarction, acute pancreatitis, acute hemolytic anemia, severe burns, acute renal disease, musculoskeletal diseases, and trauma. MDA is one of the major oxidation products of peroxidized polyunsaturated fatty acids and increased MDA content is an important indicator of lipid peroxidation. Glutathione has an ability to promote detoxification, resist proteolysis and serves as an antioxidant. Glutathione exists either in the reduced, antioxidant form designated as GSH; or the oxidized, antioxidant-spent form, usually designated GS-SG in the aqueous phase of cellular systems. The aminotransferases, ALP levels are found to be elevated in exposure group are correlated with the studies of Yaqub Surajudeen et al., (2014); Quazi et al, (2012); Saxena and Garg (2010); Simoniello et al, (2010); Manel Araoud et al, (2010); Ismail and Rohlman et al, (2010); Hariprasad et al, (2009); Durak et al, (2008); Bhalli et al, (2006); Hernandez et al, (2006); Zama et al, (2005); Patil et al, (2003); Vidyasagar et al, (2003); Altuntas et al, (2003); Catagol et al, (2003); Prakasam et al, (2001); Gultekin et al, (2000).

CONCLUSION

Sprayers in agricultural practices exposed to pesticides must be educated to take safety precautions while spraying in the fields. Immediate medical checkups can maintain the biochemical indices, ignorance can't be bliss.

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