

Original Article**Cypermethrin-Induced Liver Histopathology in Rat: Protective Role of Jamun Seed and Orange Peel Extracts**Babita Deep Srivastava¹, Manish Srivastava², Sunil Kumar Srivastav¹, Nobuo Suzuki³, Ajai Kumar Srivastav^{*1}

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ABSTRACT

Background: We evaluated ameliorative effects of Jamun seed and orange peel extracts on liver toxicity in cypermethrin exposed rat.

Methods: Rats were given following treatments daily for 30 d:

Group A: Control

Group B: Rats received cypermethrin

Group C: These rats received cypermethrin and Jamun seed extract simultaneously

Group D: These rats received cypermethrin and orange peel extract simultaneously

Group E: Rats received orange peel extract

Group F: Rats received Jamun seed extract

In respective groups dose of cypermethrin, Jamun seed and orange peel extract were 25 mg/kg body wt, 200 mg/kg body wt and 200 mg/kg body wt, respectively. Liver was fixed for light and electron microscopic studies.

Results: After 15 d cypermethrin or cypermethrin+JSE or cypermethrin+OPE treatment hepatocytes exhibited increased cell size, nuclei became more hyperchromatic and hypertrophied. Degeneration of nuclei and dilatation of sinusoids have been noticed. After 30 d cypermethrin treatment hepatocytes exhibit more pronounced hypertrophy with hyperchromatic nuclei. Few hepatocytes exhibit nuclei with irregular boundaries. Hepatocytes show foamy cytoplasm and few vacuoles. Focal necrosis visible at certain places. Binucleated cells are also encountered. In cypermethrin+JSE and cypermethrin+OPE treated rats, hepatocytes exhibit hypertrophy, hyperchromatic nuclei and dilatation of sinusoids. Degeneration of hepatocytes are seen at some places, however, focal necrosis is not seen in these groups. No noticeable histological alterations are seen in orange peel extract (group E) and jamun seed extract (group F) treated rats.

Conclusion: Cypermethrin induced hepatic toxicity can be protected by treatment with Jamun seed and orange peel extract.

Keywords: Cypermethrin, Hepatocytes, Pyrethroid, Jamun Seed Extract, Liver, Orange Peel Extract Liver.

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INTRODUCTION

Pesticides are used to eradicate pests, but their use provoked adverse effects in non-target organisms. Pyrethroids are synthetic analogues of natural pyrethrins which occur in *Chrysanthemum cinerariaefolium* and related species [1].

In recent years synthetic pyrethroids are most commonly used insecticides and preferred over organochlorines, organophosphates, and carbamates because of possessing high effectiveness against wide range of insects, rapid biodegradation and low toxicity to non-target organisms [2]. Cypermethrin

is a common synthetic pyrethroids used to control pests including moths in cotton, fruits and vegetable crops as well as in public and animal health programmes [1, 2]. Although cypermethrin has been considered as non-toxic to mammals cypermethrin affects physiological, biochemical and histopathological alterations in experimental organisms [2-6].

Plants including fruits, herbs, spices, and vegetables are rich source of phytonutrients/phytochemicals. In recent years, phytonutrients have been reported to lessen the threat of pesticides as these phytonutrients have

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been associated with their antioxidant characteristics as they scavenge free radicals. Jamun, botanically named as *Syzygium cumini* belongs to family Myrtaceae. Jamun fruits and seeds contain antioxidant compounds i.e. flavonoids, phenolic acids and anthocyanins [7, 8]. Citrus, *Citrus sinensis* is important fruit crop having rich source of phytochemicals such as flavanones, polyphenols, anthocyanins and hydroxycinnamic acids. The peel of citrus fruit contains abundant flavanones and polymethoxylated flavones which are very rare in other plants. Orange peel extract has been reported to possess antioxidative potential [9, 10]. Although few studies have been performed to evaluate the liver toxicity after cypermethrin exposure in mammals [4-6] there is lack of information regarding the protective effects of Jamun seed and orange peel extracts on liver toxicity induced by cypermethrin. Hence, in the present study, we evaluated the ameliorative effects of Jamun seed and orange peel extracts on the liver toxicity in cypermethrin exposed rat.

MATERIALS AND METHODS

Male Wistar rats weighing approximately 115-130 g were used in this study. The animals were housed in polypropylene cages under natural photoperiod. Food and tap water were given during the study period. All treatments were started after almost 2 wk of acclimatization of the rats in the laboratory. Rats were maintained on the standard laboratory feed and water *ad libitum* throughout the acclimation and experimental periods. The animals were randomly divided into six groups -- A, B, C, D, E, and F, each consisting of 20 animals. Following treatments were given daily to these groups at 08:00 each day throughout the experiment (30 d):

Group A: Control

Group B: Cypermethrin-treated: Rats received daily cypermethrin (25 mg/kg body wt)

Group C: Cypermethrin+jamun seed extract: These rats were given daily cypermethrin (25 mg/kg body wt) and jamun seed extract (200 mg/kg body wt) simultaneously

Group D: Cypermethrin+orange peel extract: These rats were given daily cypermethrin (25 mg/kg body wt) and orange peel extracts (200 mg/kg body wt) simultaneously

Group E: Orange peel extract: Rats received daily orange peel extract (200 mg/kg body wt)

Group F: Jamun seed extract: Rats received daily Jamun seed extract (200 mg/kg body wt)

The seeds of Jamun, *Syzygium cumini* were purchased from commercial supplier (M/S SVM

Naturals, Tamilnadu, India). The fruits of orange, *C. sinensis* were purchased locally and peels were separated. The jamun seeds and orange peels were washed thoroughly with water and dried in hot air oven at 40 °C. After drying, these were crushed to small pieces and powdered. Powdered Jamun seeds and orange peels were mixed with 90% ethanol in 1:20 ratio (w/v) and kept on an orbital shaker for 48 h. Then the samples were filtered with Whatman grade No.1 filter paper. The filtrates were dried in rotary evaporator at 40 °C. The dried residue was weighed and kept at -20 °C for further use. Soxhlet extraction was not used in this extraction process to prevent denaturation of heat-labile compounds present in Jamun seeds and orange peels. For use, the dried residues of Jamun seeds and orange peels were reconstituted with ethanol to provide desired dose to be given to rats. Rats (10 from each group) from all the groups were sacrificed 24 h after last dose on 15th and 30th day after initiation of the experiment under light ether anesthesia. Animals were fasted overnight before sacrifice.

Animal handling and sacrifice were carried out following the guidelines provided by Ethics Committee of the University.

Liver was fixed in Bouin's fluid for light microscopy. These fixed tissues were dehydrated in an ethanol gradient, treated with a clearing agent, infiltrated and embedded in paraffin, sectioned at 6 µm, floated on a heated water bath and mounted to glass slides. After drying overnight, paraffin was removed with a clearing agent; tissue was rehydrated in an ethanol gradient and then stained with hematoxylin and eosin (HE) for light microscopic examination. Photomicrographs were taken with the aid of Olympus CH 20i microscope and Olympus E 420 camera.

For Electron Microscopic studies, small pieces of liver were fixed in paraformaldehyde and glutaraldehyde mixture for 4 h at 4 °C. Then these tissues were washed with phosphate buffer and stored at 4 °C.

These tissues were processed at Sophisticated Analytical Instrument Facility, All India Institute of Medical Sciences, New Delhi, India.

RESULTS

The liver of control rats is composed of cords which extend from the central vein to the portal triads. Cords are separated from each other by sinusoids. Cords contain hepatocytes which are polygonal cells with a large centrally located spheroid nucleus having chromatin structure and a distinct nucleolus (Figs. 1, 2). The cytoplasm of hepatocytes is faintly granular (Fig. 1).

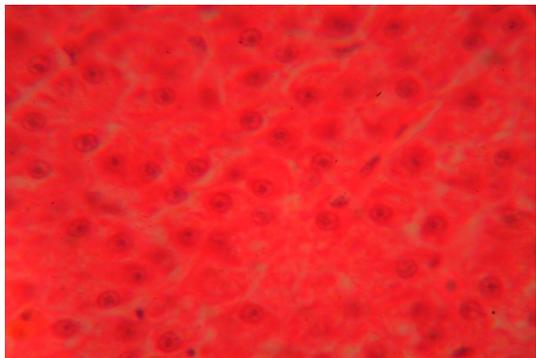


Figure 1. Liver of control rat. Hematoxylin-eosin X 500.

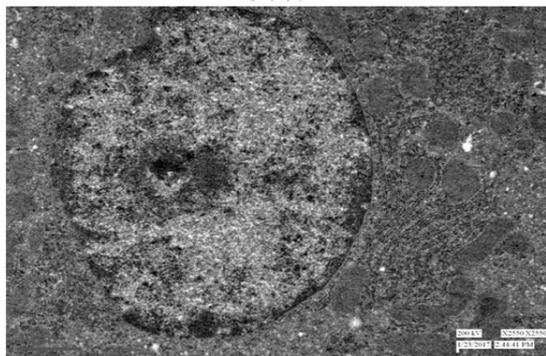


Figure 2. Electron micrograph of liver of control rat.

After 15 d cypermethrin (group B) or cypermethrin+JSE (group C) or cypermethrin+OPE (group D) treatment hepatocytes exhibited increased cell size. In these groups, nuclei became more hyperchromatic and hypertrophied (Fig. 3). Moreover, degeneration of nuclei has been encountered (Figs. 4, 5). Dilatation of sinusoids has been noticed. In group E and group F, no noticeable histological changes are observed.

After 30 d exposure to cypermethrin (group B), the hepatocytes exhibit more pronounced hypertrophy (Fig. 6) with hyperchromatic nuclei (Fig. 7). There are noticed hepatocytes having nuclei with irregular boundaries (Fig. 6, 8). The hepatocytes are seen having foamy cytoplasm (Fig. 9) and few vacuoles. Mitochondrial swelling is more pronounced (Fig. 10). Sinusoids are more dilated (Fig. 11) and filled with glycogen at few places. Degeneration of hepatocytes is more thus focal necrosis is seen at certain places (Fig. 12). Binucleated cells are also encountered (Fig. 13). In cypermethrin+JSE (group C) and cypermethrin+OPE (group D) treated rats, the hepatocytes exhibit hypertrophy, hyperchromatic nuclei, and dilatation of sinusoids. Degeneration of hepatocytes are seen at some places, however, focal necrosis is not seen in these groups. No noticeable histological alterations are seen in orange peel extract (group E) and jamun seed extract (group F) treated rats.

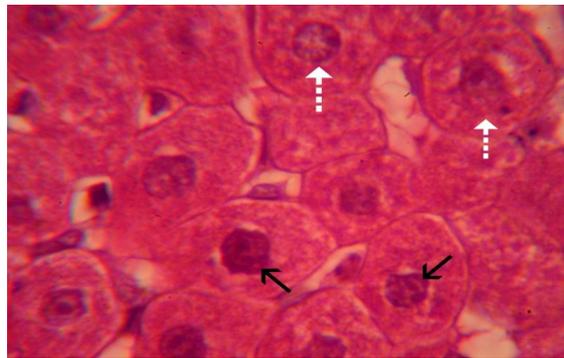


Figure 3. Hypertrophy of hepatocytes (broken arrows) and hyperchromatic nuclei (arrows) in 15 d cypermethrin treated rats. Hematoxylin-eosin X 500.



Figure 4. Degeneration of hepatocytes (arrows) in 15 d cypermethrin treated rats. Hematoxylin-eosin X 500.

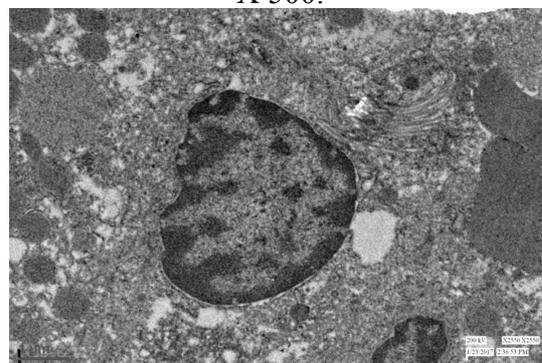


Figure 5. Electron micrograph of hepatocyte of 15 d cypermethrin treated rat showing nuclear degeneration.

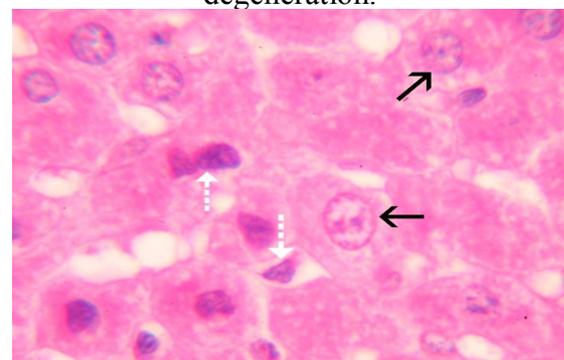


Figure 6. Pronounced hypertrophy of hepatocytes (arrows) in 30 d cypermethrin treated rat. Also note degenerating cells showing irregular boundaries (broken arrows). Hematoxylin-eosin X 500.

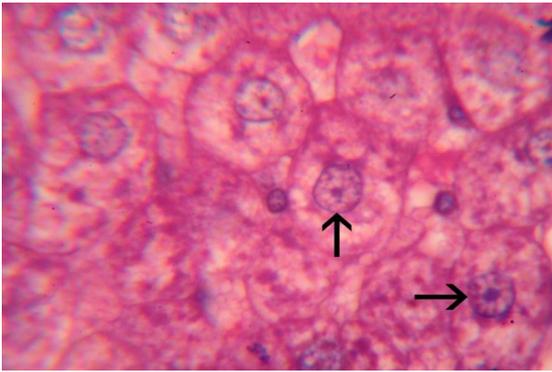


Figure 7. Hyperchromatic nuclei (arrows) in 30 d cypermethrin treated rat. Hematoxylin-eosin X 500.

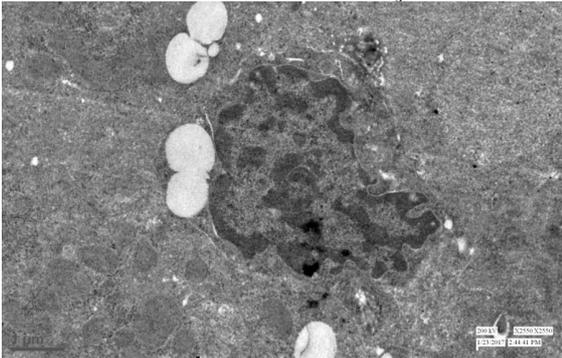


Figure 8. Electron micrograph of hepatocytes of 30 d cypermethrin treated rat showing irregular nuclear boundary and degeneration.

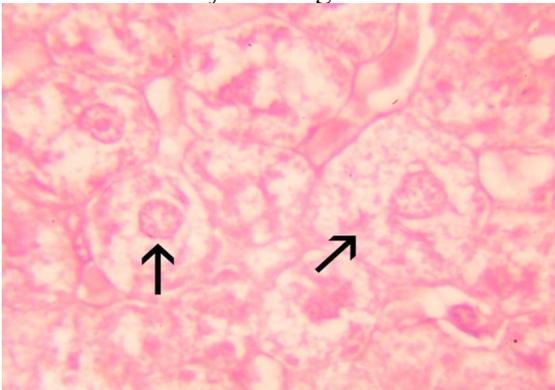


Figure 9. Foamy cytoplasm in hepatocytes (arrows) in 30 d cypermethrin treated rat. Hematoxylin-eosin X 500.

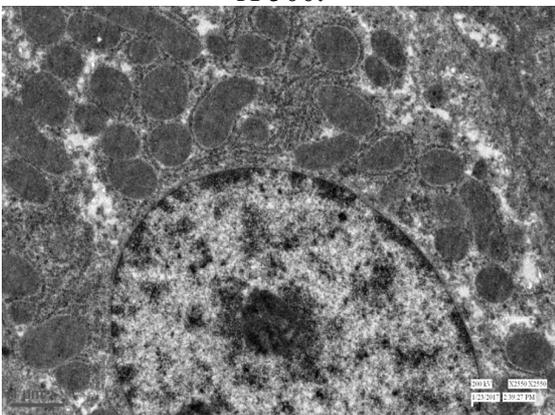


Figure 10. Electron micrograph of hepatocytes of 30 d cypermethrin treated rat exhibiting mitochondrial swelling.

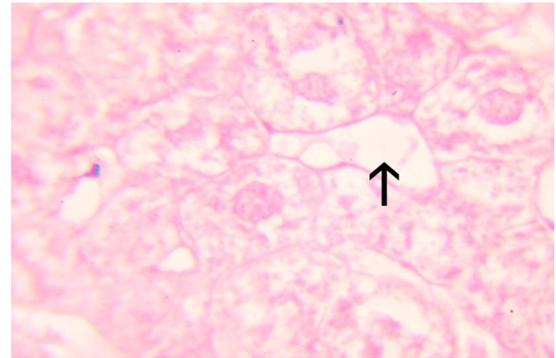


Figure 11. Sinusoidal dilatation in the liver of 30 d cypermethrin treated rat. Hematoxylin-eosin X 100.

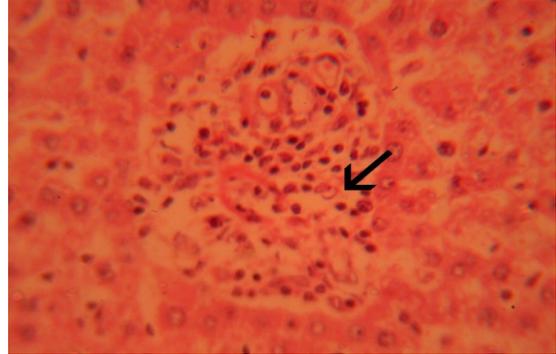


Figure 12. Focal necrosis (arrow) in liver of 30 d cypermethrin treated rat. Note the infiltration of inflammatory cells in necrotic tissue. Hematoxylin-eosin X 100.



Figure 13. Binucleated hepatocytes (arrows) in 30 d cypermethrin treated rat. Hematoxylin-eosin X 500.

DISCUSSION

Cypermethrin provoked histopathological changes in liver of rats which are hyperchromatic and hypertrophied nuclei, degeneration of hepatocytes, nuclear degeneration, hepatocytic vacuolation, sinusoidal dilatation and focal necrosis. Hypertrophy of hepatocytes and hyperchromatic and hypertrophied nuclei has also been noticed after treatment either with cadmium (rats – [11]) or chlorpyrifos (rats – [12]).

Cytoplasmic vacuolation and degeneration of hepatocytes have been noticed in cypermethrin

challenged rats. This derives support from the studies of other workers reported similar findings from toxicants treated mammals [4, 11-19], birds [20], amphibia [21] and fish [22, 23]. Few investigators have not observed changes in liver after exposure to chlorpyrifos [24] and FYROL [25]. Mild changes in liver of rats have been reported by exposure to alpha-cypermethrin [26] and diazinon [27]. Liver vacuolization points towards an imbalance between the rate of synthesis of substances in parenchymal cells and rate of their release into circulation [28].

Sinusoidal dilatation noticed in cypermethrin challenged rats is in accordance with the reports of Suput [29] and Tripathi and Srivastav [11, 12] also observed similar change in liver of toxicant exposed animals.

In cypermethrin treated rats focal necrosis was observed. In past few workers were noticed focal necrosis in liver of toxicant treated animals [11, 12, 30-32]. Binucleated cells in liver were encountered in cypermethrin challenged rats. This derives support from the observations of other investigators reported similar findings from different animals by using various toxicants [11, 12].

In the present study foamy cytoplasm was noticed in the liver of cypermethrin treatment to rats. The presence of porous/foamy cytoplasm in liver cells of rats was noticed after treatment with alpha-cypermethrin [26]. Vacuoles of different sizes and lipid-like bodies were present in empty spaces [26]. Lipid infiltration of liver cells was noticed after dichlorvos treatment [33]. Foamy cytoplasm in liver cells was encountered in rats after treatment with chlorpyrifos [11] and cadmium [12].

Degenerative changes were noticed in the liver of cypermethrin+jamun seed extract and cypermethrin+orange peel extract treated rats. However, these changes were less pronounced as compared to cypermethrin challenged rats. In cypermethrin+jamun seed extract and cypermethrin+orange peel extract treated rats, no focal necrosis has been noticed which is indicative that treatments with Jamun seed extract and orange peel extract protected the liver to some extent as compared to severe changes noticed in cypermethrin exposed rats. These extracts possess the ability to neutralize the free radicals produced by cypermethrin treatment.

In present study, there are no noticeable alterations in the liver histology of Jamun seed extract and orange peel extract treated rats. However, ballooning degeneration of hepatocytes, focal necrosis, and lymphocytic infiltration was reported in *Eugenia jambolana* seed extract

exposed rats [34]. Alloxan-induced diabetic rats when exposed to seed extract of *Syzygium cumini* for 15 d, exhibited hydropic degeneration in liver, however, after 30 d the liver became normal [35]. High level of blood sugar was noticed in these rats.

CONCLUSION

This study confirms the risk of organisms to cypermethrin as this pesticide induced severe histological lesions in liver-shrunken nuclei, degeneration of hepatocytes, obliteration of sinusoids, infiltration of inflammatory cells, focal necrosis, and mitochondrial swelling. Cypermethrin can provoke environmental stress and may pose a potential health hazard to including human via drinking water and/or food chains.

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