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Studies on Antioxidant Enzymes of Sprayers in Agricultural Practices Exposed to Organophosphorus Pesticides in the Nuzvid Area

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ABSTRACT

Organophosphates (OP) pesticides are commonly used worldwide in agricultural and in pest control. The primary defense is offered by enzymatic and non-enzymatic antioxidants which have been shown to scavenge free radicals and reactive oxygen species (ROS). The antioxidant enzymes like *Superoxide dismutase* (SOD), *Catalase* (CAT) and *Glutathione peroxidase* (GPx) have been shown to be significantly affected by pesticides. The intracellular enzymes such as RBC SOD, GPx, CAT, effects as changes are studied in human beings exposed to organophosphorus pesticides during agriculture practices of spraying in the areas of Nuziveedu, Krishna district, A.P, INDIA. The results in control age group exposed group divided as healthy group and symptomatic group showed elevated levels of antioxidant enzymes and but the duration of exposure resulted showed decrement in antioxidant enzymes as acute and chronic exposure but a significant increment of these enzymes showed according to type of groups I, II, III as ethylated, methylated and mixed variation. Significant levels of changes are observed in methylated OP insecticide sprayed individuals only rather than non-methylated OP compounds rather than the both as mixtures. It results in tiredness, weakness, nausea, dizziness, headache, sweating, tearing, vomiting, limited vision, diarrhea, polyuria, muscle trembling, hypertension and breathing disorders further accelerated lipid peroxidation, cell damage & death (oxidative stress).

INTRODUCTION

Organophosphorous (OP) pesticide poisoning is a major global health problem with thousands of deaths every year. India, a developing country, basically agriculture being the main profession and at present, we echo globalization, industrialization and such things. The food production is maximum not only in India, but also in Andhra Pradesh which is considered as 'Rice bowl', spraying of agrochemicals is a part of their routine affair annually.

While spraying the pesticides, whatever is going to happen, must be a crystal clear for all those involved in spraying. In order to focus the same concept a part of Andhra Pradesh, in Krishna district, a place known as Nuziveedu is selected as a working spot, where the agro crops, as well as gardens, a quite a lot of people, are engaged in chemical spraying. The high-risk groups exposed to pesticides include the production workers, formulators, sprayers, mixers, loaders and agricultural farm workers. During manufacture and formulation, the possibility of hazards may be more because the processes involved are not risk-free. In industrial settings, the workers are at increased risk since they handle various toxic chemicals including pesticides, raw materials, toxic solvents and inert carriers. The main routes of OP exposure are people are exposed to OPs via ingested food, water and by breathing polluted air (WHO, 2001). The exposure to workers in closed areas and of agricultural workers or people living near farms is also very important (Gupta, 2006). Such as Superoxide dismutase (SOD), whose substrate is free radical (superoxide anion; $O_2.^-$) catalyzes dismutation reaction resulting in the generation of hydrogen peroxide which is decomposed to water and molecular oxygen by the action of *catalase*. When the radical production overwhelms the endogenous levels decreased, they cause considerable cell damage/death. All the major biomolecules such as lipid, protein and nucleic acids may be attacked by free radicals, of which lipids are probably most susceptible. The cells have different mechanism to alleviate and repair damaged macromolecules.

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 are throughout the body and the biochemical enzymes analyzed are SOD, Catalase, GPx.

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 organophosphorus 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 organophosphorus 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.

Collection of samples:

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 separated. The cells were washed with normal saline and RBC's were subjected to lysis. Activity of erythrocyte *superoxide dismutase* (SOD) was measured by the method of Marklund and Marklund (1974). 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.

The Catalase activity was determined by the method described by Sinha, (1972). Dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 with the formation of per chromic acid as an unstable intermediate. The chromic acetate thus produced was measured calorimetrically at 570-610 nm.

Erythrocyte *Glutathione peroxidase* (GPx) was assayed by Paglia and Valentine method (1967). GPx catalyzes the oxidation of glutathione by cumene hydroperoxide. In the presence of *glutathione reductase* and NADPH (reduced nicotinamide adenine dinucleotide phosphate), the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured.

RESULTS

The mean values of various antioxidant enzymes in the controls and exposed workers were presented in table 1 and also depicted as figure 1. The mean value of RBC SOD increased significantly in the exposed group as compared to RBC SOD level measured in the control group. The comparison of SOD activity between the healthy and symptomatic exposed workers showed greater increase as compared to the healthy group. Increase levels of SOD served as an index of the antioxidant status of the exposed workers. The increased activity of SOD observed in the OP pesticide-exposed group reflected an activation of the compensatory mechanism through the effects of pesticides on progenitor cells, and its extent depended on the magnitude of the oxidative stress. The elevated activity of CAT observed in the exposed workers could be due to the adaptive response to the generated free radicals, indicating the failure of the total antioxidant defense mechanism to protect the tissues from mechanical damage caused by exposure to OP pesticides, which is evidenced by increased LPO. GPx activity was also found to be significantly increased in pesticide sprayers when compared to controls ($p<0.001$). The increased activity of GPx could be due to the significant production of hydrogen peroxide in pesticide–induced toxicity

The cellular enzymes status in relation to period of exposure were presented in table 2 and also depicted as figure 2. As seen from the results, the mean values of RBC SOD, GPx, CAT, were significantly decreased in the workers belonging to the chronic exposure group as compared to acute exposure group.

The mean values of the enzymes measured in the three subsets of the exposed group were presented in table 3 and also depicted as figure 3. The workers involved in spraying the mixtures of methylated and ethylated OP pesticides (group–III) showed significantly elevated values of RBC SOD, CAT, GPx, in comparison to the values obtained in group–1 and group –II.

Table-1. The levels of intracellular enzymes in the controls and exposed group

Enzymes	Controls (n=50)	Exposed group	
		Healthy (n=75)	Symptomatic (n=50)
RBC SOD (I/U)	59.7±6.6	97.6±5.8*	117.6±7.1**
GPx (Unit/mg Hb)	11.37±4.16	21.57±5.05**	33.27±4.75**
Catalase (Unit /mg Hb)	61.89±16.7	120.31±17.72***	141.55±16.58***

Mean ± SD, n=50, n=125; *p<0.05; **p<0.01; ***p<0.001.

S D is indicated as (±); Statistical comparison: Group I compared with Group II

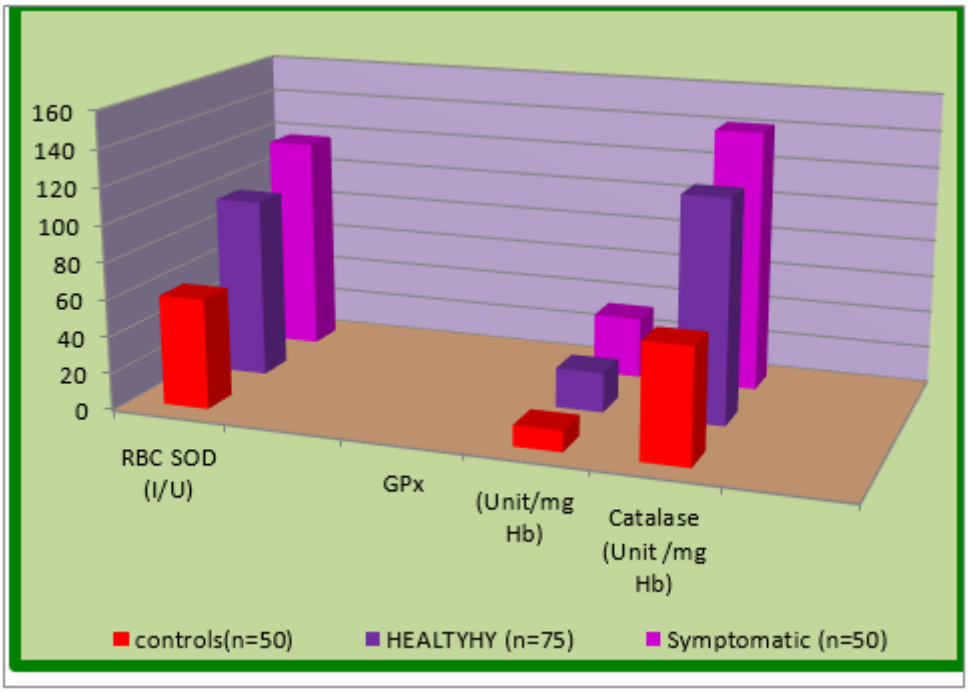

Figure: 1 Levels of Intracellular enzymes in Controls and Exposed Group

Table-2. Levels of intracellular enzymes in relation to duration of exposure

Enzymes	Exposed Workers (n=125)	
	Acute Exposure Group (n=75)	Chronic Exposure Group(n=50)
RBC SOD (I/U)	103.7±8.6	84.2±6.4*
Catalase (Unit /mg Hb)	131.27±14.68	107.59±15.20**
GPx (Unit/mg Hb)	15.96±2.21	9.65±2.31**

Mean ± SD, n=50, n=125; *p<0.05; **p<0.01; ***p<0.001.

Standard Deviation is indicated as (±); The Values are statistically significant when compared between the two exposure groups.

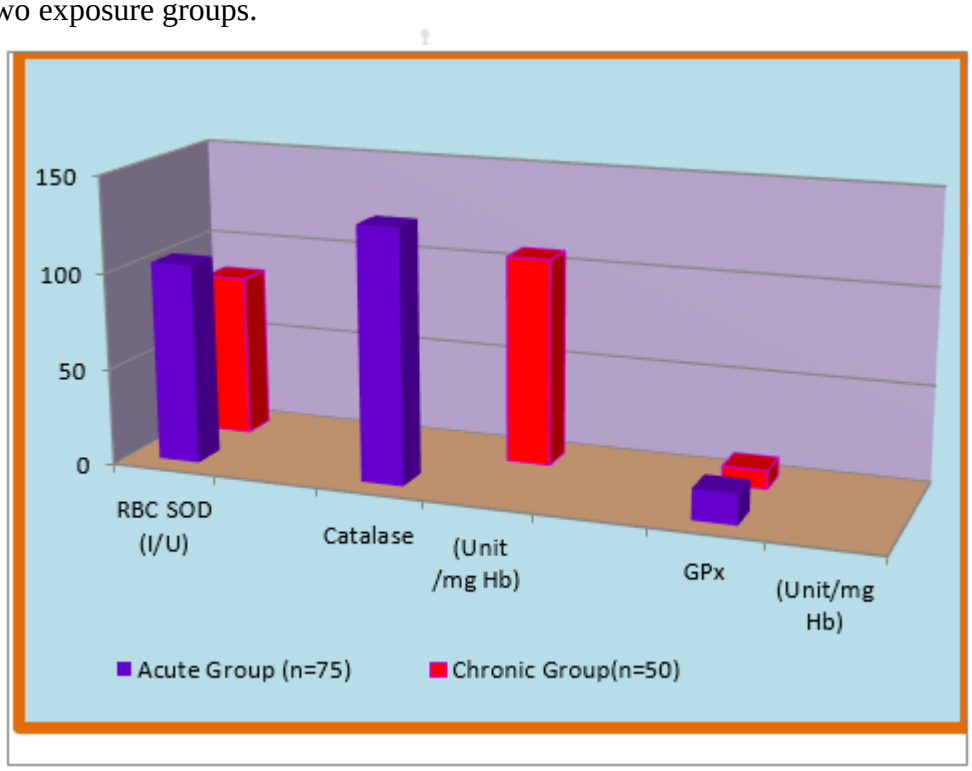

Figure: 2. Levels of intracellular enzymes in relation to duration of exposure

Table-3. The levels of different enzymes in various pesticide-exposed groups

Enzymes	Methylated Group-I (n=39)	Ethylated Group-II (n=41)	Mixed Group-III (n=45)
RBC SOD (I/U)	113.54±8.6	121.59±7.9	149.6±9.9*
(GPX, Unit/mgHb)	23.67±5.1	31.75±5.05	37.59±6.01*
Catalase (Unit/mgHb)	130.51±18.62	143.95±17.69	157.59±18.21*

Mean ± SD, n=50, n=125; *p<0.05; **p<0.01; ***p<0.001.

Standard Deviation is indicated as (±); The Values are statistically significant when compared between the two exposure groups.

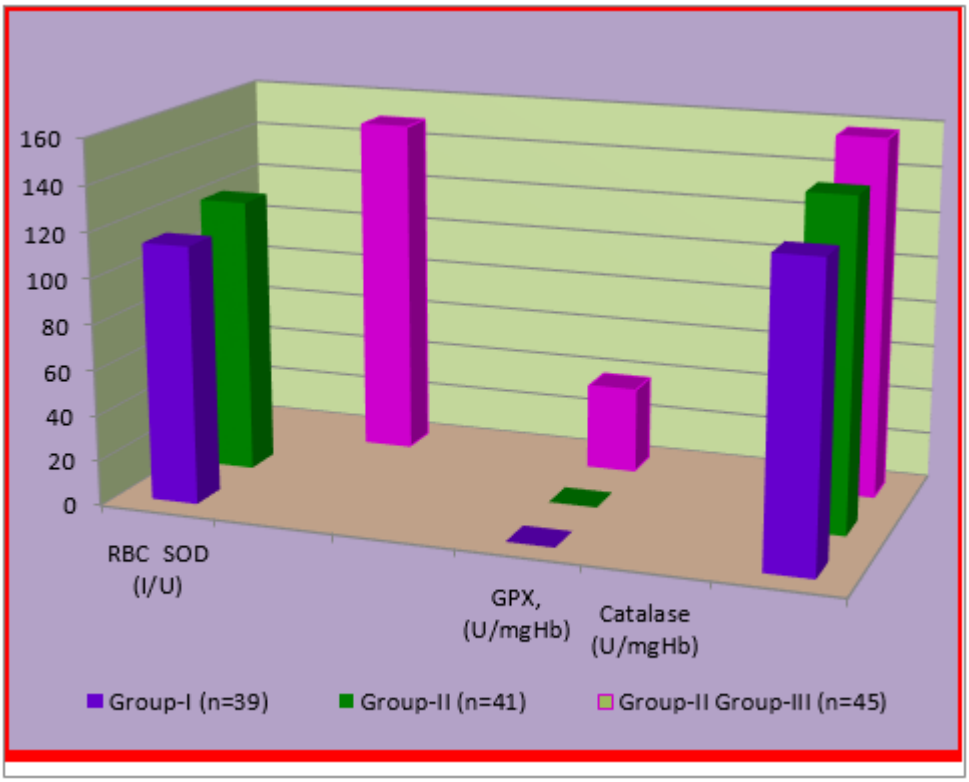

Figure-3. Levels of different enzymes in various pesticide-exposed groups

DISCUSSION

In the present study of Superoxide dismutase (SOD) values (in table: 1, 2 and 3 and also as figure 1, 2 and 3) were in the levels of intracellular enzymes SOD activities are increased significantly in the exposed group, in symptomatic exposed workers in particular time duration, and also in the mixed pesticide-exposed group.

In blood, normal erythrocyte function depends on the intactness of cell membrane which is the target for many toxic factors including pesticides. *Erythrocyte Superoxide dismutase* (SOD) and *Catalase* (CAT) are efficiently scavenging toxic free radicals and partly responsible for protection against lipid peroxidation due to acute or chronic pesticide exposure (El- Sharkawy et al, 1994; Agrawal et al, 1991).

The antioxidants enzymes SOD, CAT limits the effects of oxidant molecules on tissues and is active on the defense against oxidative cell injury by means of their being free radical scavengers (Jalaili et al, 2007; Kyle et al, 1987). The results of these antioxidants are well correlated with the earlier studies of Ayokulehin Kosoko, et al. (2014), Awad et al., (2014), Mishra, et al., (2013), Gargouri et al, (2011); Durak et al, (2008); Lopez et al, (2007); Verma and Srivastava, (2003); Vidyasagar et al, (2003). The above studies showed elevated levels of RBC-SOD activity with a parallel increase in the severity of poisoning showed that more the stress, more the free radicals are generated. The free radicals production will be so high that it even overwhelms the elevated antioxidant (SOD), failing to check lipid peroxidation, which partially effective in combating the oxidative damage. This call for the investigation of the involvement of other antioxidant enzymes such as *Catalase (*CAT); *Glutathione Peroxidase (GPX),* mainly in OP poisoning.

CONCLUSIONS

With the increase of the scientific knowledge, a mechanic revolution finding sprayers using machines needs to be revolutionized and agro-engineering must take care of a minimum exposure by the human beings so that contamination can be reduced to a maximum extent.

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