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Research Article

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2025.56.3.476-481

Protective effects of pomegranate peel extract against fipronil-induced pulmonary toxicity in Wistar albino rats

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Citation: Nakul, P., Sujatha, K., Anand Kumar, A. and Vijayalakshmi, S. 2025. Protective effects of Pomegranate Peel Extract (PPE) against fipronil-induced lung toxicity in Wistar Albino Rats.
J. Vet. Anim. Sci. **56** (3):476-481

Received: 26.04.2025 Accepted: 18.07.2025 Published: 30.09.2025

Abstract

Fipronil is a broad-spectrum phenyl pyrazole insecticide commonly used in agricultural, veterinary, and household practices. The present study was carried out to investigate the histopathological alterations induced by fipronil in the lungs of male Wistar albino rats and its amelioration with pomegranate peel extract. A total of 24 rats were divided into four groups with six animals in each group. Group I served as a control group provided with adlibitum feed and distilled water. Group II animals were treated with 10 mg/kg b. wt. fipronil orally for 45 days, group III animals were treated with pomegranate peel extract at 200 mg/kg b. wt. and group IV animals were treated with both fipronil (@ 10mg/kg b. wt.) and pomegranate peel extract (@ 200 mg/kg b. wt.) during the experimental period. Histopathological examination of lungs of fipronil-treated rats showed severe degenerative changes like hyperplastic and hyaline arteriosclerotic changes, degenerated and desquamated bronchial epithelium, perivascular oedema, mononuclear aggregates, etc. These changes were alleviated after administering Punica granatum peel extract along with fipronil which clearly indicates the protective effect of pomegranate peel extract against pulmonary injury induced by fipronil.

Keywords: *Fipronil, histopathology, pulmonary toxicity, male Wistar albino rats, pomegranate peel extract.*

Pesticides are chemical agents designed to control or eliminate pests such as insects, rodents, fungi, and unwanted plants. This broad category encompasses insecticides, herbicides, fungicides, nematicides, molluscicides, rodenticides, plant growth regulators, and other related substances (Zhan *et al.,* 2020; Bhatt *et al.,* 2021). These compounds play a crucial role in managing vector-borne diseases, safeguarding crops, preserving food and supporting various commercial and industrial sectors including aquaculture, agriculture, food manufacturing and storage (Mieldazys *et al.,* 2015; Sharma *et al.,* 2019). However indiscriminate use of pesticides will evoke harmful side effects in humans and animals. Fipronil is one among the new class of emerging phenyl pyrazole insecticides, which is ubiquitous in the environment and food due to its broad spectrum and persistent characteristics (Chen *et al.,* 2021). It blocks gamma-aminobutyric acid (GABA)-gated chloride channels, which results in excessive neural stimulation and neuronal toxicity in animals (Abou-Zeid *et al.,* 2021). Fipronil also undergoes environmental degradation, resulting in the formation of several metabolites such as fipronil desulfinyl, fipronil sulfone, and fipronil sulfide. Prolonged exposure to fipronil has

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been associated with adverse effects on organs like the liver, kidneys, and thyroid (Singh *et al.,* 2021). Emerging research suggests that oxidative stress is a key contributor to fipronil-induced toxicity, primarily through disruption of the antioxidant defense mechanisms, leading to lipid and protein damage (Deiu *et al.,* 2021).

Recent medical studies have validated the therapeutic and protective benefits of traditional herbal medicines leading to growing public interest in their use (Anquez-Traxler*,* 2011). Among these natural remedies, pomegranate stands out due to its safety as a dietary component and its emerging significance in scientific research (Ajaikumar *et al.,* 2005). Over the past several decades, extensive investigations have highlighted the potent antioxidant capabilities of pomegranate, particularly its peel, which exhibits the highest antioxidant activity (Gil *et al.,* 2000). The primary constituents responsible for these antioxidant effects and anti-inflammatory effects in pomegranate peel extract (PoPx) are punicalagin (PC) and ellagic acid (EA) (Gil *et al.,* 2000). While the toxic effects of fipronil on various organs have been well-documented, there is a noticeable lack of research concerning its impact on the lungs. Therefore, the present study aims to investigate the pulmonary toxicity induced by fipronil and check the protective role of *Punica granatum* peel extract in mitigating these toxic effects induced by fipronil in the pulmonary tissue of Wistar rats

Materials and methods

The study was conducted in 24 healthy male Wistar rats weighing around 120 grams which were procured from Sri Venkateshwara Enterprises, Bangalore. Animals were grouped into four groups with six rats in each group. Group I served as negative control and was provided with adlibitum feed and distilled water, group II was fipronil control group treated with fipronil @ 10mg/kg b. wt. orally in distilled water. Group III was treated with *Punica granatum* orally in distilled water @ 200 mg/kg body weight (daily) and group IV rats were treated with both fipronil (@ 10mg/kg b. wt.) and *Punica granatum* orally in distilled water @ 200 mg/kg body weight (daily) for checking the ameliorative effects (Jyothi *et al*. 2021). Approval of the institutional animal ethical committee was obtained prior to the commencement of the experiment (Vide order no 281/ go/ ReBi /S/2000/CPCSEA/ CVSc/ TPTY/ 010/ Veterinary Pathology/ 2023 dated: 08.05.2023). 99 per cent pure technical grade fipronil (FIP92B5266) was procured from Gharda Chemicals Ltd. Mumbai and *Punica granatum* peel extract powder (Dadim LC 23030077) was procured from Chemiloids Life Science Pvt. Ltd Vijayawada, Andhra Pradesh.

Animals were euthanised by chloroform inhalation followed by exsanguination via the retro-orbital plexus after the end of the experimental period (45 days) and a detailed postmortem was conducted. Representative tissue pieces from lungs were collected from all experimental rats and preserved in 10 per cent neutral buffered formalin followed by routine paraffin embedding technique for histopathological studies. Sections of 5-6 µ thickness were cut and stained with routine Haematoxylin and Eosin (H&E) method (Culling, 1974).

Result and discussion

Histopathology of lungs of group II rats revealed dilated, thickened, and congested blood vessels with moderate to severe infiltration of mononuclear cells (MNCs) and eosinophils in peribronchial and perivascular spaces (Fig. 1,2,3). Thickened interstitial space with proliferation of fibroblasts, infiltration of MNCs and eosinophils along with narrowing of alveolar lumen (Fig. 4) were evident in all fipronil-treated rats. Hyperplastic and hyaline types of arteriosclerotic changes (Fig. 5) along with perivascular oedema (Fig: 6), haemorrhages (Fig. 7), and mononuclear cell aggregates were evident

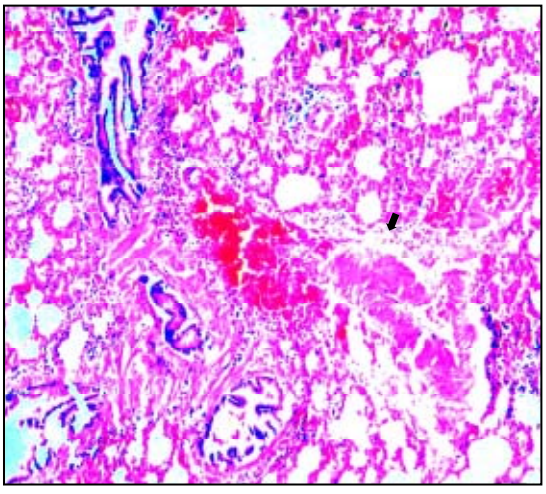

Fig. 1. Lung: Group II : Section showing hyaline type arteriosclerosis (arrowed) and congested blood vessels, perivascular and peri-bronchiolar infiltration of MNCs. H & E: x 40

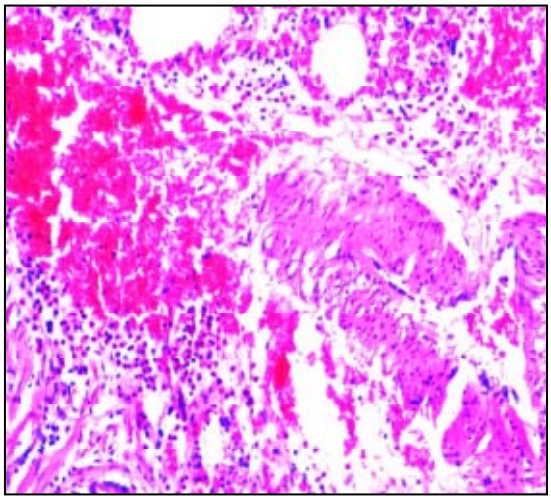

Fig. 2. Lung: Group II : Note pockets of haemorrhage with infiltration of eosinophils. H & E: x 100

in majority of animals. Bronchial and bronchiolar epithelial cells showed severe degeneration and desquamation with the presence of desquamated cellular debris in the lumen (Fig. 8) being more conspicuous. In some rats, hyperplasia of the bronchial and bronchiolar epithelium (Fig. 9) was also noticed. Bronchiectasis with peribronchiolar and perivascular adipose tissue deposition was observed in some rats (Fig. 10). In multifocal areas, the thickened alveolar septa contained multinucleated giant cells (Fig. 11), alveolar macrophages, eosinophils, lymphocytes, and fibroblasts in some rats. Emphysematous changes and peribronchial lymphoid hyperplasia were observed by the end of 6th week. These findings were in agreement with that of Prarabdh *et al.* (2014), Merkowsky *et al.* (2016), Khalil *et al*. (2019) and Bassit (2023). The changes in the current study, mainly inflammatory changes might be attributed to increased production of NO and expression of genes associated with inflammatory cytokines (Khalil *et al.,* 2019). Fipronil could also induce severe eosinophilic infiltration by greatly increasing histamine, IL-12, IL-4 and IgE production, which can cause allergic inflammatory reactions in organs (Aldayel *et al.,* 2021). The presence of adipose tissue in lungs might be due to the adipogenic nature of fipronil through the activation of AMPKα mediated pathway (Sun *et al.,* 2016). Histopathological alterations induced by fipronil in soft tissues might be due to the generation of excess free radicals and increased lipid peroxidation of the lipid membrane. Fipronil primarily modifies NADPH oxidase activity in the inner mitochondrial membrane. This will ultimately disturb the functioning of electron transport chain and lipid peroxidation in cell membranes. It also damages the DNA structure, lipids and protein configuration and causes complete histologic alterations of soft tissues including lungs (Walmsley and White, 1994; Adbdel-Daim and Abdeen, 2018). Microscopically, the lungs of group IV rats showed only mild changes like mild congestion, minimal thickening of interalveolar septae, mild infiltration of MNCs in perivascular and peribronchial

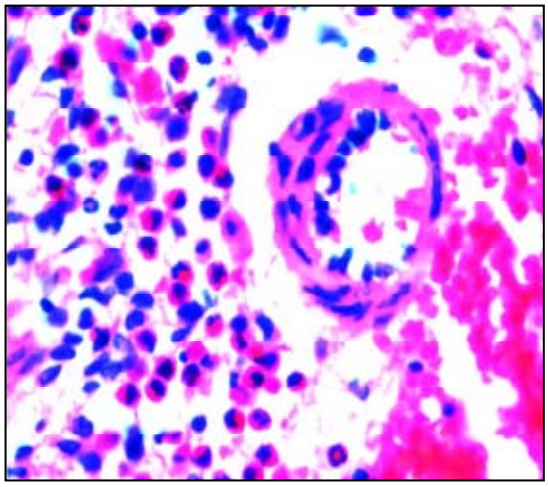

Fig. 3. Lung: Group II : Section showing perivascular infiltration of eosinophils. H & E: x 400

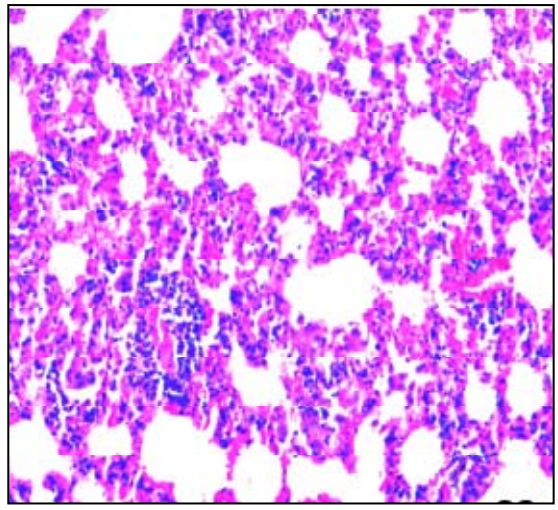

Fig. 4. Lung: Group II : Section showing thickened interstitial space with narrowed alveolar lumen. H & E: x 100

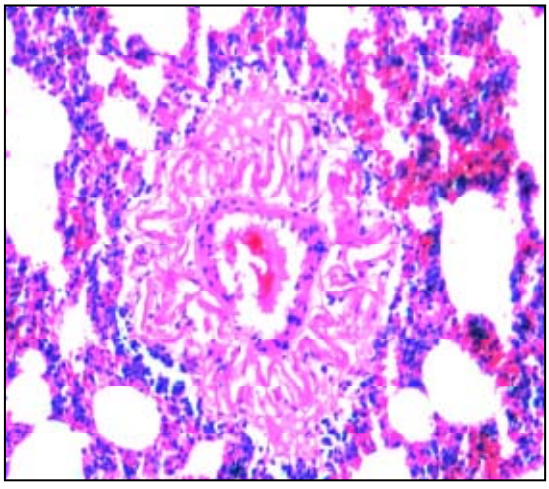

Fig. 5. Lung: Group II : Note thickened blood vessels with hyperplastic type of arteriosclerotic changes. H & E: x 100

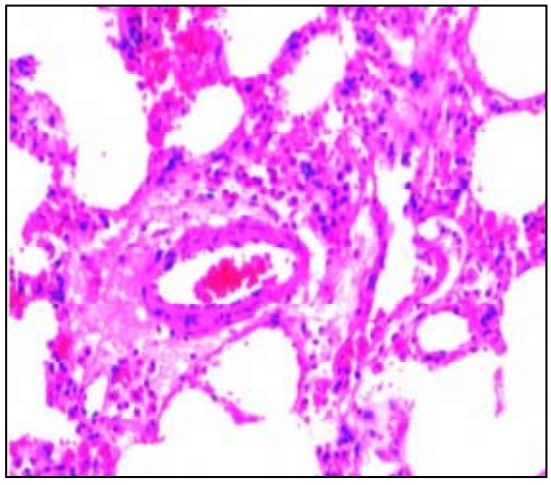

Fig. 6. Lung: Group II : Section showing perivascular edema with infiltration of eosinophils and MNC's. H& E: x 100

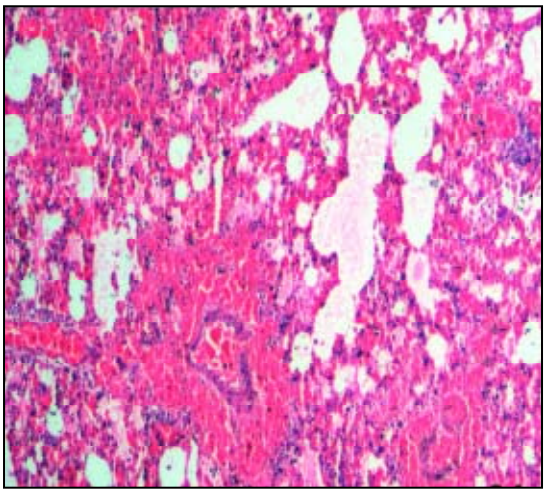

Fig. 7. Lung: Group II : Section showing perivascular and interstitial hemorrhages. H & E: x 40

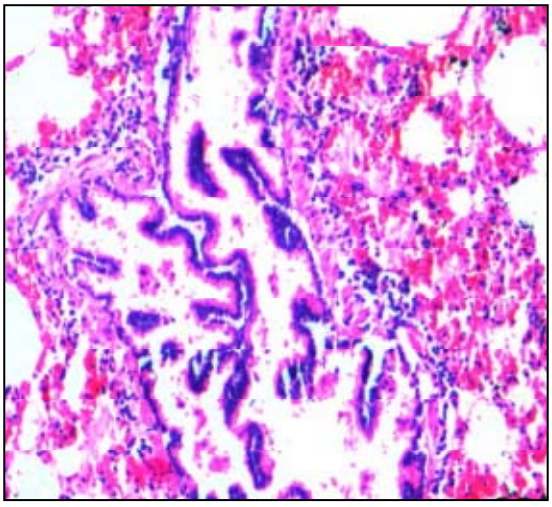

Fig. 8. Lung: Group II : Section showing degenerated and desquamated bronchial epithelium with presence of desquamated cellular debris in the lumen. H & E: x 100

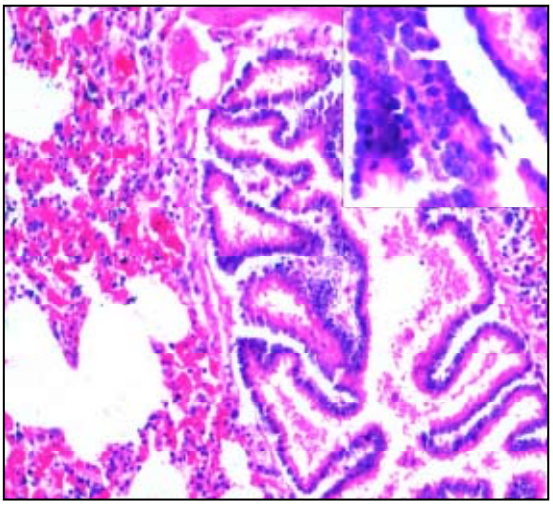

Fig. 9. Lung: Group II : Section showing hyperplasia of bronchial epithelium. H & E: x 100 (Insight: Hyperplastic epithelium. H&E: x 400)

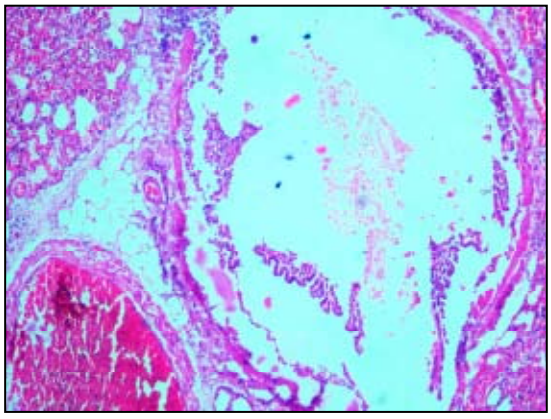

Fig. 10. Lung: Group II : Section showing bronchiectasis with peribronchial adipose tissue deposition (arrowed). H & E: x 40

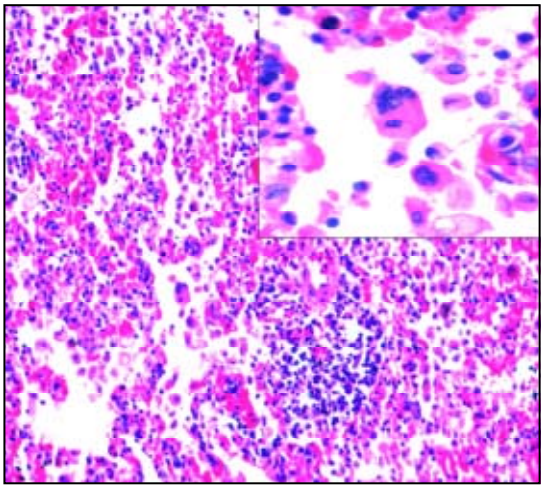

Fig. 11. Lung: Group II: Note the presence of multinucleated giant cells and MNCs in alveolar lumen. H & E: x 100, (Insight microphotograph x 400)

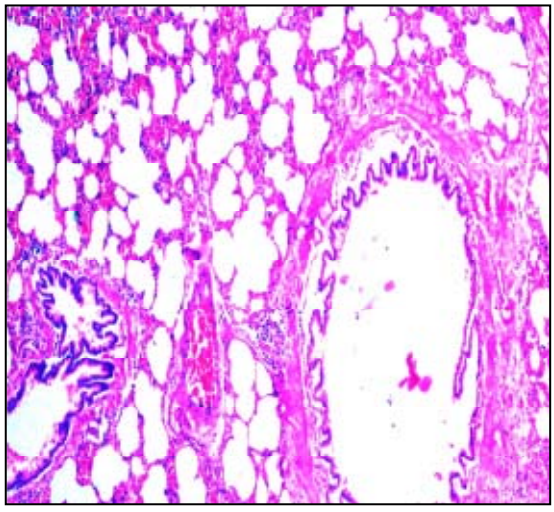

Fig. 12. Lung: Group IV : Section showing normal bronchial epithelium with less degenerative changes. H& E: x 40

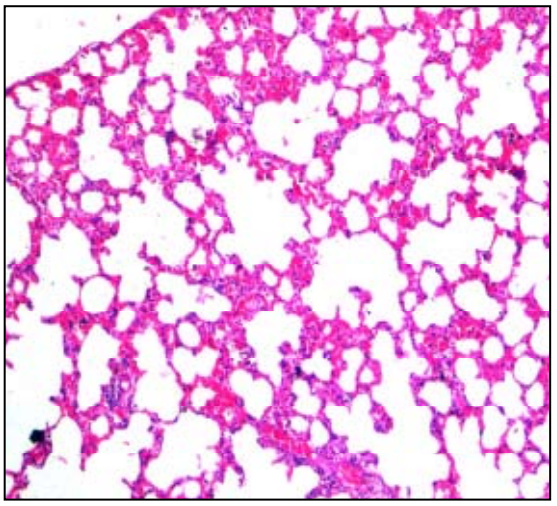

Fig. 13. Lung: Group IV : Section showing near to normal in appearance of lung. H & E: x 40

spaces, less degenerative changes in bronchial epithelium (Fig. 12) and lung was found to regain near to normal architecture (Fig: 13) when compared to fipronil treated (group II) rats. This might be due to the anti-inflammatory and antioxidant properties of *Punica granatum* (Moneim, 2012; Dkhil, 2013 and Hasan *et al*., 2016) which reduces the inflammatory changes and oxidative damage in the lungs. These reduced lung alterations might be due to high polyphenolic compounds, especially ellagitannins present in the pomegranate peel extract, which can easily pass through the mitochondrial membrane and decrease the production of excess free radicals that damage the lung tissue (Gil *et al.,* 2000; Rosenblat *et al.,* 2015).

Conclusion

The present study demonstrates that fipronil exposure induces significant lung toxicity in Wistar albino rats, as evidenced by marked histopathological alterations. However, co-administration of pomegranate peel extract (PPE) exhibited a notable protective effect, mitigating these adverse changes. The antioxidant and anti-inflammatory properties of PPE play a crucial role in preserving the structural and functional integrity of lung tissue. These findings suggest that PPE may serve as a potential natural therapeutic agent against pesticide-induced pulmonary toxicity. Further studies are warranted to explore its molecular mechanisms and potential clinical applications.

Acknowledgments

The authors are thankful to the Sri Venkateswara Veterinary University, Tirupati for providing various facilities to carry out the study.

Conflict of interest

The authors declare that they have no conflict of interest.

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