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Short Communication

Induction of oxidative stress and lipid peroxidation in rats chronically exposed to cypermethrin through dermal application

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Present study was undertaken to study the effect of cypermethrin on oxidative stress after chronic dermal application. The insecticide was applied dermally at 50 mg/kg body weight in different groups of Wistar rats of either sex weighing 150~200 g. Significant ($p < 0.05$) increase in catalase activity was observed after 30 days of exposure. However, the superoxide dismutase activity declined significantly after 60 days of exposure. The activity of glutathione peroxidase and blood glutathione levels declined significantly ($p < 0.05$) after 30 days of cypermethrin dermal application. However, the activity of glutathione S-transferase increased significantly ($p < 0.05$) in all groups after 60 days of dermal exposure. Significant increase in lipid peroxidation was observed from 30 days onwards and reached a peak after 120 days of application.

Keywords: chronic dermal application, cypermethrin, lipid peroxidation, oxidative stress

Pesticides have detrimental effects on mammals and their persistency in the environment is a serious public health concern [8]. However, cypermethrin as well as other pyrethroids have hepatotoxic, neurotoxic and immuno-suppressive potential in mammals and insects [7,36,37].

Accidental exposure with pyrethroids in humans and animals result from its advertent use [23]. The cytotoxic and genotoxic potential has also been reported in higher vertebrates [12,14] due to the induction of oxidative stress and free-radical-mediated lipid peroxidation [18]. Oxidative stress reduces the activity of ATP-dependent Na^+ channels [5]. In parasites like *Paramecium tetraurelia*, pyrethroids increases intracellular concentration of Ca^{++} ions [34] or energy deficits resulting in the inability of cells to remove cytosolic Ca^{++} ions [31]. Increased cytosolic Ca^{++} ions lead to free radical-mediated cell damage or cytotoxicity [17].

Oxidative stresses induce diverse pathological conditions varying from aging to Parkinson's disease due to the surplus release of reactive oxygen species [20,25,35]. The mammalian body has endogenous enzymatic defenses to fight oxidative stress such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione S-transferase (GST) and non-enzymatic components like reduced glutathione (GSH), ascorbic acid, vitamin E, *etc* [3]. Therefore, the present study was undertaken to study the effect of cypermethrin on the oxidative stress and lipid peroxidation following its chronic dermal application in rats.

Wistar rats (150~200 g) of either sex were procured from Indian Institute of Integrated Medicine Jammu (CSIR, India). The animals were fed a commercial diet and provided water *ad lib*. The animals were divided randomly into five groups with each group comprising of 6 rats. Group A served as the control group and received no treatment while groups B, C, D and E had cypermethrin applied dermally at the dose rate of 50 mg/kg b. wt. at inter-scapular region [28] daily for 30, 60, 90 and 120 days, respectively. The selected daily dose was 1/10th of reported dermal LD_{50} for cypermethrin [21]. Blood was collected from retro-orbital sinus in sterile heparinized tubes 24 h after the last dose.

Erythrocyte lysate was used at 1% for the CAT, SOD, GSH-Px, and GST assay, and 33% for the determination of lipid peroxidation. The activities of SOD and CAT were measured as per the method described by Marklund & Marklund [22] and Aebi [1], respectively. The GSH-Px and GST activities were assayed by the methods described by Hafeman *et al.* [15] and Habig *et al*. [13], respectively. The extent of lipid peroxidation was estimated as the concentration of thiobarbituric acid reactive product malondialdehyde (MDA) by the method of Ohkawa *et al*. [26]. Whole blood was used for the estimation of blood glutathione as per method described by Beutler *et al*. [4].

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Table 1. Effects of chronic dermal application of cypermethrin on enzymes, blood glutathione and lipid peroxidation in Wistar rats

Parameters	Days after dermal application							
	Control	30 days	Control	60 days	Control	90 days	Control	120 days
CAT (µmol of H_2O_2 decom.min^{-1} mg.Hb^{-1})	16.23 ± 2.44^a	35.52 ± 6.18^b	14.53 ± 2.23^a	45.25 ± 7.24^b	19.23 ± 2.64^a	59.51 ± 13.27^b	19.42 ± 2.65^a	65.27 ± 10.45^b
SOD (Umg.Hb^{-1})	0.025 ± 0.005^a	0.266 ± 0.021^b	0.035 ± 0.004^a	0.026 ± 0.009^c	0.031 ± 0.006^a	0.015 ± 0.012^c	0.028 ± 0.004^a	0.014 ± 0.010^c
GSH-Px (Umg.Hb^{-1})	7.70 ± 0.65^a	3.35 ± 0.37^c	8.60 ± 0.55^a	2.93 ± 0.19^c	5.50 ± 0.23^a	2.71 ± 0.11^c	8.75 ± 0.45^a	2.59 ± 0.23^c
GST (µmol of conjugate of min^{-1} mg.Hb^{-1})	0.0054 ± 0.001^a	0.0057 ± 0.0032^a	0.0051 ± 0.002^a	0.0286 ± 0.009^b	0.0054 ± 0.003^a	0.2207 ± 0.008^b	0.0054 ± 0.002^a	0.2692 ± 0.0345^b
GSH (nmol.mL^{-1})	105.79 ± 14.74^a	27.76 ± 7.45^b	100.56 ± 15.74^a	26.35 ± 6.59^c	112.79 ± 18.34^a	21.57 ± 5.12^c	98.79 ± 17.77^a	18.85 ± 2.67^b
LPO (nmol of MDA gm Hb^{-1} h^{-1})	1.35 ± 0.31^a	3.34 ± 0.68^b	1.65 ± 0.42^a	4.06 ± 0.96^b	1.79 ± 0.43^a	3.93 ± 0.89^b	1.99 ± 0.42^a	5.05 ± 0.33^b

Values are expressed as mean ± SE. (n = 6). [a,b,c]Means with different superscripts are significantly different between groups ($p < 0.05$). CAT: catalase, SOD: superoxide dismutase, GSH-Px: glutathione peroxidase, GST: glutathione S-transferase, GSH: reduced glutathione, LPO: lipid peroxidation, MDA: malondialdehyde .

Statistical analyses were done using one-way ANOVA followed by Dunnet's test with $p < 0.05$ as a limit of significance.

A significant increase ($p < 0.05$) in the catalase activity was observed in all groups (Table 1). Also, a significant increase ($p < 0.05$) in SOD activity was observed in group B, but the activity was reduced significantly ($p < 0.05$) in the other groups compared to control. GSH-Px activity was significantly reduced ($p < 0.05$) in all groups compared to the control group. Similar finding have been reported in other study during oxidative stress [24]. No significant changes in GST activity was seen up to 30 days, but thereafter, a significant increase was noticed up to 120 days. There was significant decrease in the GSH after 30 days and similar pattern followed up to 120 days ($p < 0.05$). Significant increase in lipid peroxidation indicated lipid membrane damage from 30 days onward.

Pyrethroids are metabolized in liver via cytochrome P450 oxidative pathways yielding reactive oxygen species [9,19]. Oxidative stress takes advantage of the available mitochondrial electron to make molecular oxygen, resulting in excess superoxide production in most tissues [2]. These superoxide anions are converted to hydrogen peroxide and water with the help of a group of SOD [10]. A significant drop in erythrocyte SOD levels indicates a decrease in the tissues' ability to handle excessive free radicals [2]. However, an increase in catalase activity enhances the scavenging ability of erythrocytes to handle the hydrogen peroxide to molecular oxygen and water [11,29].

GSH-Pxs catalyze the peroxides and reduce the glutathione to form oxidized glutathione and water [30]. A significant reduction in GSH-Px activity may be due to over production of free radicals [24]. Similarly, GST catalyzes the conjugation of the reduced glutathione to electrophiles and protects cellular components from oxidative damage [16]. Increased activity of GST was reported in *Drosophila melanogaster* after insecticide exposure [27]. Elevated GSTs were reported in *Nilaparvata lugens*, a pyrethroid insecticide resistant strain of insect [38]. GST levels were also increased significantly after 30 days of exposure to protect RBCs from oxidative damage. Further significant decreases in GSH levels in our study may be due to either the inhibition of GSH synthesis or increased utilization of GSH for detoxification of toxicant induced free radicals [33]. The decrease in SOD, blood GSH and GSH-Px suggests that the dermal exposure of cypermethrin may lead to excessive free radical generation. These free radicals might be attacking the thiol group of cysteine residuse and poly-unsaturated fatty acids of biological membranes [6]. Free radical-induced lipid peroxidation resulting in the deterioration of biological membranes [32].

In conclusion, the changes suggest that the accumulation of excess free radicals may be responsible for the increased lipid peroxidation which sensitizes the cells to various degenerative diseases.

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