THE TOXICITY INFLUENCE OF ORGANOPHOSPHATE PESTICIDE CHLORPYRIFOS ON ANTIOXIDANT ENZYMES IN DIFFERENT TISSUES OF ALBINO MICE

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ABSTRACT: ***Pesticides such as organophosphorus and organochlorine compounds commonly used in agriculture for achieving better quality products are toxic substances and lead to generation of Reactive Oxygen Species (ROS) which have harmful effects on human health. In the present study Healthy adult mice of same age (100±10 days) and weight (75±10 g) were divided into four groups having ten animals each. Albino mice were treated with sub acute concentration (1/10th LD_{50}) 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. The experimental mice exposed to chlorpyrifos showed statistically significant (P<0.01) increase of Xanthine oxidase(XOD), where as significant (P<0.01) decrease in Superoxide dismutase (SOD) activity and significant (P<0.01) decrease of Catalase activity, levels in Heart, Liver, Kidney and Muscle respectively. The activity levels were dose and time dependent manner in chlorpyrifos treated mice. The inhibition of SOD and Catalase (CAT) activities were 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.***

Key Words: Chlorpyrifos, XOD, SOD, Catalase, Heart, Liver, Kidney and Muscle, Albino Mice

I. NTRODUCTION

Organophosphates are considered as the safest of highly potent insecticides. Higher rates of enzymatic biotransformation, detoxification and excretion are responsible for their lower toxicity for mammals. In insects this potency is five fold less compared to mammals due to the lower body temperature and partly slower enzymatic detoxification. The small body size gives less time for the compound to be detoxified before reaching the target site**.** Organophosphate toxicity alters the antioxidant enzymes due to cell stress with the involvement of free radical intermediates of organophosphates. 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 (Castillo *et al.*, 1992; Cabre *et al.*, 2000)**.** 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 (Halli well., 1997; Giardiano, 2005). 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 (Sies, 1991). 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 (Kehrer *et al*., 1990). Under normal condition, excessive formation of free radicals and concomitant damage at cellular and tissue concentrations is controlled by cellular defense systems. These preventive defense systems can be accomplished by enzymatic and non–enzymatic mechanisms including vitamin E and Glutathione. The antioxidant enzymes such as XOD, SOD and CAT may also have an important function in mitigating the toxic effects of ROS (Adali *et al*., 1999).

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 (1990) 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. Which maintain the lowest possible levels of reactive oxygen metabolites inside the cell (Sies, 1997)

Xanthine is formed from the degradation of ATP and reoxygenation. Xanthine oxidase path way is one of the important sites of free radical production. Xanthine oxidase generates oxygen radicals and uric acid from xanthine (MC Cord, 1993). Xanthine oxido reductase under physiological conditions acts as a dehydrogenase (XDH). The dehydrogenase form of xanthine oxido reductase under metabolic stress like hypoxia and ischemia converts to an oxidase form (XOD).

Xanthine oxidase involved in major pathway of purine nucleotide catabolism in animals (Bray *et al.,* 1996). It converts hypoxanthine and xanthine to uric acid. It is thought that xanthine oxidase has an important role in reperfusion injury. One of the mechanisms proposed is that under hypoxia conditions the depletion of cell ATP results in an elevated cytosolic concentration of AMP which is catabolized to adenosine, inosine and then hypoxanthine concomitantly the conversion of XDH in to XOD occurs by a protease, probably activated by an elevated cytosolic calcium concentration during ischemia. When, reperfusion takes place, the return of oxygen takes place to a production of Super oxide (Pasquier *et al*., 1989).

Xanthine oxidase (XOD) is reported to play an important role in cellular oxidative status, detoxification of aldehydes, oxidative injury in ischemia reperfusion, and neutrophils mediation . For example, XOD may serve as a messenger or mediator in the activation of neutrophil, T cell, cytokines or transcriptions in defense mechanisms rather than as after radical generator of tissue damage. Emerging evidence on the synergistic interactions of O_2^- a toxic product of XOD and nitric oxide. Another illustration of XOD involvement in tissue injury and cytotoxicity in an emergent condition such as ischemia or inflammation.

Super oxide dismutase (SOD) is the primary antioxidant enzyme in the cell and cellular defense against superoxide radicals is provided by the enzyme Super oxide dismutase. Among, other antioxidants enzymes, SOD considered as front line of defense against the potentially cytotoxic free radical oxidative stress. The SOD catalyzes the dismutation of two superoxide (O_2^-) radicals in to hydrogen peroxide (H_2O_2) and oxygen. These enzymes obey first order reaction kinetics and the forward rate constants are almost diffusion limited. This results in steady state concentration of Super oxide radicals in tissues that may vary directly with the rate of Super oxide generation and inversely with the tissue concentration of scavenging enzymes (Enghild *et al*., 1999, Fattman *et al*., 2003).

SOD is the most important antioxidant enzyme because it is found virtually in all aerobic organisms. SODs are a family of metalloenzymes that converts O_2^- to H_2O_2 according to the following reaction. The transition metal of the enzyme reacts with O_2^- taking its electron. O_2- is the only known substrate for SOD (Ray and Hussain, 2002).

$$O_2^- + O_2^- \xrightarrow[SOD]{2H} H_2O_2 + O_2$$

The Super oxide dismutase (SOD) enzyme catalyzes the dismutation of two superoxide radicals in to hydrogen peroxide and oxygen. The Hydrogen peroxide is further oxidized by enzymes. These enzymes obey first order reaction kinetics and the forward rate constants are almost diffusion limited. This results in a steady state concentration of super oxide in tissues that varies directly with the rate of super oxide generation and inversely with the tissue concentration of scavenging enzymes (Enghild *et al.,* 1999).

Catalase is one of the most important antioxidant enzyme, which can function either in the catabolism of hydrogen peroxide (H_2O_2) or in the peroxidative oxidation of substances, such as pesticides. Catalase has four sub units each sub unit contains a heme group; heme consists of a proto porphyrine ring and a central (Fe) atom. The iron either in the ferrous (Fe^{2+}) or the ferric (Fe^{3+}) oxidative state this heme group is responsible for carrying out catalase activity. To maintain catalytic activity CAT requires $Fe2^+$ as a co factor (Powers and Lennon, 1999, Temel *et al.,* 2002).

Catalase is widely distributed enzyme in the body compartments, tissues and cell. In many cases the enzyme is located in sub cellular organelles such as, peroxisomes and cytosol (Lesiuk *et al.,* 2003). Catalase is a tetrameric peroxidative enzyme which converts the hydrogen peroxide to water and molecular oxygen and whose gene expression is regulated by H_2O_2. Catalase plays an important role in ROS metabolism and an adaptation to oxidant stress (Vaziri *et al*., 2003). Catalase catalysis the destruction of hydrogen peroxide in to water and oxygen by the following reaction.

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

H_2O_2 is produced in the cells by a number of enzymatic reactions including those catalyzed by SOD, which converts super oxide anion radical to hydrogen peroxide and water (Fredovich, 1995).

II. EXPERIMENTAL DESIGN

Species: Mice

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

Concentration selected: Tenth fold ($1/10^{th}$) lower concentration of LD_{50} was selected for sublethal treatment to the experimental mice.

Course of study: Single, double and multiple doses with 48 hours interval.

Route of administration: Oral

Tissues selected: Heart, liver, kidney, muscle, intestine, testes and blood.

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.

Selection of sublethal treatment to the experimental model: As the acute oral LD_{50} value of chlorpyrifos was determined, tenth fold lower ($1/10^{th}$) concentration was selected as sublethal to study the effect of chlorpyrifos. Healthy adult mice of same age (100±10 days) and weight (75±10 g) were divided into four groups having ten animals each. The 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 1^{st} day) was administered orally by gavage method. To the third group of animals double doses were given i.e. on 1^{st} and 3^{rd} day. Similarly multiple doses i.e., 1^{st}, 3^{rd}, 5^{th} and 7^{th} day were given to the fourth group of animals. The first group of animals was considered as controls.

Isolation of tissues: The control and experimental animals after the stipulated period (i.e. on 9^{th} day) were sacrificed and the tissues were isolated, cleaned in physiological saline and processed immediately for microscopic analysis. The tissues were also quickly isolated under ice cold conditions and stored in deep freezer at -80ºC for biochemical analysis.

Procurement of experimental animals

Healthy wistar strain mice of the same age group 100±10 days and weight 75±10 grams were selected as experimental animals for the present study. The mice were collected from Indian Institute of Science (I.I.Sc.), Bangalore. Prior to experimentation the animals were acclimatized according to the instructions given by Behringer (1973).

Maintenance of animals

The mice were maintained at laboratory conditions in the animal house at 25±2°C with a photoperiod of 12hrs light and 12hrs darkness throughout the course of the present study. The mice were fed with standard pellet diet supplied by Sai Durga feeds and foods, Bangalore and water *ad libitum*.

Pesticide selected

Chlorpyrifos, an organophosphate insecticide was selected for the present investigation.

Chlorpyrifos O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate with 95.30% purity was used as the test chemical for the present study. Technical grade chlorpyrifos was obtained from Nagarjuna Agri. Chem Limited, Ravulapalem Mandal, East Godavari District, A.P., India. Chlorpyrifos has a wide applicability and safety compared to other compounds of its class. Hence this pesticide was selected for the present study.

III. TOXICITY EVALUATION OF CHLORPYRIFOS

Lethal dose of chlorpyrifos was determined by "Probit method" of Finney (1971). Mice were treated with different concentrations of chlorpyrifos by oral intubation. Dose and mortality were noted and a graph was plotted between chlorpyrifos concentration and probit kill. LD_{50} was the dose at which 50% of the test animals were killed.

Since the toxicity of a chemical depends upon many biotic and abiotic factors. The general conditions such as temperature, humidity, food and water supply etc., were maintained constant to the maximum possible extent during experimentation.

III. BIO CHEMICAL INVESTIGATION OF DETOXIFICATION ENZYMES:

1. Estimation of xanthine oxidase

Xanthine oxidase activity was estimated by the dye reduction method of Srikanthan and Krishnamoorthy (1955). 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 37ºC 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.

2. Estimation of superoxide dismutase

The activity of SOD was 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 (1971). Different 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 ºC 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 (750mM) 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.

3. Estimation of catalase activity

Catalase activity was measured by a slightly modified version of Aebi (1984) at room temperature. Different 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 ºC 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 $H_2 0_2$ (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 $\mu c.m^{-1}$ was used to determine Catalase activity. One unit of activity is equal to the moles of H_20_2 degraded/ mg protein/ min.

Statistical treatment of the data

The mean, standard deviation (SD), percent change and one – way analysis of variance (ANOVA) (Steel and Torrle, 1960) were performed using the SPSS package programming techniques on "Intel Core 2 Duo Processor" personnel computer. Probability values less than 0.05 were considered significant (Snedecor and Cochran, 1968).

IV. RESULTS:

Xanthine oxidase (XOD)

The results of xanthine oxidase levels of the control and experimental mice under chlorpyrifos are given in (Table 1 and Figure 1). The experimental mice exposed to chlorpyrifos showed statistically significant ($P<0.01$), an increase of Xanthine oxidase levels in heart, liver, kidney and muscle respectively. The increase in xanthine oxidase levels were dose and time dependent manner in chlorpyrifos treated mice.

In experimental conditions the tissues have shown increased xanthine oxidase levels in heart (136.12%) followed by kidney (75.5%), liver (59. 9%), and muscle (55.99%) in multiple doses. The maximum increase was observed in multiple doses compared double and single dose chlorpyrifos treated mice. The lyotrophic series of increased XOD content in multiple dose chlorpyrifos treated mice is as follows:

Heart > Kidney> Liver > Muscle

Superoxide dismutase (SOD)

The results of superoxide dismutase activity levels of the control and experimental mice under chlorpyrifos are given in (Table 2 and Figure 2). The experimental mice exposed to chlorpyrifos showed statistically significant ($P<0.01$), and decrease in superoxide dismutase activity

levels in heart, liver, kidney and muscle respectively. The decrease in superoxide dismutase activity levels were dose and time dependent manner in chlorpyrifos treated mice.

In experimental conditions the tissues have shown decreased superoxide dismutase activity levels in heart (42.00%), followed by kidney (30.99%), liver (20.65%) and muscle (17.59%) in multiple doses. The maximum decrease was observed in multiple doses followed by double and single dose chlorpyrifos treated mice. The lyotrophic series of superoxide dismutase activity decreased in multiple doses chlorpyrifos treated mice is as follows:

Heart > Kidney > Liver > Muscle

Catalase (CAT)

The results of catalase activity levels of control and experimental mice under chlorpyrifos are given in (Table 3 and Figure 3). The experimental mice exposed to chlorpyrifos showed statistically significant ($P<0.01$) decrease of catalase activity levels in heart, liver, kidney and muscle respectively. The decrease in catalase activity levels were dose and time dependent manner in chlorpyrifos treated mice.

In experimental conditions the tissues have shown decreased catalase activity levels in liver (58.48%) followed by kidney (42.69%), heart (39.47%) and muscle (32.05%) in multiple doses. The maximum decrease was observed in multiple doses followed by double and single dose chlorpyrifos treated mice. The lyotrophic series of catalase activity decrement in multiple doses chlorpyrifos treated mice is as follows:

Liver > Kidney > Heart > Muscle

V. DISCUSSION

Xanthine Oxidase (XOD)

In the present investigation the xanthine oxidase levels were increased in all the tissues of mice in single dose, double dose and multiple dose. 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 (Dellacorte and Stripe, 1972). During the apoptosis in rat mammary gland, the mitochondrial XOD activity was increased (Rus *et al.,* 2007).

The elevated levels of xathine oxidase in the present investigation indicates the over production of superoxide anions $(O_2^{\bullet -})$ in the different tissues of mice in response to chlorpyrifos treatment. In *Boleophthalmus pectinirostris* liver the heavy metal cadmium (Cd^{2+}) caused an increased XOD activity levels (Liu *et al.,* 2006).

Superoxide dismutase (SOD) and Catalase (CAT)

Superoxide dismutase (SOD) and catalase (CAT) are involved in the detoxification of reactive oxygen species generated during the chlorpyrifos administration, the SOD and catalase activities were estimated in different tissues of mice. Both the enzyme activities were decreased with increase of doses. The decreasing activity reflects the oxidative status of different tissues. Because of the high concentration of polyunsaturated fatty acids (PUFA) and aerobic metabolic activity of different tissues, increase the susceptibility of these organs to peroxidative damage induced by reactive oxygen species after chlorpyrifos administration. So due to chlorpyrifos impact different tissues (heart, liver, kidney, muscle) undergo damage through free radicals. This oxidative stress produces depleted activity of both the antioxidant enzymes such as superoxide dismutase and catalase (Table 2 to 3). Some workers were also observed the decreased levels of SOD and catalase in different animal models under toxic stress conditions.

Superoxide dismutase and catalase are generally involved in the detoxification of superoxide anion radical generated by xanthine oxidase. In the present study the superoxide dismutase activity was decreased according to the doses. This result was in agreement with the result of Manna *et al.,* (2004). According to Manna *et al.,* (2004) the superoxide dismutase and catalase levels were decreased. During repeated dose toxicity of deltamethrin in rats, the superoxide dismutase and catalase activity levels were depleted significantly in different tissues (Manna *et al.,* 2005).

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 (Ryrfeldt *et al.,* 1992). Hexachlorohexane (HCH) effect on immature chick tissues decreased SOD activity (Seth *et al.,* 2000). SOD activity was significantly inhibited in both the brain and liver of albino rat during the development of behavioral tolerance to organophosphate compound phosphomidon (Venkateswara Rao, 1993).

A gradual decrease in catalase activity was observed after Isoproterenol administration in to the tissues of rats (Rathore *et al.,* 2000). The hepatic tissue catalase activity was consistently decreased in response to the chlordane dose administration when compared to the control animals (Vani, 1991). On the other hand some workers observed a recovery trend in depleted catalase activity. According to Sudhakar Reddy (2003) the hepatic catalase activity of fish, crab and snail was gradually decreased under the sublethal exposure of copper and mercuric chloride with increased time of exposure. After 15 days of post exposure, all the animals were recovered from decreased catalase activity. Free radicals cause cell injury when they are generated in excess or when the antioxidant defense is impaired. Both these processes seem to be affected in schizophrenia. Rukmini *et al.,* (2004) reported that the superoxide dismutase and catalase activities were decreased in schizophrenia due to cell damage by free radicals.

Catalase activity decreased significantly in the cyfluthrin treated tissues of albino rats. This means hydrogen peroxide generation plays important role in the toxicity of cyfluthrin (Omotuyii, 2006). Effects of some environmental parameters on catalase activity measured in the mussel (*Mytilus galloprovincialis*) exposed to lindane (Khessiba *et al.,* 2005). Ferrari, (2007) 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. Verma Radhey and Srivastava (2003) reported decrease in CAT in liver kidney and spleen. The data provide evidence for induction of oxidative stress on Chlorpyrifos exposure.

According to Alka Gupta *et al.,* (1999) in albino rat's continuous exposure of quinolphos up to PND 45 the SOD and catalase activities were decreased as 63% and 31% respectively. A synthetic, pyrethroid deltamethrin significantly decreased the SOD and catalase activities in

albino rats (Manna *et al.*, 2005). Several studies with liver, brain and tests indicate that lindane and Endosulfan causes Oxidative stress. (Junqueira *et al.*, 1998; Dorval *et al.*, 2003; Frederick and Panemangalore, 2003 ; Abdollahi *et al.*, 2004).

VI. CONCLUSION:

The present investigation clearly stated that Xanthine oxidase (XOD) activity levels were elevated in all the tissue with increase of dose. The elevated levels of XOD activity indicates the over production of oxygen free radicals in response to the chlorpyrifos toxicity. Superoxide dismutase (SOD) activity was assayed to observe the levels of detoxification of superoxide anion radicals. SOD activity was decreased with increase of doses. The SOD activity reflects the oxidative status of tissues. So, due to the chlorpyrifos impact, the cells of different tissues damaged by free radicals. This oxidative stress produces depletion activity of SOD. Catalase activity (CAT) was estimated to assess the hydrogen peroxide reduction potential of different tissues. The CAT also depleted like SOD with the increase doses of chlorpyrifos. It is due to cell damage resulting in oxidative stress leading to the depletion of CAT activity. It can be stated that alteration in antioxidant enzyme activities were more pronounced in the tissues of mice dose and time dependent manner and the chloropyrifos exposure causes for induction of oxidative stress.

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

Name of the tissue	Control	Single Dose	Double Dose	Multiple Dose
Heart				
Mean	0.2425	0.4033	0.452	0.5726
SD	±0.0150	±0.0141	±0.0142	±0.0148)
PC		(66.3092)*	(86.3917)*	(136.1237)*
Liver				
Mean	0.7271	0.9288	1.1138	1.155
SD	±0.0156	±0.01809	±0.0150	±0.0155
PC		(28.6248)*	(54.2445)*	(59.9501)*
Kidney				
Mean	0.6428	0.7233	0.904	1.1146
SD	±0.155	±0.0157	±0.0163	±0.0128
PC		(12.5233)**	(40.6347)*	(75.5121)*
Muscle				
Mean	0.317	0.383	0.4241	0.4945
SD	±0.0151	±0.0162	±0.0136	±0.0125
PC		(20.8201)*	(33.7854)*	(55.9936)*

All the values are mean ± SD of six individual observations.
SD – Standard Deviation.
PC – Percent change over control.
* Significant P<0.001
** Significant P<0.05
*** Significant P<0.01

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

Name of the tissue	Control	Single Dose	Double Dose	Multiple Dose
Heart				
Mean	1.9226	1.8243	1.523	1.1151

SD	±0.01452	±0.01240	±0.01483	±0.0136
PC		(-5.1128)***	(-20.7843)*	(-42.000)*
Liver				
Mean	4.329	4.6925	3.9828	3.4348
SD	±0.1571	±0.10324	±0.1837	±0.1331
PC		(-8.3968)***	(-7.9972)***	(-20.6560)*
Kidney				
Mean	4.318	3.9156	3.4193	2.9795
SD	±0.1380	±0.1232	±0.1399	0.1758
PC		(-9.3191)***	(-20.8128)*	(-30.9981)*
Muscle				
Mean	1.9853	1.9898	1.8286	1.636
SD	±0.1869	±0.1726	0.1368	0.1292
PC		(-0.2266)***	(-7.8930)***	(-17.5973)**

All the values are mean ± SD of six individual observations.
SD – Standard Deviation.
PC – Percent change over control.
* Significant P<0.001
** Significant P<0.05
*** Significant P<0.01

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

Name of the tissue	Control	Single Dose	Double Dose	Multiple Dose
Heart				
Mean	0.1748	0.1633	0.1441	0.1058
SD	±0.0118	±0.0144	±0.0136	±0.0161
PC		(-6.5789)***	(-17.5629)**	(-39.4736)*
Liver				
Mean	0.2963	0.2541	0.1635	0.123
SD	±0.01927	±0.0140	±0.0149	±0.0153
PC		(-14.2423)**	(-44.8194)*	(-58.4880)*
Kidney				
Mean	0.1991	0.1735	0.1531	0.1141
SD	±0.0171	±0.0152	±0.01467	±0.0108
PC		(-12.85788)*	(-23.1039)*	(-42.6921)*
Muscle				
Mean	0.1538	0.1356	0.1175	0.1045
SD	±0.01431	±0.01348	±0.01298	±0.01429
PC		(-11.8335)**	(-23.6020)*	(-32.0546)*

All the values are mean ± SD of six individual observations.
SD – Standard Deviation.
PC – Percent change over control.
* Significant P<0.001
** Significant P<0.05
*** Significant P<0.01

Fig.1 Xanthine Oxidase levels in different tissues of mice exposed to chloropyrifos

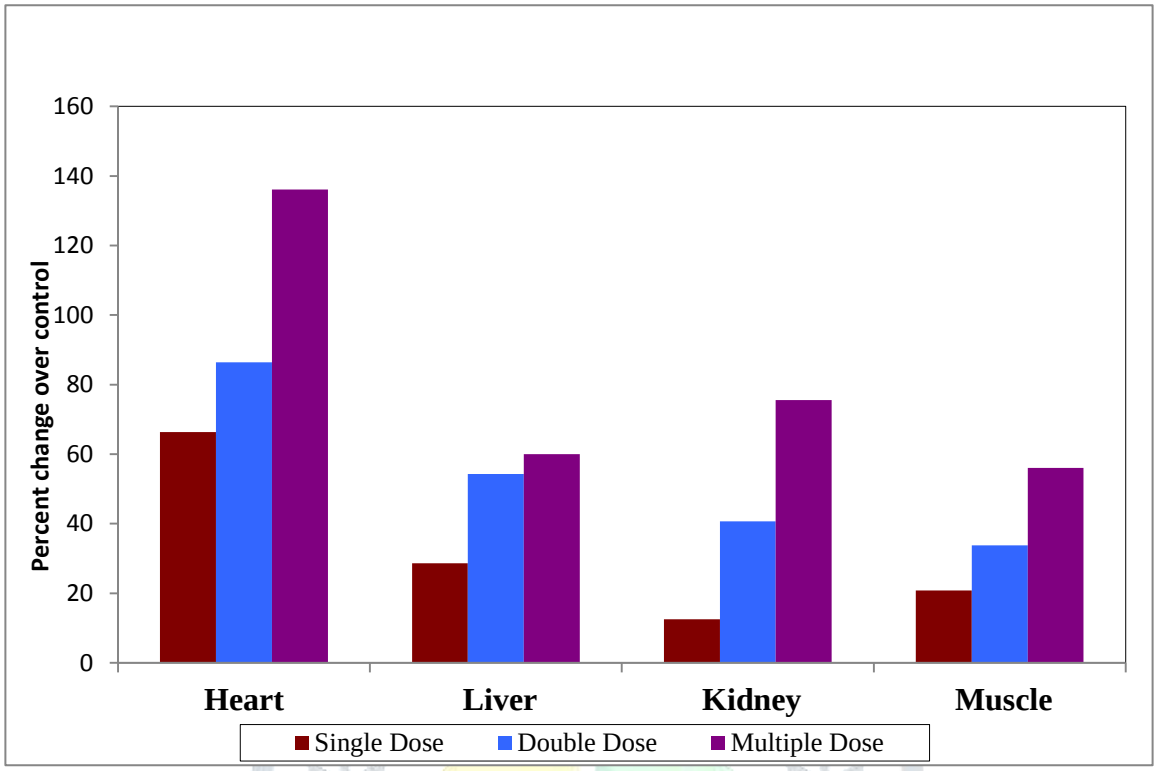
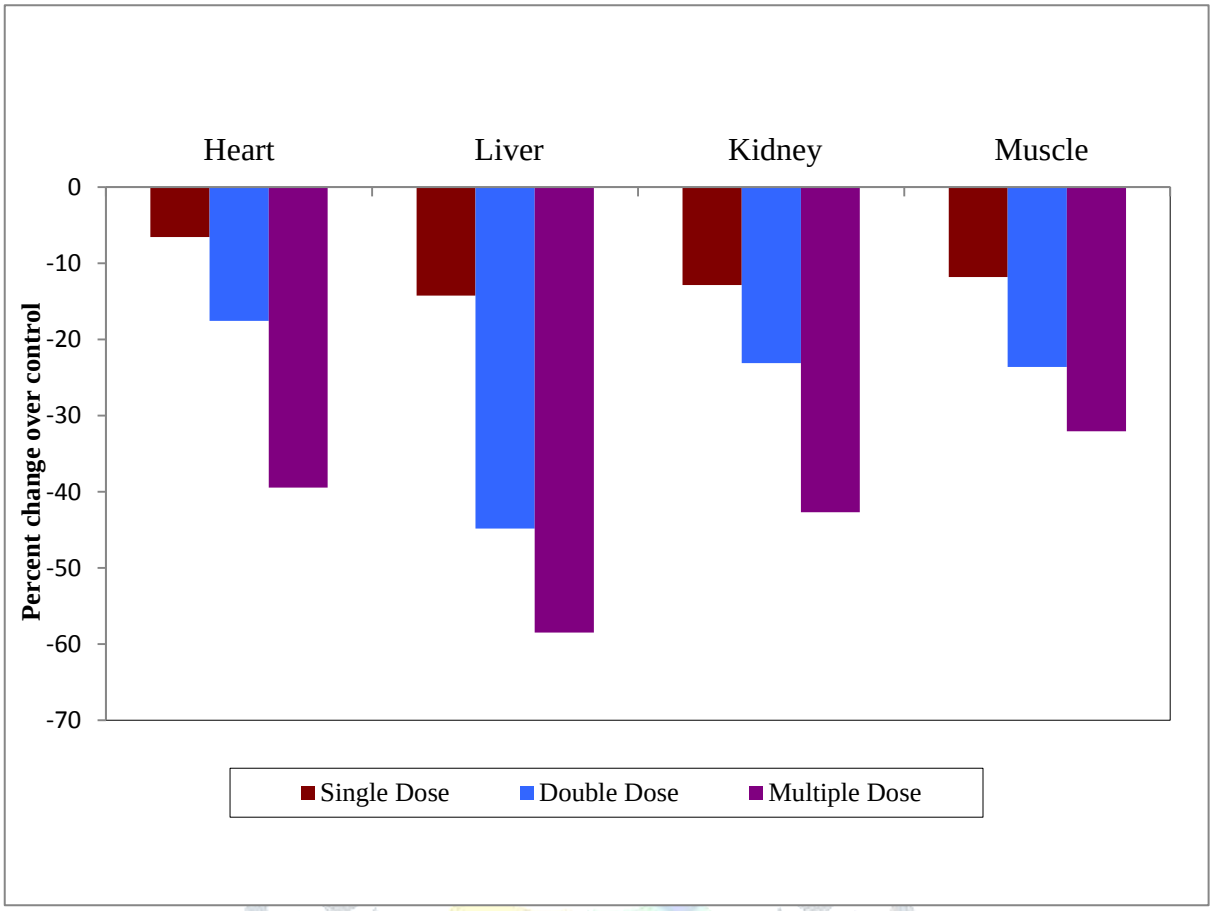


Fig.2 Superoxide dismutase activity levels in different tissues of mice exposed to chloropyrifos

Fig.3 Catalase activity levels in different tissues of mice exposed to chloropyrifos



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