RESEARCH ARTICLE

Amelioration Potential of Piperine on Cypermethrin-Induced Immunotoxicity in Wistar Rats

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Abstract

The aim of the present study was to evaluate the immunemodulatory potential of piperine against cypermethrin-induced immune dysfunction in a rat model. Thirty rats were divided into five groups of six each. Group I was kept as control and group II was used as vehicle control. Groups III, IV and V were administered orally with cypermethrin (25 mg/kg, b.wt., per oral), piperine (50 mg/kg, b.wt., per oral) and both daily for 4 weeks, respectively. Subchronic exposure to cypermethrin significantly reduced body weight, total leukocyte count, lymphocyte count, serum total protein, serum albumin, serum globulin, antibody titer against sheep red blood cells, and cell-mediated immunity. Simultaneous piperine administration restored the changes in the body weight, haematological parameters, and serum biochemical indices and significantly increased the antibody titer and cell mediated immunity. These results suggest that simultaneous piperine treatment has restore the number of factors known to be involved in the cypermethrin induced immunotoxicity which indicates that piperine can be used as a safe and effective therapy for environmental contaminants induced immunotoxicity.

Key words: Cypermethrin, Immunomodulation, Piperine, Rats

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Introduction

Cypermethrin is a synthetic pyrethroid insecticide used worldwide in agriculture, home pest control, protection of foodstuff and disease vector control (Afolabi *et al*., 2019). Even though reported to have low mammalian toxicity, cypermethrin bioaccumulation and persistence in mammalian tissues are of toxicological significance (Abdul-Hamid *et al*., 2017). Being highly hydrophobic compounds their action in biological membranes might be related to association with integral proteins and with phospholipids (Burns and Pastoor, 2018). Studies have revealed that cypermethrin exposure with toxic manifestation in several systems of the body, including reproductive toxicity, hepatotoxicity, neurotoxicity, immunotoxicity, genotoxicity (Burns and Pastoor, 2018). Several studies have shown that cypermethrin toxicity is linked to different mechanisms, including reactive oxygen species generation and oxidative stress (Afolabi *et al.,* 2019).

There is a growing public health concern regarding the possibility that occupational and environmental exposure to pesticides can cause transient and permanent changes in the immune system resulting in the onset of serious disorders such as neurodegenerative inflammatory bowel disease, cancer, rheumatoid arthritis and other inflammatory disorders, as well as conditions related to metabolic syndrome, through the activation of oxidative stress (Gangemi *et al.,* 2016). It has been well-documented that a galaxy of pesticides to which humans and animals are acutely or chronically exposed may potentially damage the immune system through a variety of distinct mechanisms (Fenga *et al.,* 2021). Khurana *et al.* (1999) have reported that cypermethrin given in feed at a concentration of 100 ppm over a period of 8 weeks caused significant depression in delayed type hypersensitivity (DTH) reaction in broiler chickens. When male Wistar rats were treated with 40 and 80 mg/kg of cypermethrin for 14 days, total white blood cell count and percentage of lymphocyte, serum nitric oxide activity and quantitative haemolysis were increased significantly whereas neutrophil percentage, total serum immunoglobulin and blood mononuclear cell proliferation and the phagocytic function of peritoneal macrophages were significantly reduced (Paramanik *et al.,* 2021). In another study, Ambwani *et al.* (2018) showed that cypermethrin treated cells displayed immunotoxic effects as observed by decrease in B and T cell proliferation.

The use of phytochemicals in addressing human health issues is increasing day by day. They are shown to possess therapeutic properties, with their use in treating dysregulation in biological systems produces little or no

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side-effects (Yoo *et al*. 2018; Vimal *et al*. 2019). Piperine, a main component of *Piper longum* Linn, is a plant alkaloid with a long history of medicinal use in Indian medicine. The compound has many pharmacologic activities such as antioxidant, immunomodulator, bioenhancer, anti-inflammatory and hepatoprotective effects (Selvendiran *et al*. 2004; Iahtisham-Ul Haq *et al*. 2021). Pathak and Khandelwal (2009) demonstrated powerful immune-protective effect of piperine in mice. Recently, it has been reported that piperine could reduce cypermethrin-induced genotoxicity in rats (Sankar *et al*. 2023). Therefore keeping in view the above facts the present study was undertaken to examine the ability of piperine in ameliorating cypermethrin-induced alterations in haematological, biochemical, and immunological parameters in rats.

Materials and Methods

Experimental Animals and Design

The study was conducted at the Division of Veterinary Pharmacology & Toxicology, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh (India). Thirty adult male Wistar rats (6-8 weeks, 100-120 g) were procured from the Laboratory Animals Resources Section of the Institute. Animals were maintained under standard management conditions and handled as per the Institutional Animal Ethics Guidelines. Prior to experiment, all the rats were kept at laboratory conditions for a period of 7 days for acclimatization.

Rats were divided into 5 groups consisting of 6 rats each. Group 1 was kept as untreated control, while Group 2 was given once equivalent amount of groundnut oil. Rats of Group 3 were administered cypermethrin (25 mg/kg, b.wt., per oral) daily for 28 days. Group 4 rats received piperine (50 mg/kg, b.wt., per oral) daily for 28 days. Group 5 rats were administered cypermethrin (25 mg/kg, b.wt., per oral) and piperine (50 mg/kg, b.wt., per oral) daily for 28 days. All the animals were observed daily for the presence of clinical signs of toxicity during the entire period of the study. Blood was collected by heart puncture in tubes rinsed with anticoagulant for haematological assay. Serum was collected for the determination of serum protein and antibody titer level from immunized rats. Haematological parameters total leukocytes, lymphocytes, monocytes, and granulocytes counts were estimated by using haematology analyser. Total protein and albumin levels in serum were estimated by Biuret method using Span diagnostic kit (Surat, India). Serum globulin was determined by subtracting albumin level from serum total protein level.

DTH Reaction

Rats were injected with 1 mg ovalbumin in 0.2 mL phosphate-buffer saline (PBS) intraperitoneally for sensitization at 19th day. The right side ear site was challenged with 50 mg ovalbumin in 50 mL PBS intradermally after 7 days post-sensitization. The left side ear site (as negative controls) was injected with PBS alone. The thickness of the ear site was measured using digital caliper at 0, 24, and 48 h after challenge.

Humoral Immune Response

SRBCs collected in Alsevier's solution, washed in PBS thrice and adjusted to a concentration of 1.2x10^6 cells/ mL were used for immunization. Six rats from each group were immunized by injecting 0.2 mL SRBCs suspension intraperitoneally 7 days prior to collection of blood. Blood was collected from retro orbital plexus on 29th day and serum was separated to determine the antibody titer by haemagglutination (HA) test. HA test was carried out by micro titration technique according to the procedure described by Beard (1980).

Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical analysis of data was performed using GraphPad InStat. Data were analyzed by analysis of variance (ANOVA) and means were compared with Tukey multiple comparison post-hoc test. A value of $p < 0.05$ was considered statistically significant.

Results and Discussion

There was no mortality in rats with any of the treatments. Cypermethrin exposed rats showed the clinical signs of toxicity such as slight nervousness, mild depression, reduced feed intake, rough hair coat, and abnormal gait. 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. The rats of other groups including those treated with cypermethrin plus piperine did not exhibit any apparent signs of toxicity. The total leukocyte counts (TLCs) in various groups are shown in Table 1. Rats exposed to cypermethrin alone showed significant decrease in TLC compared to control. Similarly, cypermethrin given orally at 55.4 and 22.2 mg kg^{-1} body wt per day for 28 days has been reported to significantly decrease the absolute TLC in rats (Paramanik *et al.,* 2021). TLC count in cypermethrin plus piperine-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 group (Table 1). Cypermethrin plus piperine-treated group showed no significant alteration in differential leukocyte count 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 serum total protein, albumin, and globulin values were found to be significantly reduced in the cypermethrin-treated group as compared to control group (Table 2). These results are in agreement with that of Grewal *et al*. (2009) and

Table 1: Effect of cypermethrin and cypermethrin plus piperine through oral route for 28 days on the TLC and DLC of male Wister rats

Groups	TLC $10^3/\mu L$	Lymphocyte (%)	Neutrophil (%)	Monocyte (%)	Eosinophil (%)
Control	9.34±0.14	78.17±0.65	17.83±1.14	4.67±0.80	0.50±0.34
Ground nut oil	8.77±0.03	77.17±0.48	18.50±0.85	4.17±0.54	0.50±0.22
Piperine	8.28±0.03	76.67±0.80	17.83±1.01	4.33±0.80	0.33±0.21
Cypermethrin	7.87±0.08[a]	69.83±0.60[a]	21.67±1.50[a]	3.83±0.60	0.50±0.34
Cypermethrin+Piperine	8.27±0.07[b]	74.67±0.42[b]	19.17±0.83	4.83±0.70	0.50±0.34

Values are mean ± SE. Significant differences are indicated by superscript a compare to control ($p<0.05$) and superscript b compare to cypermethrin group ($p<0.05$).

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 piperine was administered along with cypermethrin.

Table 2: Effect of cypermethrin and cypermethrin plus piperine through oral route for 28 days on serum protein profile of male Wister rats.

Groups	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
Control	6.0±0.06	3.78±0.05	2.30±0.04
Ground nut oil	6.0±0.03	3.84±0.04	2.20±0.04
Piperine	6.15±0.04	3.80±0.08	2.28±0.07
Cypermethrin	5.33±0.08[a]	3.30±0.09[a]	1.90±0.3[a]
Cypermethrin +Piperine	6.08±0.04[b]	3.95±0.02[b]	2.10±0.03

Values are mean ± SE. Significant differences are indicated by a compare to control ($p<0.01$), b compare to cypermethrin ($p<0.01$).

In this study, a significant decline in antibody titer in cypermethrin-treated group was observed suggesting cypermethrin-induced immunosuppression (Table 3). Similarly, El Elaimy *et al.* (2013) showed that cypermethrin inhibited the lymphocyte proliferation, phagocytic index, level of immunoglobulin G in rats. Increased antibody titer in rats that received piperine 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 (Table 4). El Elaimy *et al.* (2013) reported *in vivo* immunosuppressive effect of cypermethrin in rats. Their results revealed significant depression of cell mediated immunity and humoral immune response in rats. In this study, we observed a significant higher ear thickness in rats treated with piperine 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 piperine. Immunomodulatory action of piperine 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 (Pathak and Khandelwal, 2009).

Table 3: Effects of cypermethrin and cypermethrin plus piperine through oral route for 28 days on antibody titer in male Wister rats.

Groups	HA titre
Control	5.33±0.21
Ground nut oil	4.50±0.22
Piperine	4.83±0.17
Cypermethrin	3.50±0.22[a]
Cypermethrin+Piperine	4.07±0.16[b]

Values are mean ± SE. Significant differences are indicated by superscript a compare to control ($p<0.05$) and superscript b compare to cypermethrin group ($p<0.05$).

Table 4: Effect of cypermethrin and cypermethrin plus piperine through oral route for 28 days on cell mediated immunity of male Wister rats.

Groups	Skin thicknes (mm)		
	0 hr	24 hr	48 hr
Control	0.30± 0.01	0.42±0.01	0.39±0.00
Ground nut oil	0.29± 0.01	0.44±0.01	0.37±0.00
Piperine	0.29± 0.01	0.41±0.01	0.38±0.00
Cypermethrin	0.29± 0.01	0.35±0.003[a]	0.31±0.02[a]
Cypermethrin +Piperine	0.30± 0.01	0.36±0.03	0.36±0.02[b]

Values are mean ± SE. Significant differences are indicated by superscript a compare to control ($p<0.05$) and superscript b compare to cypermethrin group ($p<0.05$).

Conclusion

Our findings demonstrated that sub-acute oral administration of cypermethrin is able to produce immunotoxicity. The immunotoxicity produced may also instigate that production of reactive oxygen species and disruption of antioxidant defence system. However, piperine administration demonstrated attenuation of all parameters associated with these cypermethrin-induced immunotoxicities, probably due to its free radical scavenging and antioxidant properties. Our findings provide evidence that piperine has potential to ameliorate immunotoxicological effects of cypermethrin.

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