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NEUROTOXIC EFFECTS OF CYPERMETHRIN IN WISTAR STRAIN RATS: DETOXIFICATION MECHANISMS

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ABSTRACT
Pyrethroids occupy a rather unique position among many chemicals that man encounters daily, so that they deliberately added to the environment for the purpose of killing some forms of life. These insecticides with alfa cyano group acts on central nervous system, ultimately induces oxidative stress. Oxidative stress is fast becoming the nutritional and medical buzzword for 21st century. As a result of oxidative metabolism aerobic organisms generate reactive oxygen species (ROS) such as superoxide anion radical, hydrogen peroxide and hydroxyl radical. To minimise the negative effects of reactive oxygen species the vertebrates posses an antioxidant defence (AD) system by utilizing enzymatic and non-enzymatic mechanisms. Some of the antioxidant enzymes are superoxide dismutase (SOD), catalases, etc. Under normal conditions ROS are cleared from the cell by the action of superoxide dismutase, catalases. The wistar strain albino rat brain tissues such as cerebral cortex, Hippocampus, Cerebellum and Pons medulla were selected to study the role of enzymatic antioxidant defense system. In the present study the main focus was done on two main antioxidant enzymes namely superoxide dismutase and catalases. This revealed that the superoxide dismutase and catalase activity was decreased with increased doses of cypermethrin pyrethroid in different parts of rat brain differently.

Key Words: Oxidative Stress, Superoxide Dismutase, Catalases, Cypermethrin, Albino Rat

INTRODUCTION
Pyrethroids are considered as the safest of highly potent insecticides (He *et al.,* 1989; Chen *et al.,* 1991; Pauluhn, 1999; Mertz, 1999; Ray and Forshaw, 2000; Bateman, 2000). Pyrethroids are the potent insecticides occupying 25% of the worldwide insecticide market (Environment Health Perspective, 2005). Because of its wide usage the pyrethroids produces reverse effects on the biochemical activities of non-target organisms. Pyrethroids are mainly acting on the nervous system of effected animals. Higher rates of enzymatic biotransformation, detoxification and excretion are responsible for their lower toxicity for mammals. In insects this potency is fivefold 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 (Kakko, 2004). Pyrethroid toxicity alters the antioxidant enzymes due to cell stress with the involvement of free radical intermediates of Pyrethroids. The imbalance between protective antioxidants and increased free radical production leading to oxidative damage is known as oxidative stress. The toxic effects of oxygen free radicals and reactive oxygen compounds can lead to a wide variety of harmful conditions, including aging, cancer, antherosclerosis and stroke (Kale *et al.,* 1999; Grazeda-Cota *et al.,* 2004).

Pyrethrins and pyrethroids are fat –soluble pesticides, and therefore they accumulate in fat deposits in the body. The highest concentration of fat in the body is in the brain due to the lipid-based myelin sheaths surrounding every nerve cell. They are recognized as nerve poisons. Therefore pyrethroids are effective as contact insecticides and to a lesser extent as stomach poisons. The synthetic pyrethroids are particularly useful against tissue borers i.e. mostly lepidopterus larvae damaging cotton, brinjal, tomato, etc. It is estimated that 45% of the world annual insecticides used for the control of tissue borers alone (Aldana *et al.,* 2001; Luty *et al.,* 2000).

Cypermethrin is a potent pesticide3 derived from natural pyrethrin of the chrysanthemum plant. It is first synthesized in 1974 and marketed in 1979 (WHO, 1989). Over ninety percent of the cypermethrin

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manufactured worldwide is used to kill the insects on cotton. Under investigate toxicology, current stress is on understanding the toxic effects of chemicals at molecular level to predict the toxicogenic potential of chemicals and identify the specific targets of toxicity (Seth *et al.,* 2000). The prevalence of pyrethroids in insecticide formulations has increased in last decade (Wolansky, 2006). Cpermethrin, like all synthetic pyrethroids, kills insects by disrupting normal functioning of the nervous system. Cypermethrin insecticide prolongs the sodium current leading to the repetitive nerve impulses instead of single nerve impulse. These nerve impulses cause the nerve to release the neurotransmitter acetylcholine and stimulate other nerves (Vijverberg and Vander, 1990; Eells *et al.,* 1992).

Cypermethrin is a moderate toxic material by dermal absorption or ingestion (Meister, 1992; Crawford *et al.,* 1981). Inspite of the low toxicity of pyrethroids, persistence of these compounds in mammalian tissues may be dangerous (Crawford *et al.,* 1981). Hence pesticide toxicology research confines to be an important thirst area for present and future investigators due to its wide usage. Due to liberal use of pyrethroids has increased the risk of intoxication for non-target species. The absorption of cypermethrin from digestive tract and its excretion takes a quick course (Ferah *et al.,* 2005; Luty *et al.,* 2000). So cypermethrin was selected as the drug for the present investigation, in view of its wide spread usage for the control of all types of insect pests all over the world. The available literature on the cypermethrin neurotoxicity and detoxification processes is very brief. So the present investigation was mainly focused on detoxification mechanisms.

MATERIALS AND METHODS

Chemical Substances: Technical grade cypermethrin (Tagros chemicals, Chennai) with 93% purity was used as the test chemical for the present study. At present, cypermethrin was chosen for the present study because of its quick absorption from the digestive tract and quick course of excretion. Cypermethrin has a broad spectrum use in agriculture, domestic and veterinary applications due to their high bioefficiency enhanced stability and comparatively low mammalian toxicity. The LD_{50} estimated for this cypermethrin drug was 205 mg/kg body weight. In this present investigation $1/5^{th}$ LD_{50} i.e. 41 mg/kg body weight was selected as a sub acute dose for analysis. Four batches of animals were treated as control, single dose, double dose and multiple dose animals for studying detoxification mechanisms in cerebral cortex, hippocampus, and 9 cerebellum and ponsmedulla regions of rat brain. All the other reagents used were of analytical reagent grade and obtained from sigma chemicals Ltd.

Animals for Investigation: Healthy wistar strain albino rats of same age group 70±5 days and weight 175±10 grams were selected as experimental animals for the present study. The rats were collected from Indian Institute of Sciences (IISC), Bangalore. This experiment was conducted on 100 male wistar strain rats. The dosage was given by oral intubation method. The animals were maintained in controlled laboratory conditions of 12 hr dark/light cycle at 25±2º C temperature with standard pellet diet (by Sai Durga feeds and food, Bangalore) and water ad libitum.

Detoxification Mechanisms

1. Estimation of Superoxide Dismutase Activity

(E.C.1.15.1.1)

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). The brain tissue was homogenized in ice cold 0.05M potassium phosphate buffer (PH 7.0) containing 0.1 mM EDTA to give 5% homogenate (w/v). The homogenate was centrifuged at 10,000 rpm for 10 minutes at 0ºC in cold centrifuge. The supernatant was separated and used fo9r enzyme assay. The reaction mixture of 3 ml contained 100mM of phosphate buffer (PH7.8), 150 ml EDTA (0.1 mM), 600 ml methionine (13 mM), 300 ml nitro blue tetrazolium (75 mM) and the enzyme source. The reaction was initiated by the addition of riboflavin (2µM) and the samples were placed under 15 watts fluorescence bulb for 30 minutes and the absorbance was read at 560 nm against reagent blank kept in a dark place. The tubes which are not

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exposed to light severe as control. A system, devoid of any superoxide radical scavenger was used as appositive control to compare the results.

The activity of the enzyme was expressed as units/mg protein.

2. Estimation of Catalase Activity

(E.C.1.11.1.6)

Catalase activity was measured by a slightly modified version of Aebi (1984) at room temperature. The brain tissue was homogenized in ice-cold 50mM phosphate buffer (PH7.0) containing 0.1 mM EDTA to give 5% homogenate (w/v). The homogenates was centrifuged at 10,000 rpm for 10 minutes at 0°C in cold centrifuge. The resulting supernatant was used as an enzyme source.10μl 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 250μl 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 μ cm^{-1} was used to determine catalase activity. One unit activity is equal to the moles of H_2O_2 degraded/mg protein/min.

Table 1: Changes in superoxide dismutase (μg/mg protein) activity levels in different Brain tissues of wistar rats exposed to sub lethal dose of cypermethrin. Values in parentheses indicate percent change from control.

S. No.	Name of the Tissue	Control	Single Dose	Double Dose	Multiple Dose
1.	Cerebral cortex	1.509	1.263[**]	1.164[**]	0.947[**]
		±0.063	±0.174	±0.060	±0.073
			(-16.308)	(-22.837)	(-37.245)
2.	Hippocampus	1.773	1.508[**]	1.336[**]	1.179[**]
		±0.038	±0,033	±0.064	±0.123
			(-14.921)	(-24.643)	(-33.509)
3.	Cerebellum	2.066	1.761[**]	1.651[**]	1.433[**]
		±0.035	±0.054	±0.038	±0.113
			(-14.747)	(-20.103)	(-30.623)
4.	Pons medulla	2.224	1.858[**]	1.701[**]	1.514[**]
		±0.025	±0.057	±0.055	±0.095
			(-16.438)	(-23.501)	(-31.909)

Two way Anova

Source of variation	Degree of Freedom	Sum of Squares	Mean Square	'F' Values
Between doses	3	0.0827	0.276	228.113[**]
Between tissues	3	0.901	0.300	248.588[**]
TD	9	0.011	0.001	
Total	**15**	**1.739**		

*Note: ***= Significant at 0.001% level ** = Significant at 0.01% level, *= Significant at 0.05% level, ns= not significant*

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Table 2: Catalase (μ moles of H_2O_2 decomposed/mg protein/hr) activity in different brain tissues of wistar rats exposed to oral sub lethal dose of cypermethrin. Values in parentheses indicate percent change from control.

S.No	Name of the Tissue	Control	Single Dose	Double Dose	Multiple Dose
1.	Cerebral cortex	1.523 ±0.054	1.299[**] ±0.030 (-14.706)	1.181[**] ±0.017 (-22.453)	0.930[**] ±0.034 (-38.932)
2.	Hippocampus	1.593 ±0.053	1.403[**] ±0.053 (-11.967)	1.256[**] ±0.055 (-21.182)	0.990[**] ±0.045 (-37.866)
3.	cerebellum	1.676 ±0.042	1.473[**] ±0.056 (-12.065)	1.331 ±0.041 (-20.579)	1.057[**] ±0.020 (-36.901)
4.	Pons medulla	1.697 ±0.024	1.510[**] ±0.031 (-11.055)	1.350[**] ±0.032 (-20.481)	1.070)[**] ±0.020 (-36.986)

Two way Anova

Source of variation	Degree of Freedom	Sum of Squares	Mean Square	'F' Values
Between doses	3	0.790	0.263	1686.110[**]
Between tissues	3	0.074	0.025	158.096[**]
TD	9	0.006	0.001	
Total	**15**	**0.870**		

Note: *******= *Significant at 0.001% level*   ****** = *Significant at 0.01% level*
    *= *Significant at 0.05% level*   *ns= not significant*
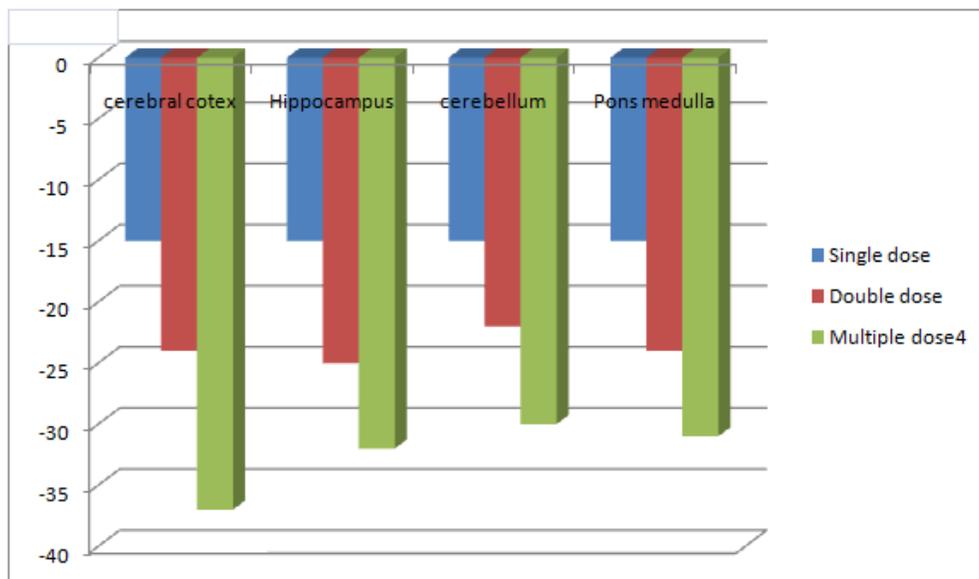
Graph 1: Changes in superoxide dismutase (μg/mg protein) activity levels in different Brain tissues of wistar rats exposed to sub lethal dose of cypermethrin. Values in parentheses indicate percent change from control.

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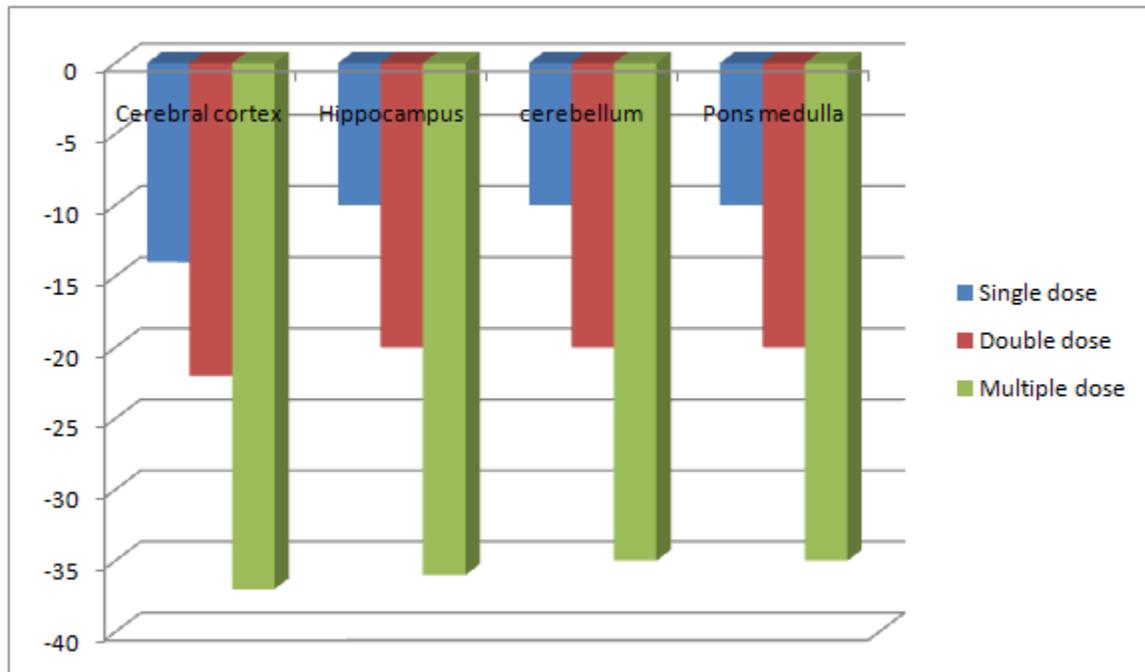
Graph 2: Catalase (µ moles of H_2O_2 decomposed/mg protein/hr) activity in different brain tissues of wistar rats exposed to oral sub lethal dose of cypermethrin. Values in parentheses indicate percent change from control.

Statistical Analysis
The mean, standard deviation, percent change and two way ANOVA and test of Significance was calculated using standard formulae. All the values "t" below 5% levels ($p < 0.001$) were designated as significant.

RESULTS AND DISCUSSION
Results
In present study the superoxide dismutase (SOD) was decreased with increase of doses. In single dose administered animals the maximum significant decreased level was observed at pons medulla (16.438) and minimum significant decrease observed at cerebellum (14.747%).This decreased tendency increased with increase of doses. In multiple dose admini9stered animals the maximum significant decrease observed at cerebral cortex (37.245%) and minimum at cerebellum (30.623%) (Table 1). In the present investigation the catalase activity levels are depleted with increase of doses. In single dose administered animals the maximum level of catalase depletion was observed in cerebral cortex (14.706%) and minimum level was observed in pons medulla (11.055%). In double dose administered animals, the maximum level of catalase inhibition was observed in cerebral cortex (22.453%) and minimum level of inhibition was observed in pons medulla (20.481%). In multiple dose administered animals significant

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maximum level of catalase depletion was observed in cerebral cortex (38.932%) and low level of inhibition was observed in cerebellum (36.986%) (Table 2).

Discussion

Superoxide dismutase and catalase enzymes 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. It has been widely recognized that hippocampus and striatum are clearly more susceptible to oxidative stress than the remaining brain regions. During oxidative stress conditions the SOD and catalase activity levels were lowered in different brain regions (Homi Hercilia *et al.,* 2002). The superoxide dismutase activity was significantly inhibited in brain and liver of albino rat during the development of behavioural tolerance to organophosphate compound phosphomidon (Venkateswara, 1993). A well known synthetic pyrethroid, Deltamethrin significantly depleted the SOD and catalase activities during repeated dose toxicity in rats (Manna *et al.,* 2005). The decreased SOD activity was observed in hypoxic-ischemia brain damage (HIBD) control rats at 24 hrs after Hypoxia-ischemia treatment (Lin *et al.,* 2006). The Hexachlorhexane (HCH) effect on immature chick tissues decreased SOD activity (Seth *et al.,* 2000). 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. An Isoproterenol administration to rats causes a gradual depletion of catalase in different tissues (Rathore *et al.,* 2000).

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