Research Article

Acute Toxicity of OP Pesticide Chlorpyrifos on Antioxidant Enzymes in Albino Rats

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Accepted on: 19-01-2016; **Finalized on:** 29-02-2016.

ABSTRACT

Albino rats were treated with sub lethal concentration ($1/10^{th}$ LD_{50} i.e., 20mg/kg body weight) of an organophosphate pesticide chlorpyrifos as single, double and multiple doses with 48 hr intervals. The toxic effect of chlorpyrifos is investigated by measuring the antioxidant enzyme activities Viz. Xanthine Oxidase (XOD), Super Oxode Dismutase (SOD) and Catalases (CAT). In the present study the activity of XOD is increased under chlorpyrifos stress, significant increased xanthine oxidase activity might be due to conversion of xanthine dehydrogenase to xanthine oxidase. The inhibition of SOD and Catalase (CAT) activities were observed, it shows that the impairment of antioxidant defense mechanism and reduction in molecular oxygen and it is due to the oxidative stress produces depleted activity of both the antioxidant enzymes.

Keywords: Chlorpyrifos, Antioxidant enzymes, Albino Rats.

INTRODUCTION

Toxicology is the study of adverse responses in biological systems that are caused by exposure to biological, chemical, or physical agents. Toxicologic research (typically performed in laboratory animals) is important for understanding the nature and mechanisms of adverse effects and their dependence on defined dose levels. Most of the pesticides are not specific in their action. They not only kill the pest but also affect the organisms whose control is not intended. Hence a general term "biocide" has been preferred by many authors in order to emphasize the overlapping ecological effects of such compounds. Based on the chemical nature of these pesticides they are classified into three general groups like Inorganic compounds including arsenicals, mercurials, borates and fluorides, Natural organic compounds derived from plants like nicotine, pyrethrum, rotenone and derris etc. and Synthetic organic compounds like organochlorides, organophosphates and carbamates[1]. Organophosphate (OP) pesticides are widely used because of their biodegradability[2].

The pesticides have been shown to cause oxidative stress, organophosphates might induce oxidative stress but the information on such ability is still incomplete[3]. Toxicity of organophosphates is caused mainly due to the inhibition of acetylcholine esterase. Cholinergic hyperactivity after the AChE inhibition initiates the accumulation of free radicals leading to lipid peroxidation, which may be the initiator of cell injury[4].

Chlorpyrifos is eliminated primarily through the kidneys in urine[5]. It is detoxified quickly in rats, dogs and other animals[6]. Animal tissues are constantly coping with high reactive oxygen species, such as super oxide anion, hydroxyl radicals, hydrogen peroxides and other radicals generation during numerous peroxides during numerous metabolic reactions[7,8]. The generation of small amount of free radicals appears to have an important biological function, but oxidative stress is caused by excess production of reactive species[9,10]. To protect cell organ system of the body against reactive oxygen species mammal cells are well equipped with a highly sophisticated and complex defense mechanism known both enzymatic and non enzymatic antioxidants.

Oxidative stress is defined as a disruption of the prooxidant - antioxidant balance in favor of the former, leading to potential damage[11]. It is a result of one of three factors: An increase in reactive oxygen species (ROS), an impairment of antioxidant defense systems or an insufficient capacity to repair oxidative damage. Damage induced by ROS includes alterations of cellular macromolecules such as membrane lipids, DNA, and/or proteins. The damage may alter cell function through changes in intracellular calcium or intracellular P^H, and eventually can lead to cell death[12]. The antioxidant enzymes such as Gpx, SOD and CAT may also have an important function in mitigating the toxic effects of ROS[13].

The first line of defense against O_2^- and H_2O_2 mediated injury are antioxidant enzymes; SOD, XOD and Catalase. The term antioxidant has been defined by Halliwell and Gutteridge[14] as "any substance that delays or inhibits oxidative damage to a target molecule". Anti oxidant enzymes together with the substance that are capable of either reducing reactive oxygen metabolites (ROM_S) or preventing their formation, form a powerful reducing buffer which affects the ability of the cell to counteract the action of oxygen metabolites. All reducing agents

there by form the protective mechanisms. Detoxification is a process of continuous reaction on particular chemical[15]. Detoxification of xenobiotics (foreign antigens) includes two major steps[16]. The primary phase involving oxidative, hydrolytic and other enzymatic pathways to produce polar end products. The secondary phase producing water soluble conjugates ready for excretion. The oxygen derived species resulting in oxidative injury is called oxidative stress[17].

Mammalian cells possess both enzymatic and non-enzymatic antioxidant defense mechanisms to cope up with oxygen free radicals. The enzymatic mechanism includes superoxide dismutase, catalase etc[18,19], where as non-enzymatic mechanism includes a variety of compounds as ascorbic acid and tocopherol etc.[20]. When the production of reactive oxygen species exceeds the ability of the antioxidant system, it results in oxidative stress. To prevent cellular damage by free radicals, free radicals mediated lipid peroxidation and tissue antioxidants are essential.

MATERIALS AND METHODS

Pesticide

Chlorpyrifos Technical (95.30%) was obtained from Nagarjuna Agri. Chem Limited, Ravulapalem Mandal, East Godavari District, A.P, India.

Pesticide stock solution

Stock solution of chlorpyrifos was prepared in acetone. Working pesticide test solutions were prepared by diluting the stock solution with distilled water.

Animal Model

Healthy adult albino rats of same age group (100±10 days) and weight (200±10 g) were obtained from the Indian Institute of Sciences (IISc) Bangalore, India. They were kept in well cleaned, sterilized cages and maintained conditions (25±2ºC and with 12 hr light, 12 hr darkness) food and water were allowed *ad libitum*.

Experimental Design

The toxicity of Chlorpyrifos was evaluated by probit method of Finney[21] and the LD_{50} of chlorpyrifos to albino rats was found to be 200 mg/kg bw. 1/10 of LD_{50} value (20mg/kg bw) was selected as sub letal dose. The animals were divided in to four groups having ten animals each. The first group animals treated as control animals. Second, third and fourth groups of animals were termed as experimental animals.

To the animals of second group single dose of pesticide (i.e. on first day) was administered orally by gavage method.

To the third group of animals double doses were given i.e. on 1st and 3rd day. Similarly multiple doses i.e. 1st , 3rd , 5th and 7th day were given to the fourth group of animals. After stipulated time the animals were sacrificed and collected the tissues like liver and kidney for the estimation of antioxidant enzyme activities.

Estimation of Antioxidant Enzyme Activities

Xanthine oxidase (XOD: CE. 1.17.3.2)

Xanthine oxidase activities were estimated by the dye reduction method of Srikanthan and Krishnamoorthy[22]. The assay mixture contained 100 mM sodium phosphate buffer (PH 7.4), 50 µ M of INT and the enzyme source. The reaction was initiated by the addition of enzyme source and incubated at 37°C for 30 minutes. The reaction was stopped by the addition of 5 ml of glacial acetic acid and the formazon formed overnight was extracted in toluene and read at 495nm against toluene blank. The activity was expressed as µM of formazon formed/mg protein/hour.

Superoxide dismutase (SOD: EC. 1.15.1.1)

The activities of SOD were assayed by the reduction of nitro blue tetrazolium. Here the superoxide was produced by riboflavin mediated photochemical reaction system. Superoxide dismutase activity was determined according to the method of Beachamp and Fridovich[23]. Liver and kidney tissues were homogenized in ice cold 50mM phosphate buffer (PH 7.0) containing 0.1 mM EDTA to give 5% homogenate (w/v). The homogenate were centrifuged at 10,000 rpm for 10 minutes at 0 °C in cold centrifuge. The supernatant was separated and used for enzyme assay. The reaction mixture contained 1.7 ml of phosphate buffer (PH 7.8), 150 ml EDTA (10 mM), 600 ml methionine (130 mM), 300 ml nitro blue tetrazolium (750 mM) and the enzyme source. The reaction was initiated by the addition of riboflavin and the samples were placed under 15 watts fluorescence bulb for 30 minutes and the absorbance was taken at 560 nm against reagent blank kept in a dark place. A system, devoid of any superoxide radical scavenger was used as a positive control to compare the results. The activity of the enzyme was expressed as units/mg protein.

Catalase activity (CAT: EC. 1.11.1.6)

Catalase activities were measured by a slightly modified version of Aebi[24] at room temperature. Liver and kidney tissues were homogenized in ice-cold 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give 5% homogenate (w/v). The homogenates were centrifuged at 10,000 rpm for 10 minutes at 0 °C in cold centrifuge.

The resulting supernatant was used as an enzyme source. 10 µl of 100% ethyl alcohol was added to 100 µl tissue extract and then placed in an ice bath for 30 min. After 30 min the tubs were kept at room temperature followed by the addition of 100 µl of Triton X- 100 RS.

In a cuvette containing 200 µl of phosphate buffer, 50µl of tissue extract and 250 µl of 0.066 M H_2O_2 (in phosphate buffer) was added and decrease in optical density was measured at 240 nm for 60 seconds in a UV spectrophotometer. The molar extinction coefficient of

43.6 µcm^{-1} was used to determine Catalase activity. One unit of activity is equal to the moles of H_2O_2 degraded/mg protein/min.

RESULTS

The results of XOD, SOD and Catalase (CAT) activities of liver and kidney tissues of control and experimental rats under chlorpyrifos toxicity were mentioned in tables 1, 2 and 3 respectively. The experimental rats showed statistically significant ($p<0.01$) enhancement in XOD activities, where as SOD and Catalase activities significant ($p<0.01$) decreased. Alterations in enzyme activities of liver and kidney tissues were in the form of a dose and time dependent manner.

Table 1: Changes in Xanthine Oxidase (XOD) activity (µ moles of formazon formed/mg protein/hr) in different tissues of control and chlorpyrifos treated albino rats. Values in parentheses indicate percent change over control.

Name of the tissue	Control	Single Dose	Double Dose	Multiple Dose
Liver Mean SD PC	0.981 ±0.033	1.160 ±0.120 (18.255)	1.514 ±0.108 (54.258)	1.861 ±0.050 (89.687)
Kidney Mean SD PC	0.788 ±0.026	1.041 ±0.054 (32.074)	1.216 ±0.097 (54.208)	1.497 ±0.024 (89.781)

All the values are mean ± SD of six individual observations; SD – Standard Deviation; PC – Percent change over control.

Table 2: Changes in Superoxide Dismutase (SOD) activity (units of superoxide anion reduced/mg protein/min.) levels in liver and kidney tissues of control and chlorpyrifos treated albino rats. Values in parentheses indicate percent change over control.

Name of the tissue	Control	Single Dose	Double Dose	Multiple Dose
Liver Mean SD PC	5.657 ±0.639	4.793 ±0.427 (-14.936)	3.657 ±0.639 (-33.675)	3.043 ±0.217 (-43.810)
Kidney Mean SD PC	3.457 ±0.425	3.165 ±0.314 (-8.446)	2.624 ±0.354 (-24.096)	2.043 ±0.224 (-40.902)

All the values are mean ± SD of six individual observations; SD – Standard Deviation; PC – Percent change over control.

Table 3: Changes in Catalase Activity (µ moles of H_2O_2 decomposed/mg protein/min) levels in liver and kidney tissues of control and chlorpyrifos treated albino rats. Values in parentheses indicate percent change over control.

Name of the tissue	Control	Single Dose	Double Dose	Multiple Dose
Liver Mean SD PC	0.311 ±0.009	0.275 ±0.011 (-11.61)	0.183 ±0.004 (-41.244)	0.151 ±0.002 (-51.507)
Kidney Mean SD PC	0.249 ±0.006	0.199 ±0.024 (-20.032)	0.147 ±0.009 (-41.105)	0.129 ±0.001 (-48.237)

All the values are mean ± SD of six individual observations; SD – Standard Deviation; PC – Percent change over control.

DISCUSSION

The basis of pesticide toxicity in the production of reactive oxygen species may be due to 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 H_2O_2 formation through dismutation reaction. Generation of free radicals probably because of the alterations in the normal homeostasis of the body resulting in oxidative stress, if the requirement of continuous antioxidants is not maintained[25].

The elevated levels of xathine oxidase in the present investigation indicates the over production of superoxide anions $(O_2^{\cdot -})$ in the liver and kidney tissues of albino rats in response to chlorpyrifos treatment. Under chlorpyrifos stress significant increased xanthine oxidase activity (Table.1) might be due to conversion of xanthine dehydrogenase to xanthine oxidase. For nitrogen balance of the tissue, xanthine oxidase is produced when the native form of xanthine dehydrogenase is altered either by sulphydryl oxidation or by limited proteolysis[26]. During the apoptosis in rat mammary gland, the mitochondrial XOD activity was increased[27]. In *Boleophthalmus pectinirostris* liver the heavy metal cadmium (Cd^{2+}) caused an increased XOD activity levels[28]. The increased XOD and decreased SOD, Catalase activities were observed in albino mice under fluoride toxicity[29].

Superoxide dismutase (SOD) and Catalase (CAT) have been detected in a wide variety of mammalian cells. Superoxide dismutase and catalase are generally involved in the detoxification of superoxide anion radical generated by xanthine oxidase. These enzymes have an important role in protecting the cell against the toxic effects of toxic pollutants[30]. Superoxide dismutase catalyzes the dismutation of the superoxide ion (O2G) to hydrogen peroxide and oxygen molecule during oxidative energy processes. The reaction diminishes the destructive oxidative processes in cells. According to Nelson and Cox[31]; Sathyanarayana[32] catalases play an important role in protection of cell from the hydrogen peroxide toxicity.

In the present study the superoxide dismutase activity was decreased (table. 2) according to the doses. This result was in agreement with the result of Manna[33]. During repeated dose toxicity of deltamethrin in rats, the superoxide dismutase and catalase activity levels were depleted significantly in different tissues[34]. Hexachlorohexane (HCH) effect on immature chick tissues decreased SOD activity[35].

Some workers were also observed the decreased levels of SOD and catalase in different animal models under toxic stress conditions. SOD activity was significantly inhibited in both the brain and liver of albino rat during the development of behavioral tolerance to organophosphate compound phosphomidon[36]. A gradual decrease in catalase activity was observed after Isoproterenol administration in to the tissues of rats[37].

Free radicals cause cell injury when they are generated in excess or when the antioxidant defense is impaired. Catalase activity decreased significantly in the cyfluthrin treated tissues of albino rats[38]. Effects of some environmental parameters on catalase activity measured in the mussel (*Mytilus galloprovincialis*) exposed to lindane[39]. Ferrari[40] reported the decreased catalase content in liver and kidney of rainbow trout. The early inhibitory effect in CAT activity may be associated with a high degree of oxidative stress. The decresed activities of SOD and Catalase (CAT) were observed in the tissue of liver, brain and kidney tissue of Channa punctaurs during sublethal concentration of triazophos[41].

Several studies with liver, brain and tests indicate that lindane and Endosulfan causes Oxidative stress[42-44]. The decreased SOD, Catalase and increased XOD activities were observed in Endurance exercise-induced albino male rats[45]. It is observed that the pesticides produce oxidative stress by inhibiting the activity of SOD.

CONCLUSION

It is observed that the organo phosphorus pesticide chlorpyrifos influences oxidative stress and antioxidant capacity in the liver and kidney tissues of albino rats. The elevated levels of xathine oxidase (XOS) in the present investigation indicates the over production of superoxide anions $(O_2^{\cdot -})$ in the liver and kidney tissues of albino rats in response to chlorpyrifos treatment. The decreased SOD activities shows chlorpyrifos produces oxidative stress by inhibiting activity of SOD and the decreased catalase activity reduces peroxidative damage in the tissues in order to modulate the levels of antioxidants. In conclusion, it can be stated that alteration in antioxidant enzyme activities were more pronounced in liver tissues than kidney tissues of rats dose and time dependent manner and the chloropyrifos exposure causes for induction of oxidative stress.

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Source of Support: Nil, **Conflict of Interest:** None.