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PROTECTIVE EFFECT OF LEUCOVERIN ON CYPERMETHRIN-INDUCED TOXICITY IN MICE

Nishal Kumar P.[1], Manjusha C.[1], Itishri Sahu[1] & Sirisha D.[1]

[1]Dept of Biotechnology, University Of Hyderabad, Hyderabad-50046, Andhra Pradesh, India

Email Id: nishal.shekar@gmail.com

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ABSTRACT

Effects of Cypermethrin and their reversal by Leucoverin following oral administration were evaluated in mice. Mature male swiss mice were orally administered cypermethrin (15 mg/kg body wt), leucoverin (50mg/kg body wt) or both daily for 4 weeks. At the end of fourth week, hematological & serum biochemical were studied. Subchronic exposure to cypermethrin significantly reduced, when the co-contaminant leucoverin administration restored the changes in the body weight, hematological parameters, and serum biochemical indices and significantly increased the antibody titer, and cell mediated immunity. These results suggest that concurrent leucoverin treatment has a beneficial role in mitigating adverse effects of cypermethrin.

Keywords: Cypermethrin, Leucoverin, oral administration

Number of Tables: 2 **Number of References**: 25

INTRODUCTION

Synthetic pyrethroids have emerged as a new class of agricultural pesticides and have found wide use over organochlorine, organophosphate, and carbamate pesticides. Currently, they have broad-spectrum use in agriculture, domestic, and veterinary applications due to their high bioefficacy, enhanced stability, and comparatively low-mammalian toxicity. There are serious concerns on the potential risks of exposure to pyrethroid insecticides with increasing production and application (Adelsbach and Tjeerdema 2003). Cypermethrin, a member of the family of synthetic pyrethroids, belongs to type II class pyrethroids and is widely used in agricultural and other domestic applications. The absorption of cypermethrin from the digestive tract and its excretion takes a quick course. It is well established that cypermethrin, both cis- and trans-isomers are metabolized to phenoxybenzoic acid and cyclopropane carboxylic acid (Lukowicz - Ratajczak and Krechniak 1991). Populations at the highest risk of high-dose exposure are producers, hygienic, and pesticide workers, and small farm owners applying cypermethrin for plant protection. Low-dose exposure originates mainly from the household application of insecticides, contaminated food, and water (Gorell et al. 1998).

It has been well-documented that a galaxy of xenobiotics to which humans and animals are acutely or chronically exposed may potentially damage the immune system through a variety of distinct mechanisms (Dean and Vos 1986). The immunotoxicological effects of cypermethrin have been investigated in different animal species. When male Wistar rats were treated with 6.25, 12.5, and 25 mg kgÀ1 of cypermethrin for 6 or 12 weeks, reduction in anti-sheep red blood cells (SRBCs) and anti-ovalbumin titer as well as the autologous rosette formation of splenic lymphocytes were observed (Desi, Dobronyi, and Varga 1986; IPCS 1989). Varshneya et al. (1992) demonstrated a dose-dependent decrease of delayed type hypersensitivity (DTH) in rats following a 2-month oral treatment with 20 and 40 mg kgÀ1 body wt perday of cypermethrin. Khurana, Chauhan, and Mahipal (1999) have reported that cypermethrin given in feed at a concentration of 100 ppm over a period of 8 weeks caused significant depression in DTH reaction in broiler chickens.

Institoris et al. (1999) reported that oral treatment with 55.4 and 22.2 mg kgÀ1 body wt dayÀ1 of cypermethrin for 28 days decreased DTH reaction in rats. These doses of the insecticide also decreased the mean cell volume of erythrocytes and white blood cell count in the peripheral blood.

Curcumin (diferuloylmethane), a yellow-orange dye derived from the rhizomes of Curcuma longa turmeric is used as a spice and food-coloring agent. Curcumin is known to exhibit a wide range of pharmacological effects such as antioxidant, antitumor, antiinflammatory, hepatoprotective, antimutagenic, antiangiogenic,

immunomodulatory, and wound healing (Naik, Mujumdar, and Ghaskadbi 2004; Maheshwari et al. 2006). Varalakshmi et al. (2008) demonstrated powerful immunomodulatory effect of curcumin in mice and rats. Owing to the subtle health problems induced by environmental pollutants like pesticides, efforts need to be expended in evaluating the immunomodulating potency of commonly available antioxidants like curcumin. Accordingly, the aim of this study was to examine the efficacy of leucoverin in ameliorating cypermethrin-induced alterations in hematological, biochemical, and immunological parameters of mice.

MATERILAS & METHODS

Selection of Animals:

The Animals (Mice) considered in the present study of genetic analysis were 8-10 weeks old random bred Swiss albino male mice with an average body weight of 22-24g, maintained under standard laboratory conditions at temp 220c ± 20c, relative humidity 50 ± 10% and 12h photoperiod. Commercial pellet diet and deionised water were fed. Around 12 mice of approximately equal weight were taken for the experiment.

Selection of test compound:

The test compound cypermethrin and leucoverin were purchased from local pharmacy shops.

Toxicity evaluation:

Naturally living organism in general protect themselves from the potentially harmful chemicals, it is their inherent tendency. A few animals may respond to low doses of toxic chemicals and others at high doses. Toxicity of the drug depends upon the concentration of the compound. The toxicity of the drug is usually expressed in terms of LD50/LC50 (for lethal dose). LD 50 (lethal dose 50) represents amount of poison/ unit weight, which killed 50% of population of animal species employed for the test. Several reports are available on the toxicity and LD50 values of drugs.

Doses selected:

Cypermethrin was administred orally for 28 days through food of dosage 15mg/kg body weight and leucoverin 50 mg /kg

All other chemicals used in the present experiment were of analytical grade. This includes, KCl from S.D fine chemie, Giemsa, Colchicines from Himedia, Fixative (Acetic Acid and Methanol).

Glass Ware:

Petri dishes, Microscopic Slides, Conical Flasks, Standard Flasks, Pasture Pipette, Syringe, 26g needles, Droppers.

Experimental Design:

Analysis of Chromosomal Aberrations in Bone Marrow of Mice

In the present study on dose effect relationship the animals were supplied with cypermethrin (15mg /kg), leucoverin (50 mg/kg) or both For Control group, 3 Mice were also maintained which received equal volume of distilled water. All the control and treated animals were sacrificed by cervical dislocation after the administration

of the test compound. 0.05% of colchicine was added to all the incubated mice 2hrs before scarifying to inhibit the spindle formation.. Animals were dissected out for femur bones and flushed out bone marrow into a Petridish containing 0.75m KCl (hypotonic) solution to get a homogenous suspension. The cell suspension was collected in clean centrifuge tubes and incubated at 370c for 45minutes. After incubation the tubes were centrifuged for 10minutes at 1000rpm. The supernatant was discarded and to the pellet 5ml of freshly prepared pre-chilled fixative (3:1 methanol and acetic acid) was added and allowed to stay at room temperature for 10minutes. This step was repeated 4 to 5 times. Finally the cells were fixed in fresh fixative.

Preparation of slides:

Air-dried slides were prepared by dropping one or two drops of the final suspension on the grease-free, pre-chilled slides with the Pasteur pipette. The slides were dried immediately by air-drying method, coded and stained in 2% Geimsa (2mL of Geimsa + 2mL of Sorenson's buffer + 46 mL of distilled water) for 10 minutes.

Scoring of Chromosomal Aberrations:

For each mice 100 well spread metaphase were screened and scored structural aberrations (gaps, breaks, fragments, exchanges and dicentrics) and numerical aberrations. However, Well spread metaphases were micro photographed with Leica CW 4000 image analyzer.

The significance of differences for the number of chromosomal aberrations between the control and treated groups was tested using 2×2 contingency chi-square ($\chi 2$) test.

RESULTS & DISCUSSION

Clinical signs of toxicity such as slight nervousness, mild depression, reduced feed intake, rough hair coat, and abnormal gait were observed in cypermethrin-exposed mice. The animals of other groups including those treated with cypermethrin plus leucoverin did not exhibit any apparent signs of toxicity. There was no mortality in mice with any of the treatments. At term, there was a significant decrease in body weight of animals treated with cypermethrin alone as compared to untreated and vehicle control groups (both the control groups hereafter referred as control). Mice treated with cypermethrin plus leucoverin showed increased body weight as compared with cypermethrin alone treated group

Table 1. Effect of cypermethrin and cypermethrin plus curcumin through oral route for 28 days on the TLC and DLC of male mice.

Group	TLC	Lymphocytes	Neutrophils	Monocytes	Eosinophipls
Contol	9.3 ± 0.1	78.1±0.6	17.9±1.1	4.6±0.8	0.5±0.3
Cypermethrin	9±0.04	60±.05	15±.04	3±0.65	0.5±0.2
Leucoverin	9.5±.02	65±.45	18±.23	5±.70	0.5±.01
Cyp+Leucoverin	9.2±.01	63±.04	17±.18	4±.68	0.5±.01

Note: *Values are expressed as mean Æ SEM of six mice*.

The value of TLC in cypermethrin plus leucoverin-treated group was significantly higher as compared to animals exposed to cypermethrin alone. Lymphocytopenia was observed in group treated with cypermethrin as compared to control groups (Table 1). Cypermethrin plus leucoverin-treated group showed no significant decrease in differential leukocyte count (DLC) as compared to control group. Significant increase in the neutrophil count was observed in rats treated with cypermethrin alone. There was no significant change in monocyte and eosinophil counts in any of the treatment groups.

The skin thicknesses in various groups are presented in Table 2. Skin thickness at 0 h showed no significant difference among the groups. Skin thickness in cypermethrin-treated group after 24 and 48 h was found to be decreased as compared to control groups. In cypermethrin plus leucoverin-treated group, there was a significant increase in the skin thickness as compared to cypermethrin alone treated group.

Table 2. Effect of cypermethrin and cypermethrin plus curcumin through oral route for 28 days on cell mediated immunity of male mice.

Treatment	Dose	0	24	48
Control	-	0.30±0.01	0.42±0.01	0.39±0.01
Cypermethrin	15	0.29±0.01	0.35±0.02	0.31±0.03
Leucoverin	50	0.31±0.01	0.44±0.01	0.40±0.01
Cyp+Leucoverin	40	0.30±0.02	0.39±0.03	0.35±0.02

Note: *Values are expressed as mean Æ SEM of six mice*

In this study, significant decrease in body weight at the end of the experimental period following the administration of cypermethrin in mice has been observed. It

may be attributed to the effect of insecticide on gastrointestinal tract resulting in decreased appetite and absorption of nutrients from gut (Venkateshwarlu et al. 1997) or might be due to direct toxicity of cypermethrin. Mice that received Leucoverin along with cypermethrin showed increase in body weight as compared to cypermethrin alone treated rats which indicate that leucoverin has appetite inducer and anti-stress effect. This finding is in agreement with observation that leucoverin treatment caused increase weight gain in rats pretreated with arsenic (El-Demerdash, Yousef, and Radwan 2009). Cypermethrin treatment caused significant decrease in TLC in rats as compared to control groups. Similarly, cypermethrin given orally at 55.4 and 22.2 mg kgÀ1 body wt perday for 28 days has been reported to significantly decrease the absolute TLC in rats (Institoris et al. 1999). No significant decrease in TLC was observed in rats that received cypermethrin along with leucoverin as compared to cypermethrin alone treated animals.

The serum total protein, albumin, and globulin values were found to be significantly reduced in the cypermethrin-treated group as compared to control groups. These results are in agreement with those of Yousef et al. (1999) on rabbits, Yousef et al. (1998) on sheep, and Baligar and Kaliwal (2001) on rats. Rivarola and Balegno (1991) reported that the reduction in plasma protein, particularly albumin, in animals treated with pesticides could be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver. The decrease in serum protein also may be due to loss of protein either by reduced protein synthesis or increased proteolytic activity or degradation (Shakoori et al. 1990). In addition, the decrease in serum protein as observed in this study could be attributed in part to the damaging effect of cypermethrin on liver cells. Reversal of these metabolic alterations in rats has been achieved when leucoverin was administered along with cypermethrin.

In this study, a significant decline in antibody titer in cypermethrin-treated group was observed suggesting cypermethrin-induced immunosuppression. Similarly, Stelzer and Gordon (1984) showed that cypermethrin inhibited the proliferation of mouse T and B cells at the 1-5 Â 10À5 M concentration range. As for the humoral immune response, Desi, Dobronyi, and Varga (1986) reported a decreased S. typhimurium antibody titer of the rats with cypermethrin treatment. Increased antibody titer in rats that received curcumin along with cypermethrin than cypermethrin alone was observed.

In vivo cell-mediated immune response was assessed by DTH reaction using ovalbumin. After secondary sensitization, a significant decrease in skin thickness in cypermethrin-treated group as compared to control suggested deleterious effect of cypermethrin on cell mediated immunity. Tamang et al. (1988) reported in vivo immunosuppressive effect of cypermethrin in mice and goats. Their results revealed significant depression of cell mediated immunity in both the species, and humoral immune response in goats. Cypermethrin given in feed at a concentration of 100 ppm for 8 weeks was

also found to cause significant depression in DTH reaction in broiler chickens (Khurana, Chauhan, and Mahipal 1999). A dose-dependent decrease in DTH has been reported in rats following a two-month oral treatment with 20 and 40 mg kgÀ1 of cypermethrin (Varshneya et al. 1992), and also in rabbits fed 300, 150, and 75 mg kgÀ1 of cypermethrin for 7 weeks (Desi, Dobronyi, and Varga 1986). In this study, we observed a significant increase in the ear thickness in rats treated with leucoverin plus cypermethrin as compared to cypermethrin alone. Our findings are indicative of increase in cell mediated immunity and effective modulation of cypermethrin-induced immunotoxicity by curcumin. Immunomodulatory action of curcumin could be attributed to its effect on various facets of the immune response, including its effect on lymphoid cell populations, antigen presentation, humoral and cell-mediated immunity, and cytokine production (Gautam, Gao, and Dulchavsky 2007). Recently, Varalakshmi et al. (2008) showed that curcumin enhanced the mitogen and antigen induced proliferation potential of T cells and also produced immunomodulatory

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