Oxidative stress and antioxidant status in acute organophosphorous pesticides poisoning cases of North Karnataka (India)

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Abstract

This study was conducted to assess oxidative stress and antioxidant status of organophosphorus (OP) poisoning cases before and after therapy with atropine and pralidoxime of north Karnataka (India). For this study 30 male OP poisoning cases (study group) and 30 male normal healthy subjects (control group) having age range 17-40 years were taken. All organophosphorus poisoning cases were diagnosed by physicians.

In OP poisoning cases plasma cholinesterase levels were significantly decreased (83%, P<0.001) as compared to controls and increased (264%, P<0.001) after treatment with atropine and pralidoxime as compared to before treatment of these drugs. Serum malondialdehyde (MDA) level was significantly increased (142%, P<0.001) as compared to controls and significantly decreased (20.47%, P<0.05) as compared to before treatment of these drugs. Plasma total antioxidant capacity was significantly decreased (18%, P<0.001) in study group as compared to controls and increased (17%, P<0.05) after treatment with atropine and pralidoxime as compared to before treatment of these drugs. Increased erythrocyte superoxide dismutase (54.70%, P<0.05), catalase (40.30%, P<0.05), and glutathione peroxidase (15.70%, P<0.001) were observed in OP poisoning cases as compared to controls. Erythrocyte superoxide dismutase and catalase were decreased after treatment with atropine and pralidoxime as compared to before treatment of these drugs which was not statistically significant but only erythrocyte glutathione peroxidase (4.9%, P<0.001) was significantly decreased.

Organophosphorus pesticides inhibit the cholinesterase, induce oxidative stress and alter antioxidant status in OP poisoning cases. The biochemical effects of organophosphorus pesticides were reduced after treatment of atropine and pralidoxime. Therefore, along with these drugs, antioxidant supplementation may be useful to reduce toxic effects in OP poisoning cases.

Key words: Atropine, Catalase (CAT), Cholinesterase (ChE), Glutathione Peroxidase (GPx), Lipid peroxidation, Organophosphorus (OP) Poisoning, Pralidoxime (PAM), Total Antioxidant Capacity (TAC), Superoxide Dismutase (SOD).

Introduction

Organophosphorus compounds (OP) are highly toxic to human beings. Poisoning caused by OP compounds is steadily increasing in India because of their easy availability and potent toxicity. Among the OP compounds the most commonly used are: Dimethoate (Roger), Monocrotophos, Chlorpyrifos, Paraoxan, Mevinphos, Triazophos.

The toxic effects of OP compounds in acute poisoning cases result from inhibition of blood ChE (cholinesterase) activity including plasma and erythrocyte. Also there is evidence that AChE inhibition correlates with OP-induced symptoms of toxicity (Ranjbar *et al*., 2005). Toxicities of OP pesticides cause adverse effects on many organs and systems such as liver, pancreas, muscles, immune system, urinary system, reproductive system and hematological system (Teimouri *et al*., 2006).

In acute poisoning the main mechanism of toxicity of OP compounds is irreversible binding of these compounds to AChE (acetylcholinesterase) and inhibiting its activity which results in accumulation and prolonged effect of ACh (acetylcholine) and consequently follows with acute muscarinic and nicotinic effects (Ranjbar *et al*., 2004). Mild poisoning includes muscarinic and nicotinic signs only, while severe cases always show central nervous system involvement. The symptoms can vary in time of onset, sequence and duration depending on the chemical, dose and route of exposure (WHO, 1986).

The imbalance between production of free radicals and antioxidant defences in the body is called oxidative stress which has important health implications. Oxidative stress is a major mechanism in the pathophysiology of several toxins and diseases. In addition oxidative stress is also a process related to xenobiotic exposure and different levels of environmental contamination (Banerjee *et al*., 1999). In such cases peroxidation of membrane lipids seems to be an unavoidable process in tissue injury and may impair antioxidant defences leading to oxidative damage by changing the balance between oxidants and antioxidants (Halliwell B. *et al*., 1999; Banerjee *et al*., 1988).

Measurement of lipid peroxidation product e.g. malondialdehyde (MDA) and total antioxidant capacity (TAC) of blood is an effective marker to study oxygen free radicals effects in the body; hence this study was conducted to evaluate the existence of oxidative stress, antioxidants and total antioxidant capacity levels in acute organophosphorus poisoning cases before and after atropine and pralidoxime drugs therapy.

Materials and methods

This study comprises 30 OP poisoning cases and 30 normal healthy control subjects. All the study and control group subjects were in the age 17-40 years range. OP poisoning cases admitted to BLDEU'S Shri. B. M. Patil Medical College Hospital Bijapur, North Karnataka (India) were taken for the study. OP poisoning cases were diagnosed by physicians by observing clinical signs and symptoms and taking detail history from family members and the patient. All the patients were given 1gm of PAM by slow intravenous injection. After that a bolus dose of atropine was administered till signs of atropinisation. Also, improvement in signs and symptoms were monitored after treatment with atropine and pralidoxime. Before the biological specimen collection, the demographic, occupational and clinical data were collected from the study group and control subjects by questionnaire and interview. The entire experimental protocol was approved by institutional ethical committee and utmost care was taken during the experimental procedure according to the Helsinki Declaration (1964).

10ml venous blood samples were collected from the OP poisoned cases under aseptic conditions.

> **Group I:** Immediately after admission to the hospital, before starting the appropriate (atropine and PAM) treatment.
>
> **Group II:** After complete recovery of the OP patient and before the patient is discharged from the hospital (i. e. on the last day of hospitalisation).

Serum and plasma were separated by centrifugation at 3,000rpm for 10 minutes, at room temperature. Then all samples were immediately placed at 4°C until they were processed to get accurate and reproducible results.

Plasma cholinesterase (ChE) was estimated by butyrylthiocholine kinetic method using standard kit of Agappe Diagnostics. Cholinesterase act on butyrylthiocholine to form thiocholine, which acts on dithio-bis-nitro benzoic acid giving pink-coloured 2-nitro, 5-mercaptobenzoate (Kendel and Bottger, 1967; Tietz, 1986).

Serum lipid peroxide i.e. MDA concentration was measured by the Satoh (1978) method. Serum proteins were precipitated by trichloro-acetic acid (TCA) and the mixture was heated for 30 minutes with thiobarbituric acid in 2M sodium sulphate, in a boiling water bath. The resulting chromogen was extracted with n-butyl alcohol and the absorbance of the organic phase was determined at a wavelength of 530nm. The values were expressed in terms of nmol/ml of malondialdehyde (MDA) using 1,1,3,3, tetra-ethoxy propane as the standard (Satho, 1978).

The plasma total antioxidant capacity (TAC) was estimated by FRAP (Ferric reducing ability of plasma) assay. The antioxidant power of plasma converts ferric ions to ferrous ions at low pH forming a pink coloured ferrous tripyridyl triazine (Fe^{III} – TPTZ) complex. Ferrous reducing antioxidant power values were obtained by comparing the change in the absorbance at 593nm in mixture with those of ferrous ion of known concentration. The TAC in plasma was expressed as nmol/ml (Iris *et al*., 1999).

Activity of erythrocyte superoxide dismutase (SOD) was measured by the method of Marklund and Marklund. Superoxide anion is involved in the auto-oxidation of pyrogallol at alkaline pH 8.5. The superoxide dismutase inhibits the auto-oxidation of pyrogallol, which can be determined as an increase in absorbance per two minutes at 420nm. The SOD activity was measured as Units/gms of Hb. One unit of superoxide dismutase is defined as the amount of enzyme required to cause 50% inhibition of pyrogallol auto-oxidation (Marklund and Marklund, 1974).

Erythrocyte catalase was measured by the method of Aebi (1983). Heparinised blood was centrifuged and the plasma was removed. The erythrocytes were washed three times with 5ml 0.9% sodium chloride and lysed in 10 volumes of cold deionised water. The whole mixture was centrifuged further for 10min at 3,000rpm. The cell debris was removed and the clear haemolysate was diluted 500 times with phosphate buffer (60mM) pH 7.4.

Catalase decomposes hydrogen peroxide (H_2O_2) to form water and molecular oxygen. In the UV range, H_2O_2 shows a continual increase in absorbance with decreasing wavelength. At 240nm, H_2O_2 absorbs maximum light. When H_2O_2 is decomposed by catalase, then the absorbance decreases. The decreased absorbance was measured at 240nm at 15 second intervals up to 1 minute and the difference in absorbance (ΔA at 240nm) per unit time was taken to be a measure of the catalase activity. The unit of catalase activity was expressed as mM of H_2O_2 decomposed/mg Hb/min (Aebi, 1983).

Erythrocyte Glutathione peroxidase (GPx) was assayed by Paglia and Valentine (1967) method. GPx catalyses

Paramaters	Controls (N=30)	OP poisoning cases	
		Before treatment (N=30)	After treatment (N=30)
I. Toxicity marker			
Plasma ChE (U/L)	6286 ± 912.53	1045.5 ± 555.6***	3802.6 ± 867***
II. Oxidative stress marker			
Serum MDA (nmol/ml)	1.05 ± 0.37	2.54 ± 0.89***	2.02 ± 0.71**
III. Antioxidant status			
Plasma TAC (nmol/ml)	1264 ± 130	1043 ± 197.8***	1215 ± 229**
Erythrocyte SOD (Units/gms of Hb)	10.14 ± 3.6	15.69 ± 6.4**	14.25 ± 3.7*
Erythrocyte CAT (mM H_2O_2 decomposed/mg Hb/min.)	15.54 ± 5.67	21.81 ± 8.1**	17.49 ± 6.5*
Erythrocyte GPx (U/L)	5351 ± 934.8	6196 ± 588.5***	5890 ± 476.8***

Table 1.0
Mean ± SD values of plasma cholinesterase, serum malondialdehyde, plasma total antioxidant capacity, erythrocyte superoxide dismutase, catalase and glutathione peroxidase in controls and OP poisoning cases before and after treatment.

** indicates P<0.05-significant, *** indicates P<0.001-highly significant and
* indicates P>0.05-Non significant as compared to controls.
ChE – Cholinesterase, MDA – Malondialdehyde, TAC – Total Antioxidant Capacity, SOD – superoxide dismutase, CAT – catalase, GPx – glutathione peroxidase.

the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH (reduced NADP+) the oxidised glutathione is immediately converted to the reduced from with a concomitant oxidation of NADPH to NADP+ (nicotinamide adenine dinucleotide phosphate). The decrease in absorbance at 340 nm is measured (Paglia and Valentine, 1967).

Statistical analysis was performed using students 't' test. The values were expressed as mean ± SD. P value less than 0.05 (P<0.05) was considered as significant.

Results and discussion

Poisoning by OP compounds is the most common cause of suicidal deaths in India. Organophosphorus compounds are irreversible inhibitors of both muscarinic and nicotinic acetyl cholinesterase and affect the central nervous system. In this study, 60% of participants were from low socio economic status. The incidence of poisoning is very common in individuals with low economic status (Agrawal, 1993).

In organophosphate poisoning cases plasma cholinesterase levels were significantly decreased (83%, P<0.001) as compared to controls and increased (264%, P<0.001) after treatment with atropine and pralidoxime as compared to before treatment of these drugs. The plasma ChE level of normal healthy control group ranged from 4,500-8,000 U/L and in OP poisoning cases 400-4,800 U/L. With an increase in the severity of poisoning there was a corresponding decrease in plasma ChE activities. This result is consistent with earlier studies (Vidyasagar *et al*., 2004; Dandapani *et al*., 2003). Inhibition of plasma ChE at the time of admission but steady recovery after treatment was found, which indicates regeneration of the enzyme and serves as an indicator of clinical improvement in the patient. If the levels do not increase, excessive ACh accumulates at synapses within sympathetic ganglia and skeletal myoneural junctions. Hence plasma ChE could be used as a parameter to monitor the prognosis of OP poisoning. Chances of recovery are greater when the patient is hospitalised at the earliest indication of poisoning.

Serum lipid peroxide (MDA) level was significantly

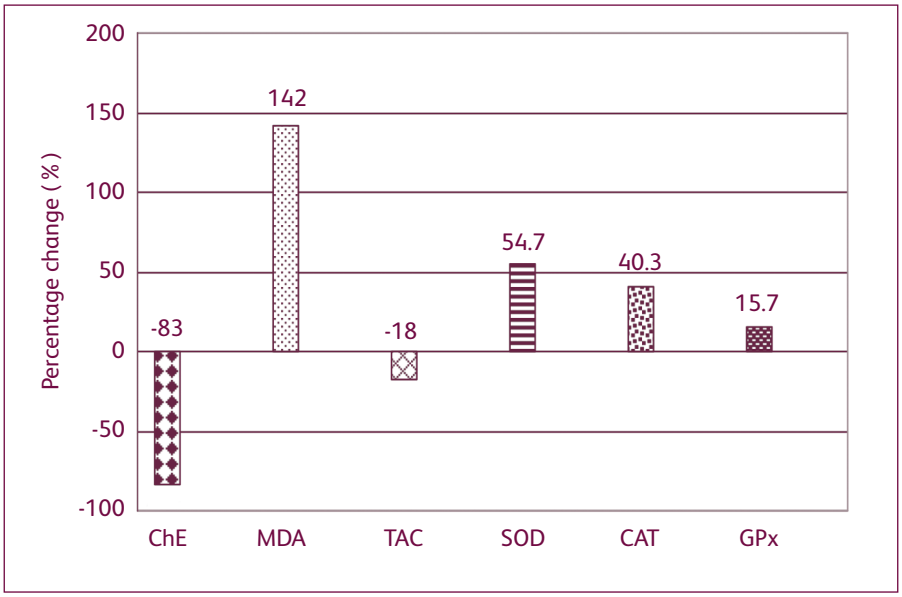

Figure 1.0
Percentage change of plasma cholinesterase (ChE), serum malondialdehyde (MDA), plasma total antioxidant capacity (TAC), Erythrocyte superoxide dismutase (SOD), catalase (CAT) and GPx of organophosphorus (OP) poisoning cases with respect to control group.

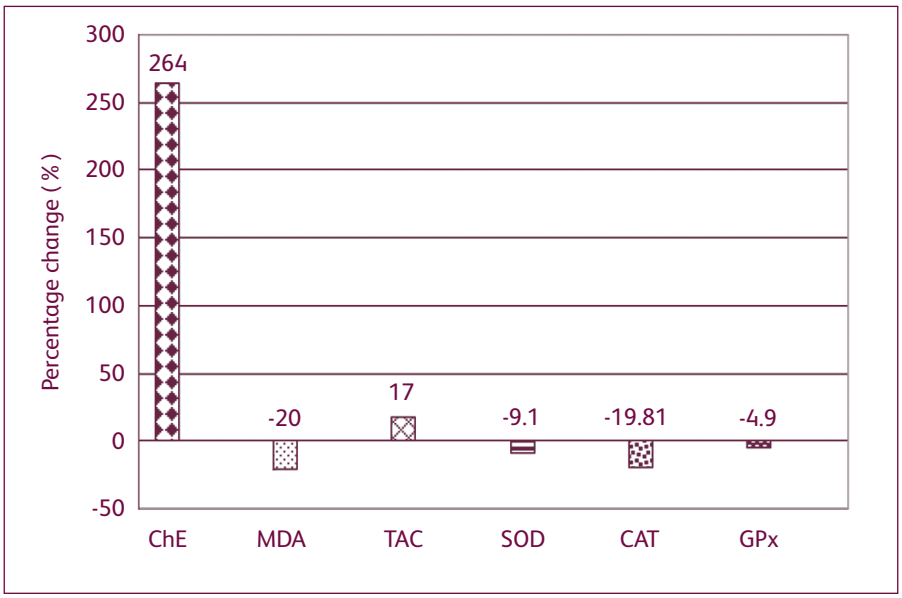

Figure 2.0
Percentage change chart of mean levels of plasma cholinesterase (ChE), serum malondialdehyde (MDA), plasma total antioxidant capacity (TAC), erythrocyte superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) of organophosphorus (OP) poisoning cases after treatment with respect to before treatment.

increased (142%, P<0.05) as compared to controls and significantly decreased (20.47%, P<0.05) as compared to before treatment of these drugs. Increased MDA level may be owing to OP poisoning, which is decreased after treatment with atropine and pralidoxime. The inhibition of ChE initiates the accumulation of free radicals leading to lipid peroxidation, which may be the indicator of cell injury. The phospholipids component of biomembranes is believed to be the site of action of OP compounds (Akhgari *et al*., 2003). Toxic manifestations induced by OP compounds may be associated with enhanced production of reactive oxygen species, which induces the oxidative process and lipid peroxidative damage in cell membranes.

Plasma total antioxidant capacity was significantly decreased (18%, P<0.001) in the study group as compared to controls and increased (17%, P<0.05) after treatment with atropine and pralidoxime as compared to before treatment of these drugs. Increased erythrocyte superoxide dismutase (54.70%, P<0.05), catalase (40.34%, P<0.05), and glutathione peroxidase (15.70%, P<0.001) were observed in OP poisoning cases as compared to controls. Erythrocyte glutathione peroxidase (4.9%, P<0.001) was significantly decreased after treatment with atropine and pralidoxime as compared to before treatment with these drugs. Erythrocyte superoxide dismutase and catalase were not altered significantly after treatment with atropine and pralidoxime as compared to before treatment with these drugs. This result indicates that the organophosphorus pesticides altered antioxidants status may be caused by more generation of free radicals.

The human body has several mechanisms to counteract the damage produced by free radicals; the basic and the most prominent defence mechanism of the human body are antioxidant agents. Antioxidants are substances that delay or inhibit the oxidative damage to a target molecule. These molecules are stable enough to neutralise free radicals by donating electrons. Thus, in acute OP poisoning, sudden overproduction of ROS (reactive oxygen species) leads to significant lipid peroxidation and consumption of antioxidant agents for which the body could not compensate in a short period. A previous study involving 22 acute malathion poisoning patients showed significant lipid peroxidation accompanied by decreased levels of TAC, total thiols and ChE activity (Ranjbar *et al*., 2005).

The susceptibility of erythrocytes and lymphocytes to oxidative stress caused by pesticide exposure is a function of overall balance between the degree of oxidative stress and antioxidant defense capability. Thus, the OP compounds may directly or indirectly modify the antioxidant defence capability of exposed subjects and therefore affect their susceptibility to oxidative stress (Banerjee *et al*., 1999). Many intrinsic radical scavenger systems involve enzymatic and nonenzymatic reactions. SOD, CAT and GPx are important components of enzymatic antioxidative systems. Generally, there is an inverse relationship between lipid peroxidation and antioxidant enzymes; however, we found significant increase in erythrocyte SOD, CAT and GPx activities as well as serum MDA concentration. Increase in SOD activities in erythrocytes of OP poisoning cases indicates an increased production of superoxide radical. Increased CAT activities in erythrocytes may be explained by their influence on hydrogen peroxide as substrate, which is formed in the process of dismutation of superoxide radicals (Shaikh *et al*., 1999). Erythrocyte SOD, CAT and GPx efficiently scavenges toxic free radicals and are partly responsible for protection against lipid peroxidation from acute/chronic organophosphorus pesticide exposure. Thus, the increase in these enzymes was probably a response towards increased ROS generation in OP toxicity.

Supporting our results, there is evidence that administration of malathion resulted in increased SOD, CAT as well as MDA concentration in RBCs and livers of rats (Possamai *et al*., 2007). Increase in erythrocyte SOD and CAT activities in dimethoate and malathion treated rats was reported by John *et al*. (2001). The increase in these enzymes was a response towards increased ROS generation in OP toxicity. Banerjee *et al*. (1999) reported that between seven and 14 days after poisoning by malathion or propoxur-altered erythrocyte of AChE, SOD, CAT, GPx levels tend to return towards normal ranges as found in control subjects, probably reflecting the fact that the normal oxidative stress status was achieved easily.

Conclusions

The present findings indicate that cells continually suffer from oxidative stress in spite of over-activity of antioxidant defence mechanism as indicated by increase in erythrocyte SOD, CAT and GPx activity. The higher levels of antioxidant enzymes may be necessary to detoxify increased concentration of lipid peroxidation products that are generated from oxidative stress because of OP toxicity. Consumption of nonenzymatic antioxidants might be so high that the body could not compensate in a short period, hence reduced TAC was observed. Therefore, in emergency treatment of acute OP poisoning, the antioxidants at suitable doses should be given in order to reduce oxidative damage, which could be effective in speedy recovery of acute OP poisoning cases. Plasma ChE estimation determines the severity of poisoning, which can be helpful for predicting the outcome in OP poisoning cases.

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