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Protective Effect of Curcumin Against Imidacloprid-induced Genotoxicity in Rats

P. R. Sakunthala Devi*, A. Gopala Reddy, G. Boobalan and C. S. V. Satish Kumar

Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Hyderabad - 500030, Telangana, India; sakunthalavet@gmail.com

Abstract

Aim: To study the effect of curcumin in imidacloprid (IMI)-induced genotoxicity by comet and micronucleus assays. **Materials and Methods:** Experiments were conducted on adult rats that were randomly divided into six groups of six rats as follows: Group 1: Sham; Group 2: Curcumin (50 mg/kg po); Group 3: IMI (20 mg/kg po); Group 4: IMI (40 mg/kg po); Group 5: IMI (20 mg/kg po) + curcumin (50 mg/kg po); and Group 6: IMI (40 mg/kg po) + curcumin (50 mg/kg po). Micronucleus and comet assays were used to study the DNA damage in all the groups. **Results:** There was a significant ($P < 0.05$) increase in the comet tail length (μm) and the frequency of micronuclei formation in the Group 4 treated with 40 mg/kg IMI when compared to other groups. Curcumin treatment in Group 6 showed a significant ($P < 0.05$) decrease in the comet tail length, and micronuclei as compared to Group 4 though the decrease was significantly ($P < 0.05$) low as compared to other groups. **Conclusions:** This study revealed that IMI has a tendency to induce the genotoxicity on exposure to 40 mg/kg b.wt. Curcumin, due to its anti-oxidant and anti-mutagenic property, alleviated the genotoxicity induced by IMI.

Keywords: Comet Assay, Curcumin, DNA, Genotoxicity, Imidacloprid

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1. Introduction

Toxicity of pesticides for nontarget organisms is of worldwide concern. Many of the pesticides are known to cause genotoxicity (e.g. endosulfan, malathion, etc.,) in nontarget species. Imidacloprid [1-(6-chloro-3-pyridylmethyl)-2-nitroimino-imidazolidine] (IMI) belongs to a major new class of synthetic insecticides (neonicotinoids) and is used widely to control the insect pests on crops and fleas on cats and dogs. Due to the selective effect on insects and apparent safety for humans, the sale of the insecticide is in fast pace.[1] Its selective toxicity results from its high affinity to insect's nicotinic acetylcholine receptors (nAChR) as compared to mammals.[2]

According to the WHO and United States Environmental protection Agency, this compound is categorized as "moderately toxic." Recently, IMI has raised concern because of its ability to cause eggshell thinning, reduced egg production, and hatching time, which are considered as the signs of possible endocrine disruption[3] and honey bee colony collapse disorder.[4] Experimental studies suggest that chronic exposure to IMI may induce neurobehavioral defects in offspring rats following *in utero* exposure,[5] and genotoxic and mutageni c alterations.[6] Therefore, it appears that this agent may not be as innocuous as reported. Reports of deleterious effects of IMI in veterinarians, veterinary technologists, dog caretakers, and owners and also of human poisoning have highlighted the need to examine the interaction of such compounds with mammalian nAChRs.[7] Despite that both animals and humans are intensely exposed to neonicotinoid pesticides, there is very little data available on the chronic toxicity of IMI to nontarget organisms, particularly about the effects of IMI on genotoxicity. Thus, increased potential neurotoxic hazards from IMI may result from the global increase in its use, persistence in crops, vegetables and fruits, and proximity to treated pets.

Curcumin, the main biologically active phytochemical component of turmeric (*Curcuma longa* L.), is nontoxic, a highly promising natural compound having a wide spectrum of biological functions. Extensive studies during the past

* *Author for correspondence*

half a century have demonstrated the protective action of curcumin in almost all the disorders in the biological system. It is also known to be a potent anti-oxidant. The molecule also possesses anti-bacterial, anti-viral, anti-inflammatory, anti-nociceptive, anti-hypertensive, anti-hyperlipidaemic, anti-fungal, anti-cancer, hepatoprotective, nephroprotective, nti-thrombotic, anti-mutagenic, anti-fibrotic activities and; thus, has a potential to treat various diseases including diabetes, asthma, allergies, arthritis, atherosclerosis, etc.[8,9]

The goal of our study was to determine the genotoxicity of IMI and its amelioration with curcumin.

2. Materials and Methods

2.1 Chemical Reagents

IMI and curcumin were administered orally by dissolving in olive oil as a vehicle.

2.2 Animals

Male albino rats of Sprague–Dawley strain weighing around 200–250 g were procured from National Institute of Nutrition, Hyderabad, Andhra Pradesh. The use of animals in this study was approved by the committee for the Purpose of Control and Supervision of Experiments on Animals.

2.3 Experimental design

The study was carried out for 3 months on 36 rats that were randomly divided into six groups of six rats in each group (OECD 408 guidelines, 1998).

- Group 1: Sham control
- Group 2: Curcumin (50 mg/kg po)
- Group 3: IMI (20 mg/kg po) once daily for 12 weeks
- Group 4: IMI (40 mg/kg po) once daily for 12 weeks
- Group 5: IMI (20 mg/kg po) + curcumin (50 mg/kg po) once daily for 12 weeks
- Group 6: IMI (40 mg/kg po) + curcumin (50 mg/kg po) once daily for 12 weeks.

3. Genotoxicity Assays

3.1 Micronucleus Assay

Micronucleus test was used for the detection of damage induced by the test substance to the chromosomes or the mitotic apparatus of erythroblasts by the analysis of erythrocytes sampled from the bone marrow of rats.[10]

3.2 Procedure

The femur bones were removed, and the bone marrow was gently flushed into tubes containing 0.5 ml Fetal Calf Serum (FCS). The serum was centrifuged at 1000 rpm for 8 min, and the sediment resuspended in a drop of the FCS. Thin smears were prepared on slides and air-dried for 24 h. Slides were fixed in absolute methanol for 3 min after which they were stained with a mixture of 12 ml Giemsa, 6 ml Leishman, and 2 ml phosphate buffer, pH 5.8, for 5 min. Afterward, the slides were rinsed with phosphate buffer and air-dried. With the oil-immersion objective lens, 1000 polychromatic erythrocytes (PCEs) per animal from the coded slides were screened for the presence of micronucleated PCE. The number of micro nucleated normochromatic erythrocytes (MNNCEs) was registered in a total of 1000 NCEs per animal.

$$\text{MN frequency (\%)} = \frac{Number\ of\ cells\ containing\ micronucleus}{Total\ no.\ of\ cells\ counted} \times 1000$$

3.3 Comet assay (Single cell gel electrophoresis)

DNA damage was carried out by the method of detection of DNA damage in single cells.[11]

3.4 Preparation of Slides

Dipped the slides in methanol and heated on a blue flame to remove machine oil/dust. When 1% normal melting point agarose was hot, dipped slides up to 1/3 frosted area, and removed gently. Wiped off the agarose under side of the slide and dried.

3.5 Preparation of Cells from Whole Blood

Blood was collected in EDTA vials and diluted with equal volumes of phosphate buffered saline. Fifty microliter, each of diluted blood and 1% low melting point agarose (LMPA) were added onto the coated slides. Placed the cover slip over it, and the slides were kept on a slide tray resting on ice packs until the agarose layer hardened (5–10 min). Gently, slided off the cover slip and added a third agarose layer (80 ml LMPA) onto the slide. Replaced the cover slip and returned the slide on the slide tray resting on ice packs until the agarose layer hardened (5–10 min).

3.6 Electrophoresis of Microgel Slides (2–4°C)

Coated slides were placed in the lysis buffer of pH 10 for 1 h, and the slides were then placed on the horizontal

gel electrophoresis chamber filled with electrophoresis buffer of pH 13. A presoaking of 20 min was done in the electrophoresis buffer in order to unwind DNA. Electrophoresis was then carried out for about 20 min (20 v/300 mA). The slides were washed thrice (5 min each wash) with neutralizing buffer and were dehydrated with 80% methanol at room temperature for about 30 min and stored until analysis. Before staining, the slides were rehydrated with chilled water or stained immediately with ethidium bromide for 5 min (add 80 ml stain) and dipped in chilled water to remove excess stain.

At least 100 cells per slide per subject were analyzed (original magnification ×200) under a fluorescent microscope equipped with a green light excitation and at 590 nm barrier filter. Comets form as the broken ends of a negatively charged DNA molecule becomes free to migrate in the electric field toward the anode. The extent of DNA damage was assessed from the length of DNA migration derived by subtracting the diameter of the nucleus from the total length of the image. The mean tail length of the damaged DNA was done using image analysis.

3.7 Statistical Analysis

The data obtained were subjected to statistical analysis by applying one-way ANOVA using SPSS® 16.0 (IBM corporation, USA) followed by Duncan test at 5% ($P < 0.05$) level of significance. The results were expressed as a mean ± standard error.

4. Results and Discussion

Genomic damage, produced by environmental exposure to genotoxins is probably the most important fundamental cause of developmental and degenerative diseases. In this study, the genotoxicity was assessed by comet and micronucleus assays. The comet assay is a simple, sensitive, and rapid geno-toxicological method used to estimate DNA damage at the individual cell level through strand breaks, open repair sites, cross-links, and alkali labile sites caused by oxidative stress.[12]

In this study, it was found that cells from the control rats had tightly compressed DNA and maintained the circular form of the normal nucleus, with little or no evidence of comet formation. In contrast, cells from the rats treated with 40 mg/kg IMI displayed an altered appearance. There was a significant ($P < 0.05$) increase in the comet tail length (mm) in the Group 4 treated with 40 mg/kg IMI when compared to other groups. Curcumin treatment in Group 6 showed a significant ($P < 0.05$) decrease in the comet tail length as compared to Group 4. However, the decrease was significantly ($P < 0.05$) low when compared to Groups 1, 2, 3, and 5 [Table 1 and Figures 1-3].

Table 1. Length of comet tail in whole blood cells of different groups of rats

Group	Tail length (µm)
1.Control	7.55±1.11
2.Curcumin control (50 mg/Kg)	7.45±1.14
3.Imidacloprid (20 mg/Kg)	7.52±1.22
4.Imidacloprid (40 mg/Kg)	15.34±2.13c
5.Imidacloprid (20 mg/Kg) + Curcumin (50 mg/Kg)	7.50±1.43
6. Imidacloprid (40 mg/Kg) + curcumin (50 mg/Kg)	10.67±1.64b

Values are Mean+SE (n=6); Duncan's multiple comparison test. b,c $P<0.05$ as compared to group 1, 2, 3, 5

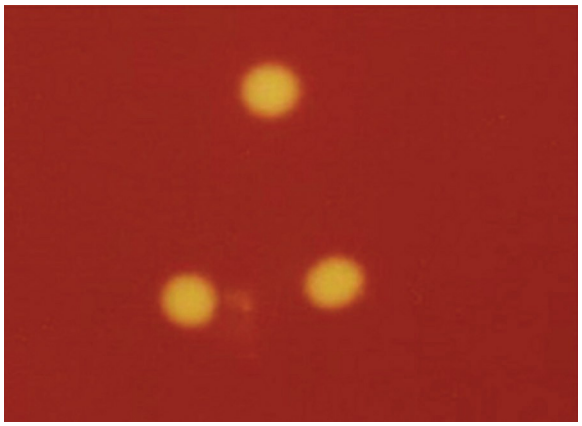


Figure 1. Comet of peripheral blood cells with ethidium bromide stain, ×65. Note the intact DNA (Group 1).

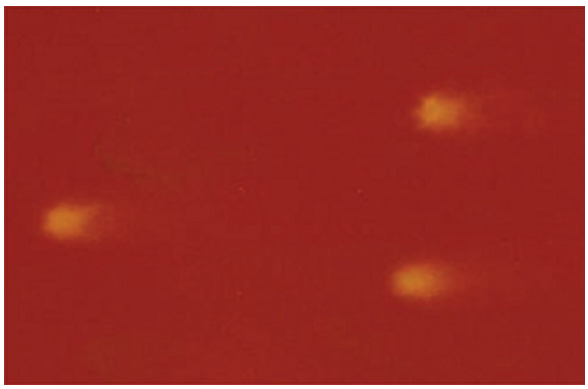


Figure 2. Comet of peripheral blood cells with ethidium bromide staining, ×65. DNA damage seen as comet (Group 4).

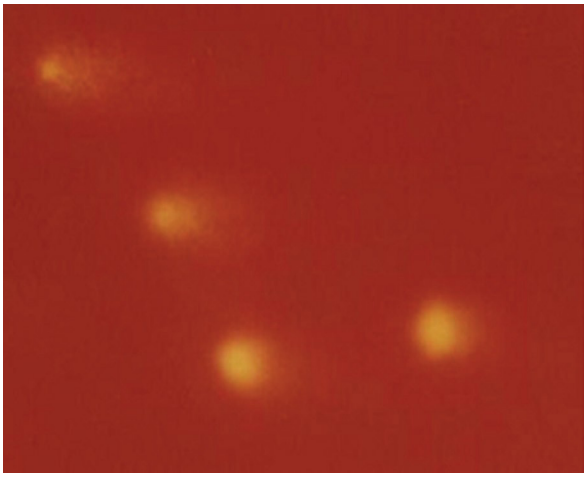


Figure 3. Comet of peripheral blood cells with ethidium bromide staining, ×65. DNA damage seen as comet (Group 6).

The basis of this altered appearance was, during alkaline unwinding (at pH 13.0) double stranded super coiled DNA was unwound and denatured. As a result, double stranded complex DNA became single stranded and relaxed. Hence, when electrophoresis was carried out, due to electric attraction, fragmentized DNA (negatively charged) was migrated toward anode leaving the intact DNA in the head region. If nucleus contains more damaged DNA, a large amount of these broken DNA (which are small in size as compared to intact DNA molecule) will migrate in the tail region. Thus, the comet-like shape was formed as evident in this study. The results of the present study suggest that IMI is associated with oxidative stress leading to oxidative DNA damage in peripheral blood cells. The results are in accordance with the previous study.[13] Therefore, DNA damage in the peripheral blood could be a suitable early bio-marker to monitor the environmental or occupational IMI toxicity. Curcumin, due to its anti-genotoxic potential, decreased the DNA damage as evinced by a decrease in comet tail length.[14,15]

Micronucleus assay is the most extensively used indicators of early biological effects associated with DNA- damaging agents. Micronuclei are acentric fragments or complete chromosomes that fail to attach to the mitotic spindle during cytokinesis and are excluded from the nuclei. Different mechanisms are involved in the formation of micronuclei including chromosome breakage (clastogenesis) and spindle disruption (aneugenesis).[16] The frequency of occurrence of micronuclei in the femoral bone marrow showed a significant ($P < 0.05$) increase in toxic Group 4 as compared to the remaining groups. Group 6 revealed the significant ($P < 0.05$) decrease in micronuclei frequency as compared to toxic group 3, but significantly ($P < 0.05$) less when compared to other groups [Table 2 and Figure 4].

Table 2. Frequency of micronuclei in bone marrow erythrocytes of different groups of rats

Group	MN frequency
1.Control	1.61±0.12
2.Curcumin control (50 mg/Kg)	1.58±0.12a
3.Imidacloprid (20 mg/Kg)	1.65±0.13
4.Imidacloprid (40 mg/Kg)	3.54±0.12
5.Imidacloprid (20 mg/Kg) + Curcumin (50 mg/Kg)	1.66±0.11
6. Imidacloprid (40 mg/Kg) + curcumin (50 mg/Kg)	2.15±0.03

Values are Mean+SE (n=6); Duncan's multiple comparison test. b,c P<0.05 as compared to group 1, 3, 4, 5,6

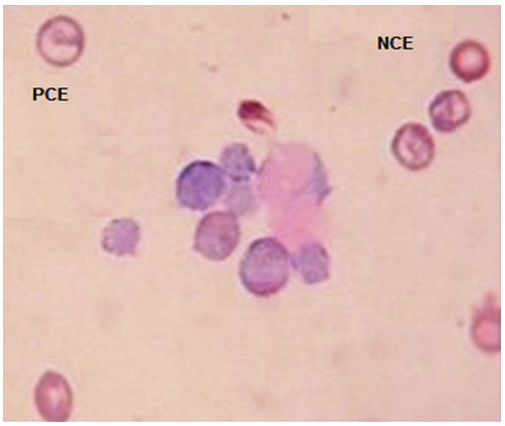


Figure 4. Bone marrow smear showing polychromatic erythrocyte and normochromic erythrocyte, ×100 (Group 4).

In this present study, the frequency of MN formation was seen with IMI at 40 mg/kg, while 20 mg/kg IMI showed negative result. These results are in agreement with previous reports.[16] Curcumin decreased MN frequency due to its anti-oxidant and anti-mutagenic potential.

5. Conclusion

The present study revealed that IMI has a tendency to induce genotoxicity on exposure to 40 mg/kg b.wt. Curcumin, due to its anti-oxidant and anti-mutagenic property, alleviated the genotoxicity induced by IMI.

6. Acknowledgements

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Nil.

8. Conflicts of Interest

There are no conflicts of interest.

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