Effect of Piperine on Cypermethrin-Induced Genotoxicity in Wistar Rats

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Abstract

The indiscriminate use of cypermethrin, a type II pyrethroid to enhance crop production has aroused great concern, because this product is likely to reach the aquatic environment, thereby posing a health concern to humans and animals. The aim of the present study was to investigate the effect of piperine on cypermethrin-induced genotoxicity in Wistar rats. Thirty adult male rats (6-8 weeks old) weighing 100-200 g were divided into five equal groups of six each. Group I was kept as the control and group II was used as vehicle control. Groups III, IV and V were administered orally with cypermethrin (25 mg/kg b.wt.), piperine (50 mg/kg b.wt.), and cypermethrin and piperine daily for 28 days, respectively. Administration of cypermethrin (25 mg/kg, b.wt.) orally for 28 days resulted in significant increase in the frequency of micronuclei formation in bone marrow cells and DNA damage. Piperine (50 mg/kg, b.wt.) oral administration caused significant reduction in micronuclei formation and marked reduction in DNA damage. The study revealed that presence of piperine could reduce cypermethrin-induced genotoxicity in rats.

Key words: Comet assay, Cypermethrin, Genotoxicity, Piperine, Rats.

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Introduction

Worldwide pesticides play a major role in agricultural production by protecting crops from pest attack (Taju *et al.,* 2014). Now-a-days, pyrethroids come under the major insecticide category, accounting for more than 30% of the world market and used against various pests due to their broad-spectrum activity and cost-effectiveness (Pietrantonio *et al.,* 2014). Cypermethrin is one of the most widely used synthetic insecticides for agricultural and domestic purposes (Taju *et al.,* 2014), which comes under the category of type II pyrethroids with the presence of cyano group. It is highly toxic to fish and bees. Indeed, humans could be exposed to cypermethrin toxicity directly through spraying or indirectly through consuming the pesticide-contaminated products like fish and honey. Both *in vitro* and *in vivo* experiments with rat peripheral blood lymphocytes showed that cypermethrin severely damages DNA and causes imbalance in the pro oxidant/antioxidant status in lymphocytes (Gabbianelli *et al.,* 2004; Suman *et al.,* 2005). Recently, genotoxic effects of cypermethrin have been reported in human peripheral lymphocytes (Chakravarthi *et al.,* 2007; Sandal and Yilmaz, 2010).

In recent years, attention has been focused on whether naturally occurring compounds can modify the effects of various mutagens and carcinogens. *Piper nigrum*, belonging to the family *Piperaceae* is one of the most widely used spices all over the world. Apart from its use as a spice, *P. nigrum* is frequently used for medicinal, preservation, and perfumery purposes. Black pepper contains 2.0-7.4% of piperine. Piperine displays numerous pharmacological effects such as antiproliferative, antitumor, antiangiogenesis, antioxidant, antidiabetic, antiobesity, cardioprotective, antimicrobial, antiaging, and immunomodulatory in various *in vitro* and *in vivo* experimental trials (Iahtisham-Ul Haq *et al.,* 2021). In view of the above facts, this study was aimed at evaluating possible *in vivo* genoprotective effects of piperine on cypermethrin-induced genotoxicity in rats.

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Materials and Methods

Experimental Animals

Thirty adult male Wistar rats (6-8 weeks, 100-120 g) were procured from the Laboratory Animals Resources Section of

the Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh (India). 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.

Experimental Design

The rats were divided randomly into five equal groups comprising of 6 rats in each. Group I and II were used as water control and vehicle control (ground nut oil), respectively. Rats of Group III were administered cypermethrin (25 mg/kg b.wt.) orally daily for 28 days. Group IV rats received piperine (50 mg/kg b.wt.,) orally daily for 28 days. Group V rats were administered both cypermethrin and piperine as above daily for 28 days. Cypermethrin and piperine were dissolved in ground nut oil. Rats of all the groups were euthanized after 24 h of last administration and bone marrow cells from both femurs and blood were collected for micronuclei and Comet assay, respectively. Micronuclei and Comet assay were performed by the method of Hayashi *et al.* (1983) and Singh *et al.* (1988) respectively

The data was analysed statistically using one-way ANOVA followed by Tukey's test. The values were expressed as mean ± SEM. p values < 0.05 were considered as significant.

Results and Discussion

In the present study, the effects of piperine on genotoxicity induced by cypermethrin was assessed by the micronucleus test and the Comet assay. The results presented in Table 1 revealed that cypermethrin given daily @ 25 mg/kg b.wt. caused significant increase in number of micronuclei formation (7.87±0.80) as compared to water control (3.11±0.48), vehicle control (3.50±0.22) and piperine alone (3.50±0.34) groups. Piperine when given along with cypermethin a significant reduction in micronuclei formation (5.83±0.31) was observed as compared to cypermethrin alone treated rats (Table 1, Fig. 1a, 1b). Our results corroborated with the earlier studies (Amer and Aboul-Ela, 1985; Suman *et al.,* 2005; Sandal and Yilmaz, 2010).

Comet assay, also known as single cell gel electrophoresis is a microgel electrophoresis technique, which detects DNA damage in individual cells. DNA tail formation is considered as the biochemical indicator of apoptotic cell death (Andrighetti *et al.,* 2006). The present study showed significant increase in comet tail length (19.33±0.18) in lymphocytes of rats exposed to cypermethrin as compared to the control rats (Table 2, Fig. 2a, 2b). Treatment of piperine with cypermethrin exposed rats showed significant decrease in comet tail length (13.08±0.14). No significant changes were observed in animals treated with piperine and vehicle alone. In agreement to the present study, many studies demonstrated that cypermethrin exposure significantly increased DNA damage and comet tail-lengths (Gabbianelli *et al.,* 2004; Ferre *et al.,* 2020). In the present study, the treatment with piperine showed the decrease in cypermethrin-induced micronuclei formation and primary DNA damage, demonstrating the protective role of piperine. Piperine is a well-known antioxidant. Addition of piperine (0.5-10 µM) significantly reduced the 1-methyl-4-phenylpyridinium-induced nuclear damage, mitochondrial membrane permeability changes, formation of reactive oxygen species and depletion of reduced glutathione in PC12 cells (Lee *et al.,* 2006). Further, Selvendiran *et al.* (2005) reported the inhibitory effect of piperine (75 mg/kg b.wt., per oral) in mice against cyclophosphamide-induced genotoxicity.

Table 1: Effects of piperine on the frequency of micronucleated polychromatic erythrocytes (MNPCEs) and comet tail length (µm) induced by cypermethrin in rats

Groups	MNPCEs/1000 PCEs	Tail length (µm)
Control	3.17±0.48	8.87±0.02
Ground nut oil	3.50±0.22	8.19±0.03
Piperine	3.50±0.34	9.15±0.04
Cypermethrin	7.87±0.08[a]	19.33±0.18[a]
Cypermethrin + Piperine	5.83±0.31[b]	13.08±0.14[b]

Means with superscript a,b differ significantly (p<0.05) within the column

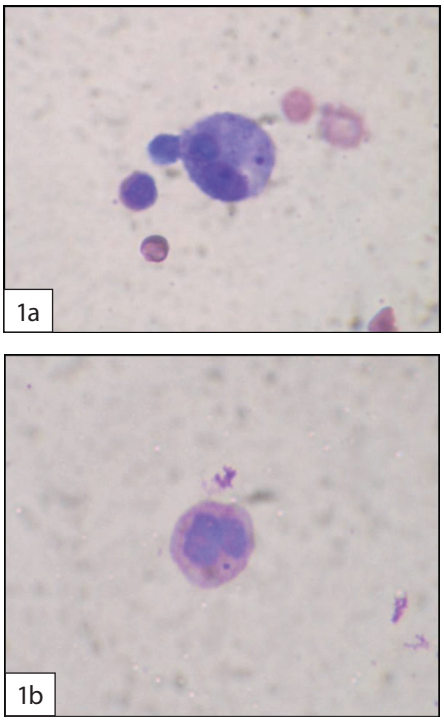

Fig. 1: Photomicrograph (1a and 1b) showing a micronucleated polychromatic erythrocyte

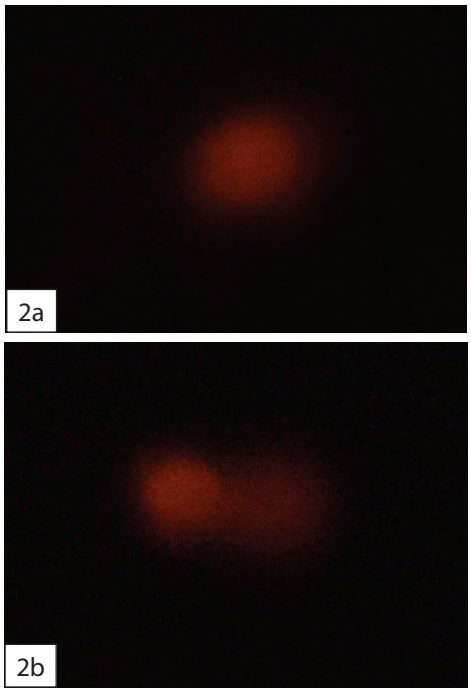

Fig. 2: Representative (2a, 2b) photomicrographs of comet in bone marrow cells

In conclusion, the oral administration of piperine could be a better remedial option against cypermethrin-induced genotoxicity. The exact mechanism by which piperine produces protective mechanism cannot be explained at present, but its antioxidant potential may be the possible reasons.

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