Histological assessment of the Effects of Pyrethroids insecticide Mortein on the Lungs of Adult Wistar Rats

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Abstract:
Introduction: Pyrethroids insecticide Mortein (M) composed of allethrin, imiprothrin and phenothrin is commonly used in the annihilation of household insects in Nigeria. These chemicals have been associated with the development of some respiratory diseases. This study was carried out to assess the histological effects of Pyrethroids insecticide “M” on the lungs of adult Wistar rats.

Materials and Methods: twenty five healthy adult Wistar rats of both sexes were acquired from the animal house of Faculty of Basic Medical Sciences, Delta State University Abraka and used for the experiment. The rats were randomly assigned into five groups. Groups I-III was orally given a sub-lethal dose of 2250mg/kg/bw of M for 7, 21 and 40 days respectively, IV was given tap water as placebo while V was given olive oil. At the end of the experiment, the rats were sacrificed, lungs harvested and fixed in 10% formal saline. The lungs tissues were processed for histology and the slides were microscopically analyzed for histological changes across the groups.

Results and Discussion: all the treated lung tissue sections showed similar histological architecture differing from those of the control groups. There was thickening of the connective tissue stroma, congestion of intervening blood vessels, presence of mixed inflammatory cells infiltrates and lung injuries. These features varied based on the period of treatment suggesting time-dependency.

Conclusion: short to long exposure of Wistar rats to sub-lethal dose of M insecticide caused an acute to chronic lung injuries which is detrimental to their health.

Keywords: Pyrethroid, Sub-lethal, Lungs, Insecticide, Rats.

I. Introduction
Pesticide toxicities can be suffered by workers during manufacturing, packaging and application or may even contaminate food during home application making humans very susceptible to their exposure. The debilitating effects of these chemicals may result to the development of diseases and thereby result in systemic degeneration. Diseases such as cancer, asthma, liver cirrhosis, and bronchitis which have been on the increase lately were observed to result from the injurious effects of toxins[12] consequently raising questions of how safe these household chemicals really are. The environmental contamination with pesticides is both regional and world-wide problem.[3] The toxic chemicals have been found in a variety of environmental samples including water, air and house dust, and their presence has also been noted in the tissues of non-occupationally exposed people, particularly in the adipose tissue, blood and urine.[4] Efforts are being made to exonerate several factors that have been previously considered carcinogen. Some studies had suggested the predisposition mostly of children exposed to some pesticide to development of diseases such as asthma, bronchitis and cancer.[5-7] Some researchers have also found an association between asthma and the use of pesticides by male farmers.[8] Although their study involved adults, concerns are raised about children’s exposure especially domestically as the lungs is yet to be fully developed. Pyrethroids which were synthesized from pyrethrins may possess toxicity which depends on systemic bioavailability as was reported by[9] that pyrethroids were not easily absorbed through the skin, but were absorbed through the gut and pulmonary membrane. One of the major concerns of using Pyrethroids pesticide is the issue of sensitization. Some studies have reported allergic contact dermatitis in sensitized and un-sensitized guinea pigs.[10,11] They further observed that after an initial exposure, the sensitized subject responds to a smaller dose than the initial dose suggesting dose-response toxicity. M insecticide which was researched on in the present study is made up of the active ingredients: d-trans allethrin(0.10%w/w), imiprothrin(0.02%w/w) and d-phenothrin (0.03%w/w). These compounds were reported to be un-harmful to humans in low doses when used individually.[12] Although few studies have been carried out to determine the toxicological effects of these Individual chemicals in experimental animals, no study involving the effects of a parent mixture of them in rats lungs have been carried out, thereby necessitating this study. The aim of this study was therefore to histologically determine the effects of M insecticide on the lungs of adult Wistar rats.
II. Materials And Methods

Experimental Animals
Twenty five (25) adult Wistar rats of both sexes were acquired from the animal house of the College of Health Sciences Delta state University Abraka. The animals were in healthy conditions and weighed 150-250g. On transfer to the experimental location, the rats were allowed to acclimatize for two weeks under favorable climatic conditions and fed with standard rat pellets and water ad libitum.

Mortein Insecticide (allethrin, imiprothrin and phenothrin)
The chemical product ‘M’ insecticide and olive oil were purchased from a local store in Abraka, Ethiope-East Local Government Area of Delta State. ‘M’ insecticide had National Agency for Food and Drug Administration Control (NAFDAC) number of 048724.

Experimentation Adopted from.[13]
Twenty five (25) rats were randomly divided into five groups of five (5) animals each. The rats in groups I-III were treated with 2250mg/kg/bw of ‘M’ insecticide dissolved in olive oil orally via an improvised cannula for a duration of 7, 21 and 40 days respectively once daily. Rats in Group IV were given tap water as placebo while those in group V received 2250mg/kg/bw olive oil once daily throughout the duration of the experiment.

Samples Collection and Preparations for Histology
At the end of the experiment, the animals were fasted overnight then sacrificed by spinal dislocation. The lungs were harvested and examined with the naked eyes for any gross morphological changes. The tissues were fixed with formal saline and standard manual tissue processing techniques were employed. Sections of about 3-5µm were cut by a Slee Medical rotary microtome and stained with haematoxylin and eosin (H&E). The slides were analyzed with the aid of a light microscope for histological changes.[14] The slides were viewed and captured on a Brunel light microscope, 20 mega pixels (Brunel SP35 Digital Trinocular).

Ethical clearance
Approval for this study was obtained from the Research Ethics Committee of the Faculty of Basic Medical Sciences Delta State University Abraka and the experiment was carried out in strict accordance with the guidelines for the care and use of animals for research.

III. Results

Fig 1 showed sections of the rat lungs from the normal control group composed of several variably sized alveoli lined by pneumocytes. There were capillaries within the loose connective tissue stroma that separates the alveoli, also seen are bronchi and blood vessels.

Fig 2 showed sections of the rat lungs treated with 2250mg/kg/bw for 7 days composed of several variably sized alveoli lined by pneumocytes. Several bronchi were seen intermixed with the alveoli. The bronchi were lined by simple columnar ciliated epithelium with abundant goblet cells. The underlying connective tissue stroma was thickened, highly cellular and composed of mixed inflammatory infiltrates. Also seen were few congested blood vessels. These features were in keeping with acute lung injury.

Fig 3 showed sections of the rat lungs treated with 2250mg/kg/bw for 21 days composed of several variably sized alveoli. The intervening stroma was studded with abundant chronic lymphoplasmacytic cell infiltrate intermixed with macrophages. The intervening blood vessels were mildly congested and the bronchi were lined by a single layer of epithelia cells with plentiful goblet cells. These features were in keeping with active chronic lung injury.

Fig 4 showed sections of the rat lungs treated with 2250mg/kg/bw for 40 days composed of several variably sized alveoli. The intervening stroma was studded with abundant chronic lymphoplasmacytic cell infiltrate intermixed with macrophages. The intervening blood vessels were mildly congested and the bronchi are lined by a single layer of epithelia cells with plentiful goblet cells. These features were in keeping with active chronic lung injury.
IV. Discussion

Pyrethroids pesticides are complex mixture and as xenobiotic, they have been reported to be highly lipophilic that can affect the lungs thereby causing respiratory dysfunction.\textsuperscript{[15]} In the index study, M insecticide was found to induce thickening of the connective tissue of the lungs of rats. This thickening was more prominent in the rats treated for 7 days suggestive of an acute lung injury (ALI). Mild to marked congestion of intervening blood vessels was accompanied with inflammation. Most of these effects usually result from the activities of mast and inflammatory cells that produces cytokines and histamine. In most cases, activities of these cells infiltration into the lung parenchyma resulting in edema as reported by \textsuperscript{[16]} with symptoms of hyperventilation and dyspnea as presented by the treated rats. Thickening of the lung connective tissue stroma is similar to desmoplastic reaction secondary to insult to the lungs.\textsuperscript{[17]} This reaction resulted probably from the injurious effects of the agent and is similar to that observed in early stages of cancer. Our findings are similar to those observed by \textsuperscript{[18]} which reported hyperplasia, clumping of cells, pulmonary edema and fibrosis in mice exposed to a pyrethroids pesticide. Symptoms presented by the rats such as hyperventilation and dyspnea are indicative of direct lung reaction to the agent \textsuperscript{[19]} or CNS predisposition in the reticular formation as reported by \textsuperscript{[20]} The abundant chronic lymphoplasmacytic cell infiltrate intermixed with macrophages observed in this study conforms and was similar to that reported by.\textsuperscript{[21-23]} Most of these histopathological changes presented in the lungs of the treated rats followed a time - dependent manner.

\textbf{Fig 1} shows normal rat lungs from control group: BV = Blood vessel, B = Bronchus, A = Alveolus; \textbf{Fig 2} shows rat lungs treated with 2250mg/kg/bw for 7 days: B = Bronchus, R = Respiratory bronchiole, BV = Blood vessel, A = Alveolus, AS = Alveolus sac, AD = Alveolus duct; \textbf{Fig 3} shows rat lungs treated with 2250mg/kg/bw for 21 days: B = Bronchus, BV = Blood vessel, A = Alveolus, Green arrow = lymphoplasmacitic cells, Black arrow = macrophages; \textbf{Fig 4} shows rat lungs treated with 2250mg/kg/bw for 40 days: B = Bronchus, Black arrow = macrophages.
V. Conclusion

We can suggest from this study that exposure to sub-lethal dose of M pesticide at longer duration than considered in the index study could lead to chronic degenerative changes in rat lungs.

Reference