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*Original Article*

# Biochemical Study of Antioxidant Enzymes and Oxidative Stress among Pesticide Sprayers

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**Abstract**

*Objective:* Comparative study of the extent of oxidative stress and antioxidant enzymes between control healthy subjects and pesticide sprayers. *Materials & Methods:* This study was conducted in 100 pesticide sprayers, who were engaged in agriculture sector and 40 healthy subjects as control. Superoxide dismutase, catalase, cholinesterase and glutathione were the antioxidant enzymes and MDA was taken as oxidative stress for biochemical investigations. Comparative study was made between healthy control subjects and pesticide sprayers. *Results and Conclusion:* There were significantly fall in cholinesterase ( AchE, BuchE ) levels in the pesticide sprayers group as compared to healthy control 18.2% and 18% respectively. Decline level of AchE and BuchE were noticed respectively in exposed sprayers with regard to healthy control. The significant increase in the level of superoxide Dismutase (SOD), Catalase (CAT) were noticed. While the level Of GSH ( Glutathione ) was diminished in the exposed sprayers compared to the control. The increase in lipid peroxidation was reflected by elevated levels of malonalaldehyde (MDA) in pesticide sprayers, indicates oxidative stress. *Conclusion:* The increased level of MDA is due to over production of free radicals in pesticide sprayers. Pesticides might be responsible for excess lipid peroxidation to produce higher level of reactive oxygen species. It is supposed that the rise in SOD, CAT because of compensatory mechanism to combat the over produced free radicals. Fall in cholinesterase and GSH might be exhaustion of these by compensating over produced oxidative stress and free radicals.

**Keywords:** Oregano Phosphorous Pesticide/ Insecticide; Cholinesterase; Oxidative Stress (MDA); SOD; CAT; GSH.

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## Introduction

Organophosphorus pesticides / insecticides are commonly used worldwide in agriculture to control pests and insects. These are biocides having capability to kill so many forms of life. They are human produced deadliest poisons, hence its presence even at low level in long term exposure could be responsible for health hazards [1].

There are so many clinical manifestations because of pesticides poisoning. Major clinical manifestations caused by excessive accumulation of acetylcholine (AchE) in synaptic vesicles of neurons. Cholinesterases are the enzyme hydrolyzes the cholinesterase. Organophosphate pesticides irreversibly inhibit the cholinesterase resulting in excessive accumulation of acetylcholine ( Ach) and butylcholine (Buch), leading to the paralysis of cholinergic transmission in the CNS, autonomic ganglion and parasympathetic nerve endings [2].

Among the pesticides organophosphorus insecticides and carbamates have been widely used as these compounds are non- persistent in the environment [3].

Exposure of pesticides/ insecticides occurs in

sprayers through the skin absorption and inhalation, as they are not in the habit of using face masks and other protective devices [4].

Most pesticides exert their toxicity on the target and non- target organs through the inhibitory action of acetyl cholinesterase in the nerve and muscle tissues [5-7]. Inhibition of butyl cholinesterase activity is taken as biomarker for pesticide exposure, henceforth the monitoring of BuchE activity in blood/ plasma could be a useful to predict and prevent health hazards of pesticides [8].

Toxicity of organophosphorus causes a lot of adverse effects on different systems of the body, inducing biochemical and hematological changes [9]. Recently it has also been studied that toxic effect of pesticides implicated to induce characteristic changes in oxidative stress and oxidant – antioxidant system balance.

Raised oxidative stress is because of over production of free radicals (ROS). All the major biomolecules such as lipids, proteins and nucleic acids may be attacked by free radicals, but lipids are probably most susceptible. When the free radical production overwhelms the endogenous level of antioxidant system decreased & excess MDA released, they cause considerable cell damage/death [10].

The cells have different mechanism to alleviate oxidative stress and repaired damaged molecules. The primary defense against oxidative stress is offered by enzymatic and non enzymatic antioxidants which have been noticed to scavenge free radicals and reactive oxygen species. The antioxidant enzymes SOD, CAT and glutathione peroxidase affected by toxicity of pesticides [11]. In view of this our study contained enzymatic and non enzymatic antioxidant parameters along with oxidative stress as lipid peroxides in terms of MDA.

## Materials and Methods

This study was conducted in the neighboring villages of lucknow and its peripheral areas as including Barabanki, at the Mayo Institute of Medical sciences Barabanki, Uttar Pradesh. The study included total rural population of 100 sprayers including 30 females also spraying a number of organophosphate pesticides in agricultural fields. A control group consisting of 40 unexposed workers, who never had any exposure to OP pesticides was taken as reference group, The OP pesticides mainly sprayed were dichlorvos chlorpyriphos, Diazinon, Methyl parathion and so on.

A pre- structured survey proforma was maintained to record detailed history including the personal and occupational history of each and every subjects participated in this study. Alcoholics and smokers were excluded from the study. The spray procedures, use of equipments, eating and drinking habits, personal cleanliness were also recorded in the proforma. It was observed among all pesticide applicators that mixing of pesticides with bare hands, leakage from the pesticide tank eating food without proper washing during and after spraying operation was found to common.

*Collection of Samples*

Venous blood was collected in a total 100 of pesticide sprayers along with 40 normal healthy subjects as a control. 10 ml of venous blood was collected from each subject in heparinized tubes and brought to laboratory for biochemical analysis.

*Biochemical Parameters*

The extent of lipid peroxidation in the blood was assayed by measuring the formation of Thiobarbituricacid reactive substances (TBARS) using the method of stocks and dormandy 1971 and expressed as nmol MDA formed / ml blood [12].

Reduced glutathione (GSH) was estimated in the blood by the method of Jallow etal 1974 and expressed µg/ml of blood [13].

Blood AChE and BuchE activity was determined by the method of Ellman et al (1961) as modified by chambers and chambers (1989) by taking acetylcholine iodide as substrate and expressed as mmols hydrolyzed/ h/ L blood ( IU/L) [14].

Catalase activity in blood was estimated by the method of Sinha (1979) using $H_2O_2$ as substrate and expressed as µmol $H_2O_2$ hydrolysed/min/ gHb [15].

Determination of superoxide dismutase activity in blood was adopted by the method of Misra and fridovich (1972). SOD activity was also expressed as µmol superoxide hydrolyzed/min/ gHb

## Observations and Results

*MDA and GSH*

The Mean ± S.D values of blood reduced glutathione (GSH) in pesticide exposed sprayers were 18.32±1.66µg/ml, which were significantly lower than their respective referents. The decrease in GSH activity could be more consumption of GSH to combat

effect of lipid per oxidation. The Mean ± S.D value MDA was observed 25.98 ± 2.01 nmol/ TBARS/ ml which were significantly higher than their respective referents.

The increase in lipid peroxidation reflected by elevated levels of MDA in pesticide exposed sprayers indicates oxidative stress.

*AchE and BuchE*

The blood AchE and BuchE levels in pesticide exposed sprayers were recorded as 531.26±18.26 IU/ L and 452.04±16.88 IU/L respectively, which were significantly lower than their respective controls.

*CAT and SOD*

The blood CAT level in pesticide sprayers was 135.±12.33µmol H2O2 hydrolysed /ml/ gHb, while it was 105.40±15.19 in respective controls. The elevated activity of CAT is because of toxic effect of organophosphate pesticides to compensate the excess lipid peroxidation (MDA) suggest an insufficient antioxidant defense.

The Mean ± SD blood SOD value were 8.04±2.47 µmol hydrolysed/ml/gHb in pesticide sprayers as compare to normal healthy control (3.29±0.78).

**Table 1:** Biochemical profiles of pesticide sprayers and healthy controls

| Biochemical Parameter | Healthy Controls (n=40) Mean ± S.D | Pesticide Sprayers (n=90) Mean ± S.D |
|---|---|---|
| MDA (nmol TBARS/mL) | 9.46 ± 0.77 | 25.98 ± 2.01* |
| GSH (µg/mL) | 30.00 ± 2.56 | 18.32 ± 1.66* |
| AchE (IU/L) | 670 ± 23.63 | 531.26 ± 18.26* |
| BuchE (IU/L) | 542.66 ± 43.28 | 452.04 ± 16.88* |
| CAT (µmole $H_2O_2$ hydrolyzed/min/gHb) | 105 ± 15.19 | 135.70 ± 12.33* |
| SOD (µmole decomposed/min/gHb) | 3.29 ± 0.78 | 8.04 ± 2.47* |

* Statistically significant P < 0.05

**Discussion**

**O**rgano phosphate pesticide toxicity is primarily a problem of developing countries like India. In the present study it was observed imbalance between oxidant and antioxidant equilibrium resulting in increase in neurological disordered symptoms were observed among the pesticide sprayers. Our findings support the previous studies of pesticides and neurological symptoms reported in farm workers [11, 16].

The study indicated that there was significant decrease in level of AchE and BchE and on increased level of MDA among pesticide sprayers with regard to referents. Our results support the earlier findings of vidyasagar et al [17].

Low activity of Acetylcholinestarase (AchE) and butyrylcholinesterase (BchE) reflects the inhibition of cholinesterase activity due to adverse effect of organophosphate pesticides. The inhibition of acetyl cholinesterase might have resulted in the accumulation of acetylcholine at the synaptic junction leading to neurotoxicity, cytotoxicity and excess lymphocyte and excess lymphocyte motility [18, 19]. Investigation of AchE activity is important in context of selection of this enzyme as a potential biomarker in pesticide exposed subjects.

In the chronic pesticide exposed sprayers the antioxidant enzymes, superoxide dismutase (SOD) catalase (CAT) and glutathione (Reduced) GSH were significantly affected. The organophosphate pesticide toxicity in sprayers lead to decreased glutathione (GSH) and elevated MDA level as well as increased activities of catalase and superoxide dismutase. Increased activities of catalase and SOD might be more to compensate increased level of oxidative stress in terms of lipid peroxidation (MDA). The increased tendency of antioxidant enzyme may be up to its threshold level after that it could be in decreasing tendency.

Reduced glutathione is a substrate of enzymes namely GPx (Glutathione peroxides) and GST (Glutathione transferase). GSH/ GSSG ratio is essential indicator of redox status of the cell [20].

It has been observed that pesticides disturb this ratio confirming the presence of oxidative stress. Our results suggest that toxicity of pesticide induce oxidative stress by depleting intracellular GSH and increasing reactive oxygen species (ROS) production. GSH is known to play a key role in regulating intracellular of levels of redox status. Organophosphate pesticides appear to disrupt this key cellular pathway perhaps by disturbing mitochondria metabolism as suggested recently by Delgado et al 2006; chan et al & sherer et al 2006 [21].

The increased activity of catalase (CAT) seen in pesticide sprayers coupled with an increase in the lipid peroxidation (MDA) suggests an insufficient weak antioxidant defense. SOD (superoxide

dismutase) is an antioxidant enzyme acts on highly reactive superoxide anion and convert it in to less reactive molecular oxygen and hydrogen peroxide ($H_2O_2$) species.

In the present study increased activity of SOD seen in pesticide sprayers might be coupled with an increase in the free radicals and (ROS) reactive oxygen species. As the pesticide sprayers predominantly developed neurotoxin symptoms on exposure to OP pesticides which is corroborated with the clinical and biochemical findings reported in this research study.

## Conclusion

An increased level of MDA, SOD, CAT and decreased level of GSH and Cholinesterase's in exposed OP pesticide sprayers is probably reflective of increased lipid peroxidation coupled with weak antioxidant defense.

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