Subash Vijaya Kumar. *et al.* / *International Journal of Pharmacy & Therapeutics*, 2(2), 2011, 68-73.

ISSN 0976-0342
2229-7456

International Journal of Pharmacy & Therapeutics

Journal homepage: www.ijptjournal.com

IJPT

EFFECTS OF ORGANOPHOSPHORUS POISONING ON DIFFERENT SYSTEMS OF THE BODY

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ABSTRACT

Organophosphate (OP) compounds have been widely used for a few decades in agriculture for crop protection and pest control. Thousands of these compounds have been screened and over one hundred of them have been marketed for these purposes. The present study is focused on the effects of OP poisoning on the body systems, cholinesterase, antioxidants and lipid peroxides. The study was conducted at the emergency care department of a tertiary care teaching hospital, which is a 1000 bedded multidisciplinary super specialities government hospital. The study was carried out for the period of five months. The patients included in the study were those who had ingested OP compound available as household or agricultural pesticide. Among 25 patients, 19 were males. The mean age was 32.16±2.67 years with a range of 18-60 years. A control group consisting of 25 unexposed pesticides, were age-matched, who never had any exposure to OP pesticides. The most predominant age group was 18-30 years age group and most commonly involved OP compound was Monocrotophos. Acetyl cholinesterase levels were found to be 99.99±5.13 U/ml, which is significantly lower (P = 0.0001) than healthy volunteers. In addition to that, MDA levels was found to be 23.52±3.15 nM/ml, which is significantly (P = 0.0476) increased compared to healthy subjects. Whereas, glutathione levels were significantly lower (P = 0.0223) and similarly, total antioxidant level in these patients were found to be significantly lower (P = 0.0305) compared to healthy subjects. Our results elaborate the significance of oxidative stress in organophosphorus poisoning and emphasis clinicians to prescribed antioxidants. However further pharmacological and histopathological studies will be necessary.

Key words- Acetyl cholinesterase, Antioxidant, Organophosphorus, Atropine, Glutathione.

INTRODUCTION

Organophosphorus (OP) compounds have been widely used for a few decades in agriculture for crop protection and pest control, thousands of these compounds have been screened and over one hundred of them have been marketed for these purposes (Vijayakumar S *et al*., 2010) Some have also been used in the medical treatment of myasthenia gravis, e.g. diisopropyl phosphorofluoridate (DFP) (Comroe JH *et al*., 1946). They are also been used as plasticizers, stabilizers in lubricating and hydraulic oils, flame retardants, and gasoline additives (De bleaker JL *et al*., 1992). These compounds inhibit the cholinesterase enzyme which leads to the accumulation of acetylcholine at synapses, causing over stimulation and subsequent disruption of transmission in both the central and peripheral nervous systems. These OP compounds induce many changes in the physiology of the organs. It is reported that OP compounds, besides their inhibitory effect on acetyl cholinesterase (AChE), also induce changes characteristic of oxidative stress. Superoxide dismutase (SOD), whose substrate is a free radical (superoxide anion; O_2•-) catalyzes dismutation reaction resulting in the generation of hydrogen peroxide (H_2O_2). This H_2O_2 is decomposed to water and molecular oxygen by the action of catalase. When the free radical production overwhelms the endogenous antioxidant levels, they cause considerable cell damage/death. All the major biomolecules like lipids, proteins, and nucleic acids may be attacked by free radicals, but lipids are probably the most susceptible. The oxidative destruction of lipids (lipid peroxidation) is a destructive, self-perpetuating chain reaction, releasing malondialdehyde (MDA), as the end product (Vidya sagar J *et al*., 2004). So, the OP poisoning is associated with enhanced lipid per oxidation, reduced Glutathione levels and elevated antioxidant status and

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increased oxidative stress. The present study is focused on the effects of OP poisoning on body systems, cholinesterase, antioxidants and lipid peroxides.

MATERIALS AND METHODS

The present study was conducted at the Emergency Departments of a tertiary care teaching hospital, i.e., Mahatma Gandhi Memorial Hospital, Warangal, Andhra Pradesh, India, which is 1000 bedded multidisciplinary super specialties government hospital. The study was carried out for the period of five months. The patients included in the study were who had undergone exposure to organophosphorus poison either by household or agricultural pesticides.

In addition to that emergency department serves residents up to a 6 mile west of the middle town area. Sample / Data collection was performed according to hospital regulations after approval by the Hospital administration. The setting was the emergency department of an inner city level trauma centre with approximately 85,000 patient visits per year. Patients between 1 and 90 years old and exposed to OP poisons were selected. The study population consisted of 25 OP poisoning cases admitted between the months of June 2010 to October 2010. A control group consisting of 25 unexposed pesticides, who never had any exposure to OP pesticides was taken as a reference group.

All subjects diagnosed as case of OP poisoning, on the basis of history of the victim or the attendant along with the clinical features like miosis, increased salivation, increased respiratory secretions, muscle cramps and abdominal discomfort were included in our study. All subjects attendee completed a detailed standardized questioners especially aimed the time and the quantity of consumption of the OP compounds. The victims were also sorted for different epidemiological factors like age, gender, marital status, socio-economic status, representative area (rural / urban) and the mode of intake (suicidal / homicidal / accidental). All patients were observed in the emergency department of our hospital by specialists in emergency medicine. The treatment included specific antidotes like oxime (pralidoxime) and Muscarinic receptor antagonist (atropine) and supportive treatment like IV fluids and an antibiotic as prophylaxis.

The outcome was compared with severity and time lapse between ingestion of compound and initiation of the therapy. If patients needed advanced treatment they were sent either to other service or the ICU in our hospital, like RICU (Respiratory Intensive Care Unit) where emergency care observations and mechanical ventilator supports are available at bedside. The Protocol of the study was submitted to the Superintendent of our hospital and to Kakatiya Medical College to obtain the Ethical Committee approval. The study began after the approval was granted.

The literature supporting the study was collected and analyzed. The different sources used to collect the literature were Micromedex drug information databases, various websites like pub med, science direct, DOAJ, Medline, etc.

Venous blood samples were collected from the patients after obtaining Informed consent from the patient or the attendee. The samples were collected in 5 ml heparinised vials (for plasma) and 5ml plain tubes (for serum). The samples were immediately centrifuged at 3000 rpm for 30 minutes and supernatant layer separated in labelled eppendroff's tubes and kept at 40ºC till biochemical analysis.

At the baseline all the patients had a complete history and physical examination. Pulse rate, blood pressure and ECG recordings were taken on arrival in the Emergency department. ECG analysis included the calculations of PR interval, QRS duration, RR interval, QT and QTc interval. QT interval was measured from the first deflection of QRS complex to the point of T wave offset, defined as the return of the T wave to the baseline of the ECG. The QTc interval was calculated using Bazett's formula ludomirsky A *et al*., (1983). QTc was considered to be prolonged when it was longer than 0.41 sec for males and 0.42 sec for females.

Estimating the Parameters

1. Acetyl cholinesterase (AChE)

The AChE levels were determined in plasma. AChE (true and pseudo) estimation was done by the colorimetric method using acetylcholine chloride as substrate. Both true and pseudo AChE would hydrolyze the substrate and produce choline and acetic acid. The change in the color of the indicator, bromothymol blue, caused by the liberated acetic acid from ACh was read by a spectrophotometer (Vidyasagar J *et al*., 2004).

Procedure: Bromothymol blue (Nice Chemicals), 0.5 ml solution was diluted with 3.8 ml of distilled water, and 0.2 ml of 15% acetylcholine chloride (Qualikems Fine Chemicals) was added. To it 100 µl of the plasma was added and the change in the color was read at 620 nm at 37ºC, after 30 minutes. A standard graph was plotted by using acetic acid 0.015N in concentrations of 10, 20, 50, 100 and 200 micromoles. The unit of AChE activity was defined as the micromoles of acetic acid liberated from 1 ml of plasma in 30 minutes at 37ºC.

2. Liver function tests - SGOT, SGPT and total bilirubin were estimated using the kits (Coral clinical systems; Ensure biotech ltd) with colorimetric methodology.

3. Kidney function tests - serum creatinine and blood urea were estimated using the kits (Excel diagnostics ltd) with colorimetric methodology.

4. Oxidative stress
Estimation of lipid peroxides
The amount of lipid per oxidation products present in the serum samples were estimated by the Thiobarbituric acid reactive substances (TBARS) method (Placer ZA *et al*., 1966).

Procedure: To 0.5 ml of plasma/serum 0.5 ml of 30% Trichloro acetic acid (TCA) was added to precipitate the proteins and vortexed for 30 sec. Clear supernatant was taken after centrifuge at 3000 rpm for 10 minutes. To the supernatant 100µl of 1%TBA solution was added and the solution was heated for 1hr at 98ºC. It was kept in ice for 10-15 minutes. Then the supernatant was collected. The absorbance of mixture which was in pink in color was read at 532 nm. The MDA content was determined from standard graph by using standard 1, 1, 3, 3 – Tetra ethoxy propane (Sigma Aldrich) in different concentrations.

5. Antioxidant status
Glutathione
Glutathione forms a coloured complex with DTNB, which is measured spectrophotometrically (Ellman GL *et al*., 1959; Beutler E *et al*., 1963).

Procedure: To 0.5 ml of citrated blood, 0.5 ml of 5% trichloroacetic acid (TCA) solution was added to precipitate the proteins and centrifuged at 3000 rpm for 20 minutes. To 0.1 ml of supernatant, 1 ml of sodium phosphate buffer and 0.5 ml of DTNB (Himedia Labs) reagent were added. The absorbance of the yellow colour developed was measured at 412 nm. The glutathione content was determined from standard graph by using pure glutathione (Himedia Labs).

Estimation of Total Antioxidant levels
For the estimation of total antioxidant status, we used a stable free radical 2, 2 – diphenyl – picryl hydrazyl (DPPH – Sigma Aldrich) at the concentration of 0.2mM in methanol (Blois *et al*., 1958; Kalpana T *et al*., 2001).
Procedure: 0.1ml of plasma was deproteinted by the addition of 1ml of methanol, vortexed for 30sec. Then centrifuge at 3000 rpm for 30 minutes to separate the proteins. To the clear supernatant 1.5ml of methanol and 0.5ml of DPPH solution were added, mixed thoroughly and absorbance was read at 517nm against blank, prepared in an identical way but without the addition of serum / plasma. Ascorbic acid (Finar Chemicals) was used as a reference standard. The standard graph was plotted using different concentrations of ascorbic acid and the antioxidant status values were expressed in terms of nM of ascorbic acid.

Statistical Analysis: The data were analyzed with unpaired t test, using the software Graph pad prism version-5. P values < 0.05 were considered significant.

RESULTS

Among 25 patients 19 were males and 6 females. The mean age was 32.16 ± 2.673 years with a range of 18 to 60 years. There was no significant difference in the mean age between genders. Majority (60%) of the patients were in the 18 – 30 age groups. The mean hospital stay was 7 days. Whereas, 1 patient was died because he was admitted to the hospital after 6 hours. However, during our study period Of 25 patients, there were 5 (20%) students, 7 (28%) farmers, 5 (20%) housewives and 8 (32%) service holders. A majority of patients had suicidal intention. In these context, 4 patients had a history of previous suicidal attempt. The patients admitted as early as 30 minutes to as long as 12 hours after ingestion of the poison with 90% of the patients admitted to hospital within 2 hours after ingestion, with the mean time interval of about 1 hour 10 min. The most commonly involved OP compound was Monocrotophos, which was implicated in 10 (40%) patients. Acetyl cholinesterase (AChE) levels were 99.99 ± 5.134 U/ml which is significantly lower (P<0.0001) compared with control (151.6±6.169). In addition to that, electro-cardiographical manifestations of acute OP poisoning found to be QTc prolongation, tachycardia, bradycardia (in few cases), ST-T changes (slight ST elevation & T wave depression). PR interval was found to be normal in our study. The liver and kidney function tests were found to be normal. The Mean ± SEM values of these parameters are given in Table 1.

Table 1. Summary of liver and kidney function tests of healthy volunteers vs patient sample

LFT	NORMAL	PATIENT
SGOT	20.96±0.938	15.68±1.512
SGPT	18.52±0.779	9.136±0.649
BILIRUBIN	0.617±0.041	0.65±0.068
SR.CREATININE	0.84±0.059	1.18±0.089
BLOOD UREA	16±1.44	25.04±2.961

The MDA level found was 23.52 ± 3.152 which is significantly (P = 0.0476) increased compared controls (16.44±1.480). The levels of glutathione were significantly lower (P = 0.0223) compared to controls (12.15±0.478). The Total Antioxidant levels in those patients were found to be significantly lower (P = 0.0305) compared controls (32.84±2.705).

DISCUSSION

Organophosphorus compound poisoning is a common, rapidly progressive and potentially fatal clinical entity. Self poisoning with organophosphorus compounds is a major health problem worldwide. Through the inhibition of acetyl cholinesterase, organophosphorus poisoning is characterized by clinical picture of acute cholinergic crisis.

Poisoning with organophosphorus compounds is the major clinical problem in the Warangal district with thousands of poisoning and hundreds of deaths every year. According to (Vijayakumar S *et al*., 2010), 2226 cases were admitted in our hospital in 2007 due to different types of poisonings, out of which 383 cases were of organophosphorus compound poisoning . In addition to that there was higher incidence of organophosphorus poisoning occurring in northern telangana region with a mortality rate of 10%.

The organophosphorus compounds besides AChE inhibition, lead to changes characteristic of oxidative stress Hai DQ *et al*., (1995). In humans, organophosphorus compounds were shown to reduce the total cholesterol and phospholipids level of RBC membrane following phosphamidon and malathion, and increase lipid peroxides level following malathion (John S *et al*., 2001). The basis of OP toxicity in the production of Oxygen free radical may be due to

a) Their "redox-cycling" activity - they readily accept an electron to form free radicals and then transfer them to oxygen to generate superoxide anions and hence hydrogen peroxide through dismutation reaction (Ryrfeldt A *et al*., 1992).

b) Generation of free radicals probably because of the alteration in the normal homeostasis of the body resulting in oxidative stress, if the requirement of continuous antioxidants is not maintained.

Organophosphorus poisoning compounds have been reported to induce production of reactive oxygen species and oxidative tissue damage. The efforts of the endogenous antioxidant enzymes to remove the continuously generated free radicals initially increase due to an induction but later enzyme depletion results, resulting in oxidative cell damage (Bagchi D *et al*., 1994). Hence, when the generation of reactive free radicals overwhelms the antioxidant defense, lipid peroxidation of the cell membrane occurs. This causes disturbances in cell integrity leading to cell damage/death. All major biomolecules like lipids, proteins and nucleic acids may be attacked by free radicals, but lipids are probably the most susceptible (Cheese man KH *et al*., 1922).

In the present investigation, a significant decrease in activity of acetyl cholinesterase and increase levels of MDA was found among patients as compared to the controls. These findings are in agreement with a study from the (Vidyasagar S *et al*., 2004; Rastogi SK *et al*., 2009) in which electrocardiographic findings are not reported by the researchers. (Vijaya kumar S *et al*., 2010) reported that of the OP patients have shown increased heart rate whereas, most of the patients showed ventricular arrhythmias, tachycardia & bradycardia and attributes of mild myocardial ischemia. In the present study the MDA (lipid peroxide) and Glutathione levels served as an index of oxidative stress and antioxidant status respectively. Total Antioxidant levels in serum were estimated and found to be significantly decreased ($P < 0.0001$) than that of controls. (Mogda KM *et al*., 2009), had reported that Profenofos (a persistent and toxic organophosphorus compound) treatment results in a significant increase in MDA concentration but a significant decrease in glutathione levels. (Guney M *et al*., 2007), had reported similar results. Similar findings have been observed in our study. Levels of MDA, a major oxidation product of peroxidized polyunsaturated fatty acids, have been considered as an important indicator of lipid per oxidation (Kalender S *et al*., 2004) was significantly increased, whereas, the glutathione level was found to be significantly decreased ($P < 0.0223$) compared to healthy volunteers. This might be due to its participation in the activation, inhibition and progression of lymphocyte and increased GST activity (Banerjee BD *et al*., 1999). Moreover, the decline in glutathione level observed could be attributed to the conjugation reactions (glutathione consumption) superseding the cell ability, to regenerate glutathione. Glutathione is the cell's natural antioxidant, which destroys free radicals formed in cells. Significant dose-dependent depletion of glutathione levels confirmed the potential of the organophosphorus compounds to induce oxidative stress (Rajeswary S *et al*., 2007).

We assessed the Liver enzymes SGOT and SGPT in organophosphorus poisoning patients, are well known diagnostic indicators of hepatic injury. Our study shows decrease in these levels and a slight increase in total bilirubin levels compared to healthy volunteers. Moreover, Our results showed significant increase in serum creatinine ($P = 0.0026$) and blood urea ($P = 0.0085$) levels when compared to normal healthy volunteers.

Organophosphate exposure produces clinical manifestations of cholinergic excess. Cardiac complications commonly occur in association with poisoning and are described primarily as a result of OP exposure (Kiss Z and Fazekas T, 1979). It is notable that 60% patients had QTc prolongation which has recently started to be considered as one of the targets of abnormalities in ECG because of prolonged QTc interval in the heterogeneous ion channel disease that can cause sudden death by lethal arrhythmia. The QT prolongation causes catastrophic polymorphic ventricular arrhythmia or ventricular fibrillation without affecting cardiac pump function. Adrenaline, premature complexes and QT/T-wave alternant are known to trigger these catastrophic cardiac mal-functions. Such lethal arrhythmia has

individual differences, and depends on the type of the ion channels with abnormality. The QT prolongation and repetitive ventricular tachyarrhythmia after OP poisoning was first described by (Luzhnikov EA *et al*., 1975) and precisely documented by (Ludomirsky A *et al*., 1982). Apart from QTc prolongation, we have also found non-specific ST-T changes like slight ST segment elevation and inverted T waves. Although the nonspecific ST-T change has generally been recognized as being not directly related to any cardiac diseases, it has been observed before starting the ST elevation caused by coronary spasm (Kumiko T*et al*., 2006).

Our results elaborate the significance of oxidative stress in organophosphorus poisoning and emphasis clinicians to prescribed antioxidants. This study needs to be validated with larger study involving greater number of subjects and possible use of antioxidant to prevent oxidative stress.

REFERENCES

Bagchi D, Bagchi M, Hassoum EA, Stohs SJ. In vitro and in vivo generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage of selected insecticides. *Toxicol*, 104, 1994, 129.

Banerjee BD, Seth V, Bhattacharya A, Pasha ST, Chakraborty AK. Biochemical effects of some pesticides on lipid peroxidation and free radical scavengers. *Toxicol Lett*., 107, 1999, 33-47.

Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. *J Lab Clin Med.*, 61, 1963, 882-8.

Blois MS. Antioxidant determination by the use of a stable free radical. *Nature*, 4617, 1958, 1198-200.

Cheeseman KH, Slater TS. Free radicals in medicine. New York: Churchill. Livingstone; 1922, 481-93.

Comroe JH, Todd J, Gammon GD, Leopold IH, Koelle GB, Bodansky O, et al. The effect of di-isopropyl-fluorphosphate (DFP) upon patients with myasthenia gravis. *Am J Med Sci*., 212, 1946, 641-51.

De Bleeker JL, De Reuck JL, Willems JL. Neurological aspects of Organophosphate Poisoning. Clin. *Neurol. Neurosurg*, 94, 1992, 93-103.

Ellman GL. Tissue Sulfhydrl groups. *Arch Biochem Biophys*, 1, 1959, 70-7.

Fareedullah Md, Vijayakumar S, Sudhakar Y, Venkateswarlu B, Ashok kumar E. Current review on Organophosphorus poisoning. *AASR*, 2, 2010, 199-215.

Guney M, Demirin H, Oral B, Ozguner M, Bayhan G, Altuntas I. Ovarian toxicity in rats caused by methidathion and ameliorating effect of vitamins E and C. *Hum Exp Toxicol*, 26, 2007, 491-26.

Hai DQ, Varga IS, Matkovics B. Effects of organophosphates on the antioxidant systems of fish tissue. *Acta Biol Hung*., 46, 1995, 39-50.

John S, Kale M, Rathore N, Bhatanagar D. Protective effects of Vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes. *J Nutr Biochem*, 12, 2001, 500-4.

Kalender S, Endosulfan-induced cardiotoxicity and free radical metabolism in rats: the protective effect of vitamin E. *Toxicol*, 202, 2004, 227-35.

Kalpana T, Karunakar N, Reddy MS, Prabhakar MC, Krishna DR. Assessment of antioxidant activity of some antileprotic drugs. *Drug Res.*, 51, 2001, 633-7.

Kiss Z. and Fazekas T. Arrhythmias in organophosphate poisonings. *Acta Cardiol*., 34, 1979, 323-30.

Kumiko T, Yoshiko A, Miwako K. Long QT and ST-T change associated with organophosphate exposure by aerial spray. *Environmental Toxicology and Pharmacology*, 22, 2006, 40-45.

Ludomirsky A, Klein HO, Sarelli P, Becker B, Hoffman S, Taitelman U, et al. QT prolongation and polymorphous (torsade de pointes) ventricular arrhythmias associated with organophosphorus insecticide poisoning. *Am. J. Cardiol*., 49, 1982, 1654-58.

Luzhnikov EA, Savina AS, Shepelev VM. Pathogenesis of disorders of cardiac rhythm and conductivity in acute organophosphate insecticide poisoning. *Kardiologia*, 15, 1975, 126-129.

Mogda KM, Afaf AI El-K, Rashed MA, Koretem KM. Oxidative and biochemical alterations induced by profenofos insecticide in rats. *Nature and Science*, 7, 2009, 1-15.

Placer ZA, Crushman LL, Johnson BC. Estimation of products of lipid per oxidation (Malonylaldehyde) in biochemical systems. *J Biol Chem*, 16, 1966, 359.

Rajeswary S, Kumaran B, Ilangovan R., Yuvaraj S, Sridhar M, Venkataraman P, Srinivasan N, Aruldhas MM. Modulation of antioxidant defense system by the environmental fungicide carbendazim in Leydig cells of rats. *Reprod Toxicol*, 24(3-4), 2007, 371-80.

Rastogi SK, Satyanarayan PVV, Ravishankar D, Sachin Tripathi. A study on oxidative stress and antioxidant status of agricultural workers exposed to OP insecticides during spraying. *Indian J Occup Environ Med.* 13, 2009, 131-4.

Ryrfeldt A, Bennenberg G, Moldeus P. Free radicals and lung disease. In: Cheeseman KH, Slater TF, editors. Free radicals in medicine. London: Church Hill Livingstone 1992, 588-603.

Vidyasagar J, Karunakar N, Reddy MS, Rajnarayana K, Surender T, Krishna DR. Oxidative stress and antioxidant status in acute OP insecticide poisoning. *Indian J Pharmacol*, 36, 2004, 76-9.

Vijayakumar S, Fareedullah Md, Mohan Rao K, Ashok Kumar E. Electrocardiographic findings of inpatients with organophosphorus poisoning. *Cardiovas Toxicol*, 2, 2011, 9104-4.

Vijayakumar S, Venkateswarlu B, Sasikala M, Vijay Kumar G. A study on Poisoning cases in a tertiary care hospital. *J Nat Sc Biol Med*, 1, 2010, 35-9.