Original Article

Effect of acute organophosphorus pesticide poisoning on oxidative stress and antioxidant status

Vinutha Bhat[1], Pruthvi Acharya[1] and Shankar M. Bakkannavar[2,*]

[1]Department of Biochemistry; [2]Department of Forensic Medicine and Toxicology,
Kasturba Medical College, Manipal Academy of Higher Education, Manipal – 576104, India.

ABSTRACT

Organophosphorus (OP) poisoning is a worldwide public health problem. Assessment of oxidative stress and antioxidant status using many biochemical parameters has become a notable research work. In this view, the serum nitric oxide (NO) levels are checked as an indicator of oxidative stress. Serum levels of Vitamin E, Vitamin C, glutathione reductase and glutathione peroxidase are checked as a marker of antioxidant property. Correlation of oxidative stress biomarker NO with other antioxidant parameters such as Vitamin E, Vitamin C, glutathione reductase and glutathione peroxidase is done in the study. The results show that vitamin C and vitamin E decreased in cases of organophosphorus poisoning whereas NO, glutathione reductase and glutathione peroxidase increased compared to controls, with statistical significance $p < 0.0001$. Nitric oxide shows a negative correlation between vitamin C and glutathione peroxidase and shows a positive correlation between vitamin E and glutathione reductase. But the positive correlation between vitamin E and glutathione reductase is statistically insignificant.

KEYWORDS: organophosphorus poisoning, oxidative stress, antioxidant property, serum markers.

INTRODUCTION

Organophosphates are the insecticides most widely used in the pest control of crops globally [1]. The most frequently used OP compounds include parathion, malathion, chlorpyrifos, and dichlorvfos [2]. Organophosphate compounds bind strongly to cholinesterase. The cholinesterase rapidly hydrolyzes the neurotransmitter acetylcholine into inactive fragments of choline and acetic acid [3]. The inhibition of the cholinesterase activity results in the buildup of acetylcholine in synapses, causing disturbance in synaptic transmission at neuroeffector junctions and skeletal myoneural junctions. This is achieved by stimulation of receptor locations of acetylcholine that results in a multitude of physiological and metabolic disturbances [4]. Certain pesticide compounds themselves causing these enzyme inhibitions, are called as 'direct inhibitors', whereas a few other compounds will get metabolized and converted to inhibitor form; such compounds are termed as 'indirect inhibitors', examples being baytex and malathion [5]. Apart from causing the changes in the cholinergic synapses, they affect certain metabolic pathways. In liver, OP compounds cause certain biochemical changes like, the alteration of the transaminases such as ALT, AST, direct bilirubin and indirect bilirubin. In addition, they also cause ineffective tissue perfusion of heart [6].

Degree of toxicity of OP poisoning is characterized as follows:

- Mild toxicity has symptoms such as impaired vision of eyes, headaches and dizziness, weakness, anxiety, etc.
- Moderate toxicity includes stomach pain, salivation, watering eyes, and muscular tremors.

*Corresponding author: shankar.mb@manipal.edu

• Severe toxicity comprises of difficulties in vision and respiration, diarrhea, convulsions, finally coma and death [7].

The imbalance state that occurs between the generation of free radicals and antioxidant defenses in the body is referred to as oxidative stress and has substantial health consequences [8]. Oxidative stress resulting from oxygen free radicals, hydrogen peroxide, hydroxyl radical, nitric oxide generation, etc. is referred to as reactive oxygen species (ROS) [9].

ROS is extremely reactive to proteins, lipids and DNA molecules that cause harm to these macromolecules and may even cause cell dysfunction or death [10]. Oxidative stress happens when the rate of ROS production exceeds the rate of removal. Elevated lipid peroxidation, changes in antioxidant activity, or imbalance in the cellular redox system can be used as an early 'marker' of oxidant stress [11].

The antioxidant enzymes' efforts to remove the continuously produced free radicals increase owing to the outcomes of induction but subsequent depletion of the enzymes occur, resulting in oxidative cell damage [7].

There are many free radical scavenger systems involving both enzymatic and non-enzymatic responses. One such enzymatic system includes glutathione peroxidase, SOD (Superoxide dismutase) and catalase. SOD converts super oxide radicals to hydrogen peroxides. Glutathione peroxidase and catalase will convert hydrogen peroxide to H_2O. The basic machinery of non-enzymatic antioxidant protection involves ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), β-carotene and vitamin A [11]. OP intoxication features will start to appear only when there is aerial oxidation to generate Oxon. These oxons will inhibit acetyl choline esterase. These oxons can be deactivated by hydrolases such as carboxylases and by A-esterases such as paraoxons. All the OP compounds except phosphates are metabolically converted to their corresponding Oxon, mediated by cytochrome P450 isoforms, monooxygenase enzymes containing flavin [12, 13].

The present study was conducted to know the early biological signs of cytotoxicity such as changes in the antioxidant activity and the oxidative stress.

The correlation of antioxidant activity and oxidative stress would help in assessing the relationship between them. This will also help in therapy such that the particular antioxidant can be given in an accurate way to prevent further oxidative damages.

MATERIAL AND METHODS

The Institutional Ethics Committee approval was received (IEC:936/2018). The study was conducted at the Department of Biochemistry, Kasturba Medical College, Manipal.

44 organophosphorus-poisoned subjects were included in the present study. The left-over samples were only collected from Manipal Poison Detection Center, Kasturba Hospital, Manipal. These samples were anonymized.

Estimation of nitric oxide

NO estimation is done by Griess reaction method [14]. In this method nitrite in the reduced form is made to react with the Griess reagent, consisting of the diazotizing reagent such as sulfanilamide (SA) in the acidic medium to form transient diazonium salt. This intermediate is then allowed to react with a coupling reagent, N-naphthyl-ethylenediamine (NED) to form a stable azo compound. The intense purple color of the product allows nitrite assay with high sensitivity and can be used to measure nitrite concentration. The absorbance of this adduct at 540 nm is linearly proportional to the nitrite concentration in the sample. The reduction of nitrate to nitrite is often accompanied by treating samples with certain reductants such as cadmium. The copperized cadmium method is one of the most popular methods to convert nitrate to nitrite, among the researchers. In this method cadmium is exposed to copper sulphate solution; copper ions (Cu_2^+) are reduced on the cadmium surface to form a porous metallic copper 'coat'. This formed copper layer facilitates electron transfer from cadmium to nitrate. The nitrate to nitrite conversion can be then achieved rapidly using a relatively small surface area. The overall reaction in the mild alkaline solution can be described as follows:

$$NO_3^- + H_2O + Cd \longrightarrow NO_2^- + Cd_2^+ + 2OH^-$$
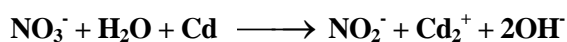
The absorbance is read at 540 nm.

Estimation of Vitamin C

To 0.4 ml of serum, 1.6 ml of 10% trichloroacetic acid was added, mixed and centrifuged at 2000 rpm. To 1.0 ml of supernatant, 0.4 ml of dinitrophenyl hydrazine reagent was added, stoppered and incubated at 37°C for 3 hours. After 3 hours it was chilled in an ice bath and 1.6 ml of cold 65% H_2SO_4 was added. After 30 min the sample was read at 520 nm. Blank contained 1.0 ml of trichloroacetic acid both for serum and plasma filtrate. The standard was composed of 0.4 ml of 1 mg/100 ml ascorbic acid and was also treated in a similar fashion [15].

Calculation:

$$\frac{\text{Absorbance of unknown sample}}{\text{Absorbance of standard}} *1 = \text{mgAscorbic acid } / 100\text{ml}$$

Estimation of Vitamin E

This method is based upon the reduction of the ferric chloride ions by tocopherols after xylene extraction of the blood samples. The ferrous ions react with α, α bipyridyl to give a red color which is measured at 520 nm [15].

$$\text{Serum tocopherol (mg/dl)} = \frac{[\text{Reading of unknown sample at 520nm- (reading of unknown sample at 460nm) (0.29]} *1}{[\text{reading of standard at 520nm}]}$$

Estimation of glutathione reductase

Principle

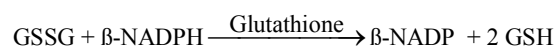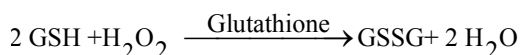$$\text{ß-NADPH+GSSG} \xrightarrow{\text{GLUTATHIONE REDUCTASE}} \text{ßNADP+ 2GSH}$$

Decrease in the absorbance is read at 340 nm [16].

Estimation of glutathione peroxidase

Principle: Glutathione peroxidase catalyses the conversion of GSH to GSSG [17].

$$2 \text{ GSH} + H_2O_2 \xrightarrow{\text{Glutathione}} \text{GSSG+ 2 } H_2O$$

$$\text{GSSG + ß-NADPH} \xrightarrow{\text{Glutathione}} \text{ß-NADP + 2 GSH}$$

Decrease in absorbance is read at 340nm.

RESULT

The study comprised of control and case groups. Non-parametric Mann-Whitney U test was performed to compare between the groups. Non-parametric tests were done as the data was not normally distributed. Spearman's correlation is done for determining the correlation between NO and other antioxidants (GR, GP, Vitamin C, Vitamin E) as shown in Table 1 & 2 and Graphs 1 to 5. The comparison was considered significant, if the p value was <0.05.

For Mann-Whitney U test p < 0.001 is considered significant. It is done for comparison between cases and controls for all the parameters.

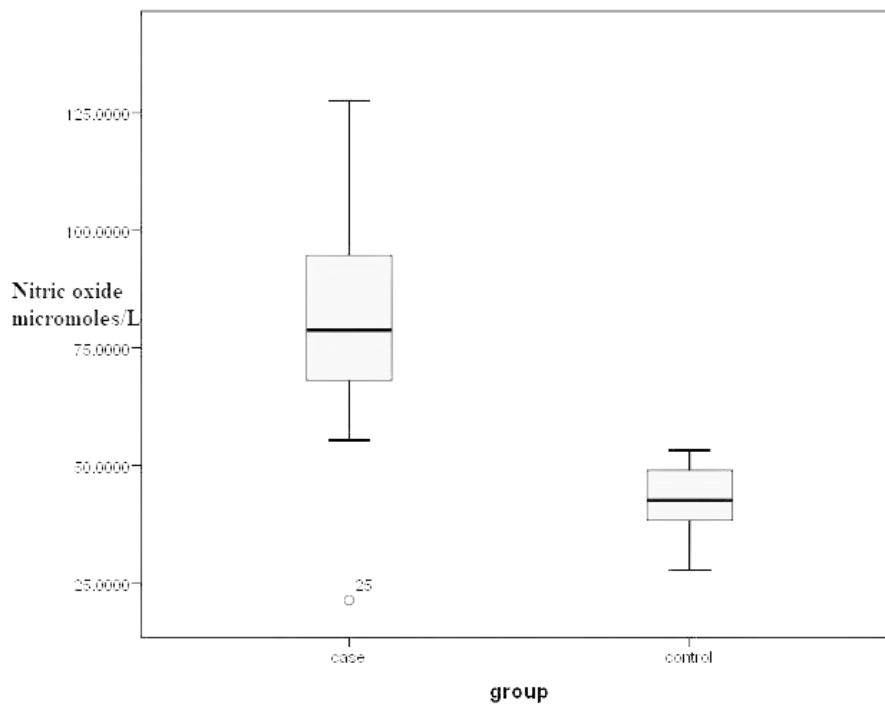**Table 1.** Comparison between cases and control for all parameters.

Parameters	N	Cases Median (Q1, Q3)	Controls Median (Q1, Q3)	*p-value
Vitamin C	44	0.24(0.14, 0.41)	1.60(1.0,1.8)	p < 0.0001
Vitamin E	44	1.41(0.91, 1.95)	3.50(1.35,3.97)	p < 0.0001
NO	44	78.72(68.08, 96.27)	42.55(38.2948.93)	p < 0.0001
Glutathione reductase	44	0.06(0.03, 0.08)	0.03(0.02,0.04)	p < 0.0001
Glutathione peroxidase	44	0.39(0.12, 0.63)	.8500(0.800.91)	p < 0.0001

*p < 0.0001 is significant.

Table 2. Spearman's correlation of nitric oxide with the following parameters.

Parameter	r value (Spearman's correlation co-efficient)	p value
Vitamin C	-0.1	p < 0.5
Vitamin E	0.01	p < 0.9
Glutathione Reductase	0.19	p < 0.2
Glutathione peroxidase	-0.2	p < 0.1

Significant at the level p < 0.05.

Graph 1. Comparison between nitric oxide levels of cases and control [nitric oxide (1)-cases, nitric oxide(2)-control].

DISCUSSION

The main objective of the study was to know the effect of organophosphorus poisoning on the oxidative stress and antioxidant status.

The nitric oxide level in cases increased when compared to that of controls, which is statistically significant at p<0.0001. The same result has been depicted in other acute OP poisoning studies. Increased nitric oxide production is due to increased ROS formation because of the OP toxicity [18].

The difference between vitamin C and vitamin E levels in cases compared to the control group was statistically significant (p<0.0001). Both vitamin E and vitamin C levels were observed to have decreased in OP poisoning. There are no studies which have done work on measuring these vitamins after OP intoxication. But the studies done in rat models have shown that these vitamins act as good antioxidants in reducing the toxic effects of various pesticides.
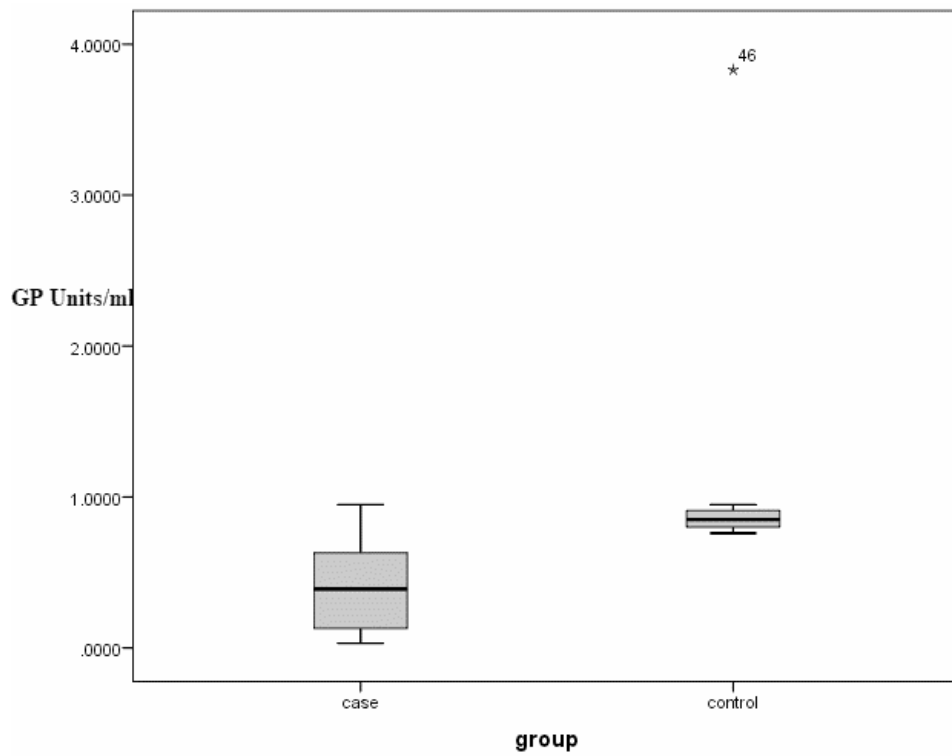
In the case group there was a significant difference in the glutathione peroxidase level
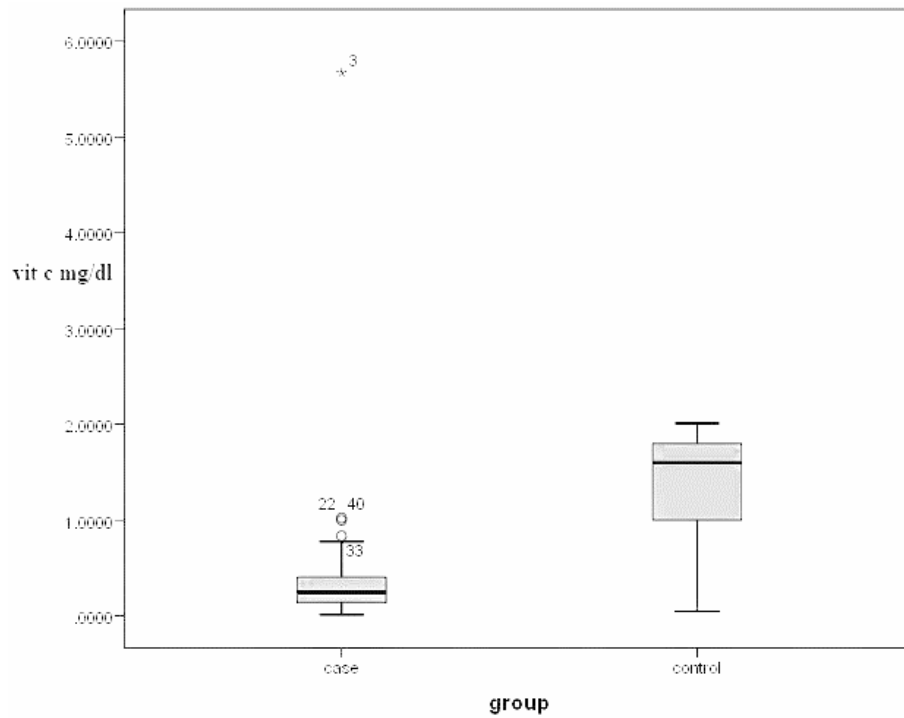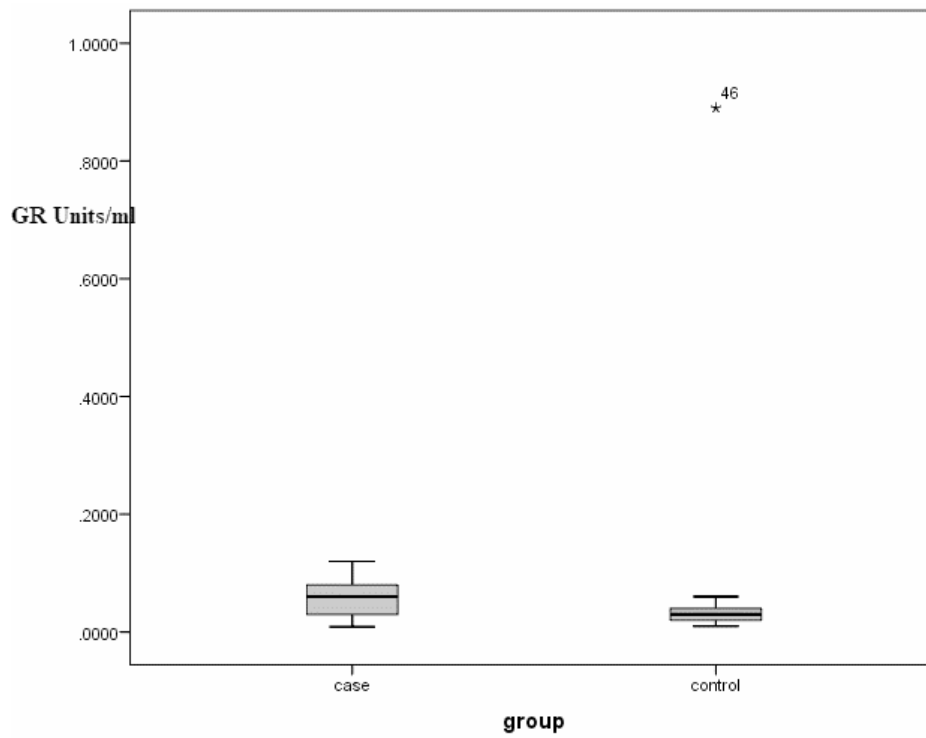
Graph 2. Comparison between vitamin C levels of cases and control [Vitamin C (1)-cases, Vitamin C(2)-control].
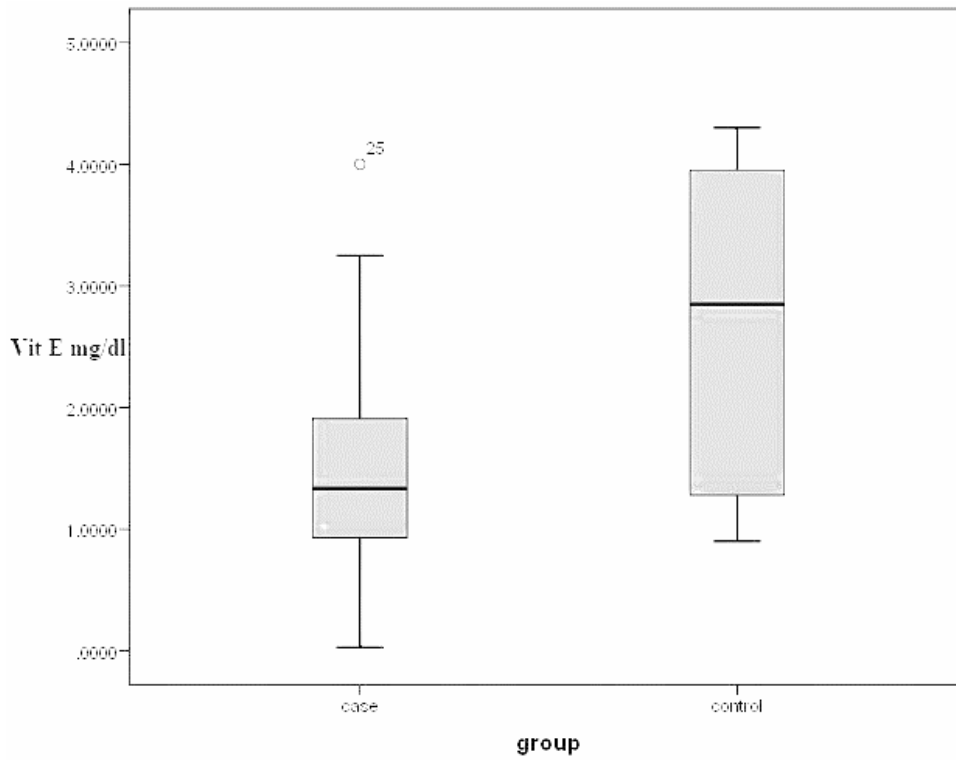
Graph 3. Comparison between Vitamin E levels of cases and control [Vitamin E(1)-cases, Vitamin E(2)-control].

Graph 4. Comparison between GP levels of cases and control [GP(1)-cases, GP(2)-control].

Graph 5. Comparison between glutathione reductase levels of cases and control [GR(1)-cases, GR(2)-control].

when compared with the control group with the p value < 0.0001. The same result has been supported by a few researchers who cited that decrease in the antioxidant enzymes may have occurred because of the indirect inhibition of the enzymes resulting from the binding of the oxidative molecules formed during the pesticide metabolism [19].

The amount of glutathione reductase is significantly increased in the case group compared to the control group with p < 0.0001. The enhanced activity of antioxidant enzymes could have resulted from the activation of the compensatory system leading to the initiation of free radical scavenging enzymes to counter the oxidative stress induced by the pesticides [19].

Nitric oxide showed negative correlation with vitamin C (with p value > 0.05) and glutathione peroxidase. It also showed a weak positive correlation with vitamin E and glutathione reductase. But it is statistically insignificant. The study can be done with larger sample size to have better outcome.

CONCLUSION

The current study showed the elevated oxidative stress indices as a consequence of the OP poisoning. The extent of oxidative stress is the reflection of the degree of OP toxicity. So, this can be used as an effective biochemical parameter in the continuous monitoring of the state of toxicity. As we already mentioned in the discussion, certain vitamins have been established as good antioxidants in reducing the cytotoxic effects of the pesticide by reducing the oxidative stress in certain rat models and even in humans. So, it is essential to measure the level of antioxidants in the OP-poisoned patients so that the vitamin E can be given in an appropriate dose to help in reviving the patient. The study can be further continued by measuring various other antioxidants to know the impact of pesticide metabolism on them. To attain a better outcome in such studies, it is necessary to have a larger sample size and long-term exposure to the pesticide. Different biochemical parameters can be measured at regular intervals to know the impact of poisoning if well tested with accuracy. Various other oxidative stress parameters can be explored for better prognosis.

FUNDING

ETHICAL APPROVAL

Obtained from Institutional Ethics Committee.

CONFLICT OF INTEREST STATEMENT

None to be declared.

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