

Histopathological Evaluation of the Mosquito Coil Emissions on the Lungs of Adult Rabbits

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Abstract

Pyrethroids are highly active insecticides composed of allethrin, cypermethrin, Pyrethrum, and imiprothrin, and these ingredients are frequently used to eradicate domesticated insects. These synthetic substances generally are correlated with several gasping diseases. Therefore, the main focus of this research was to check out the possessions of histology of Pyrethroid insecticides "Mortein smoke" on the respiratory organ of mature rabbits. Sixteen adult rabbits of same age group with 1.5kg to 2.5kg weight of both sexes were used for an experiment and divided into four groups (the experiment was laid out in a Completely Randomized Design (CRD). Out of four groups, one group (C) acted as a control group and the remaining three were experimental groups (group1, group2, group3). Each group comprises four rabbits, including two male and two female rabbits. The control group was kept at room temperature without any chemical exposure. In comparison, the experimental group of animals faces exposure to the mosquito coil smoke containing synthetic pyrethroid insecticide, such

as allethrin for 3hrs, 5hrs, and 8hrs, respectively, for 30 days. All these animals were dissected on the exact 31st day. In order to ascertain the effects of mortein coil emissions on the pulmonary organs (lung) of mature experimental rabbits, conduct histological procedures. All of them assess the lung tissue passage intimate unique histological configurations that are incompatible with the animals in the control group. Thickening of the alveolar wall, growth of blood vessels, emphysema, macrophages, congestion in the lumen of the bronchus, and accumulation of inflammatory cells were all seen. This type of analysis is dependent on how long the medicine is used.

Introduction

Mortein coils utilize a slow-smoldering formula to release synthetic insecticides like pyrethroids, creating mosquito-detering smoke (Liu et al., 2022). Their controlled combustion in enclosed spaces aims to reduce human-mosquito contact, thereby helping to prevent diseases like malaria and dengue (Xiong et al., 2023). However, recent studies emphasize ensuring adequate ventilation to mitigate potential respiratory risks from prolonged indoor smoke exposure (Wei et al., 2024). The most common element of Mortein coils is a pyrethroid. It is an abundantly accessible element for depurate mosquitoes and is generally used in Asia (Chang and Lin, 1998). The term 'pyrethroid' is commonly used to elect artificial insecticides obtained structurally from the natural pyrethrins (Soderlund et al., 2002). Pyrethroid insecticides are brownish-yellow in color. It has an irritating smell and is mainly used to control agricultural insects. Regular use of pyrethroids insecticide could make insects obtain immunity and the inhalation of pyrethroid smoke may cause lung damage (Chalap et al., 2019). The naturally available pyrethrins are used as a representation to build artificial pyrethroids from petroleum derivatives (Rathore et al., 2018). The most widely used synthetic pyrethroids are ecto-parasiticides and it is less poisonous to animals than other insecticides (Mishra et al., 2012). The frequently used insecticide is cypermethrin to control varying pests, like pests of cotton, moths, a pest of fruit and vegetable crops, and used for spot treatment, fracture to an oversight insect pest in stores (Ullah et al., 2006). It causes muscular tremors, ataxia, and limbs weakness, coma, convulsions, and death from pulmonary sadness in animals due to the consumption of high doses of cypermethrin; it also causes skin irritation, while its coetaneous contact in the facial part may give rise to a subjective sensation of innovative (Sandhu and Brar, 2000). Mosquito coils are often used overnight in the sleeping zone. In this position, high exposure may transpire; parents and their Based on the stated study rationale, the use of Mortein coils is justified by their critical public health role, despite documented risks. The histological investigation in rabbits is essential because these coils release a complex emission of fine particles and vapors that are deeply inhaled into the alveolar region, posing a potential pathological threat to lung tissue (Liu et al., 2022). Furthermore, their widespread use continues in developing nations due to their affordability and effectiveness as a primary defense against mosquito-borne diseases like malaria and dengue (WHO, 2023). Therefore, this research is vital to scientifically quantify the specific health trade-offs, balancing the proven epidemiological benefits against the potential for chronic pulmonary damage (Xiong et al., 2023). children are usually uncovered to this synthetic compound (mosquito coil smoke) containing small molecules, vapors, and metal fumes (Liu and sun, 1988). These particles enter the alveolar part of the lungs by inhalation (Cheng et al., 1992). Mosquito mortein coils can be fire desperate. The use of mortein coil has expanded countless accidental fires. The annoying smell from the smoke may also stay; penetrate fabric and furniture (Trumbull and Charles, 2000). Although the inhaling smoke of mosquito mortein coil may have the capability of a dverse health possessions, large populations of developing countries still use this synthetic mortein coil in their routine lives (Graba et al., 2007).

Therefore, this research was designed to investigate the histopathological outcome of inhaling mosquitocidal mortein coil smoke in the lungs of adult rabbits.

MATERIALS AND METHODS

Experimental model:

A total of sixteen adult rabbits of the same age and gender, weighing between 1.5 and 2.5 kg, were used in this investigation. Ten of the animals weighed 1.5 kg, and six weighed 2.5 kg. The rabbits were housed in cages maintained at a temperature between 29°C and 32°C, with clean water and fresh green grass available at all times. All animals were procured from a nearby market.

Insecticide exposure:

The experimental animals were placed 7 inches away from a Mortein mosquito coil to replicate real-world exposure conditions, simulating human living spaces where coils are typically placed at a similar distance. To ensure adequate circulation and containment of the pesticide smoke, the cages were completely enclosed. Different groups of animals were exposed to the smoke for varying durations.

Experimental design:

The experiment followed a completely randomized design (CRD). Groups of rabbits were exposed to coil smoke for 0, 3, 5, or 8 hours. The experimental units (rabbits) were first arranged into four homogeneous groups, each containing two males and two females. Treatment exposures were then randomly assigned to these groups. No secondary structuring element, such as time or location, was included, confirming the use of a CRD rather than a randomized complete block design (RCBD).

Random assignment of all experimental units to treatment groups is the foundation of a CRD. Accordingly, the 16 rabbits were divided into four groups at random (Control, Group 1, Group 2, and Group 3). The only treatment factor—exposure duration to coil smoke—was assigned at four levels (0, 3, 5, and 8 hours per day). Although equal distribution of sexes (two males and two females per group) was maintained to ensure baseline homogeneity, this did not function as a blocking factor. Therefore, the design qualifies as a CRD, with treatment duration as the sole structured variable.

Framework:

The variables studied included behavioral and morphological changes, body weight (morphometric analysis), and histological alterations. Laboratory instruments such as microtome, microscope, and weighing balance were used for analysis. Permanent markers were used for tagging, and Mortein mosquito coils with matchboxes were employed for exposure. For lung sample collection, a complete dissection kit, chloroform, 90% ethanol, cotton, and biopsy jars were utilized.

Morphometric investigation:

A scientific laboratory weighing balance with a legibility range of 1 mg to 1 g and a maximum capacity of 14 kg was used to measure successive changes in body weight caused by exposure to Mortein mosquito coil smoke. After exposure, the initial and final body weights, as well as the percentage change in weight of experimental rabbits, were recorded for each group. Each value was calculated repeatedly, and mean values were obtained to determine weight variations between the control and experimental groups.

Sample assemblage and histological preparations:

At the end of the experiment, all experimental animals were fasted overnight, anesthetized using chloroform, and dissected from the ventral side to carefully isolate the lungs. The harvested lungs were examined macroscopically for gross morphological changes. The tissues were fixed in formal saline solution and processed manually using standard histological techniques. Tissue sections of approximately 3–5 μm thickness were prepared using a microtome and stained with hematoxylin and eosin (H&E). Histopathological alterations in lung tissues were examined and photographed under a light microscope, following standard analytical procedures in toxicological pathology (Zheng & Ochoa, 2023).

Statistical analysis:

Data obtained from morphometric and histological analyses were subjected to statistical evaluation using one-way analysis of variance (ANOVA). The results were expressed as mean $\pm$ standard deviation (SD), and significance was determined at $P < 0.05$. Statistical analysis was performed using SPSS software version 26.

RESULTS

Gross Observations

There was no statistically significant difference ($P > 0.05$) in food intake among all experimental groups (rabbits) compared to the control group. However, animals in the test groups exhibited a noticeable reduction in body weight across all exposure durations. In addition, reduced physical activity and lethargy were observed in the experimental animals compared to the control group.

Morphological Changes

Rabbits exposed to pyrethroid-containing Mortein coil smoke appeared morphologically weak and displayed signs of laziness, skin irritation, and general discomfort. Exposure to coil smoke caused wooziness and hair loss on the legs. Urine from rabbits exposed for 5 hours/day and 8 hours/day appeared more concentrated and had a pungent odor compared to the 3-hour exposure group. The nostrils of exposed animals exhibited discoloration that increased with exposure duration: Group 1 (3 hours/day) showed mild yellowing, Group 2 (5 hours/day) showed a deeper yellow color, and Group 3 (8 hours/day) exhibited a dark yellow to brownish appearance. By the end of the experiment, rabbits in the 8-hour exposure group were extremely weak, showing difficulty in standing and walking (Figs. 1 a-d).

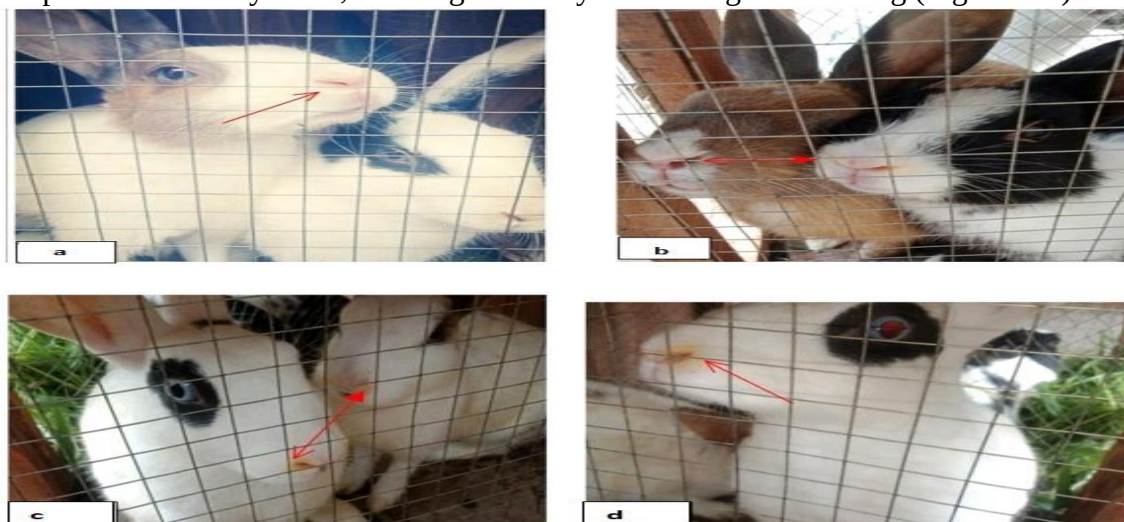


Fig 1: Showed the Nostrils of **a.** Control group animals; **b.** Group-1 animals exposed to the smoke of coil for 3hrs/day for 30 days; **c.** Group-2 animals exposed to the smoke

of coil for 5hrs/day for 30 days; **d.** Group-2 animals exposed to the smoke of coil for 8hrs/day for 30 days.

Morphometric Observations

The mean body weight of rabbits in the control group increased slightly during the experiment, while a gradual decline was recorded in the exposed groups. The decrease in body weight was exposure-dependent, with Group 3 (8 hours/day) showing the highest reduction, followed by Group 2 (5 hours/day) and Group 1 (3 hours/day). Statistical analysis using one-way ANOVA indicated a significant difference in final body weight among the groups ($P < 0.05$) (Table 1).

Table 1 Effect of Mortein coil smoke exposure on mean body weight of adult rabbits

Exposure Duration	Initial Body Weight (g)	Final Body Weight (g)	Weight Difference (g)	% Weight Change
0 h / 30 days	1509 $\pm$ 24.1	1535 $\pm$ 21.3	+26 $\pm$ 3.1	1.72% increase
3 h / 30 days	1250 $\pm$ 19.5	1239 $\pm$ 18.6	-11 $\pm$ 2.7	0.88% decrease
5 h / 30 days	1308 $\pm$ 22.7	1278 $\pm$ 20.4	-30 $\pm$ 3.5	2.30% decrease
8 h / 30 days	1380 $\pm$ 23.2	1308 $\pm$ 20.1	-72 $\pm$ 4.3	5.22% decrease

Values represent mean $\pm$ SD ($n = 4$). Statistical significance determined using one-way ANOVA followed by Tukey's post hoc test; groups differ significantly at $P < 0.05$.

Recorded Lung Weight

The lung weight of male rabbits in the experimental groups increased significantly compared to the control group ($P < 0.05$), while that of female rabbits decreased significantly in comparison to the control. The variation in lung weight was exposure-dependent (Table 2).

Table 2 Effect of Mortein coil smoke exposure on lung weight of treated rabbits

Group	Male Lung Weight (g)	Female Lung Weight (g)
Control	7.2 $\pm$ 0.3	9.0 $\pm$ 0.2
Group 1 (3 h/day)	8.3 $\pm$ 0.2	6.7 $\pm$ 0.3
Group 2 (5 h/day)	10.4 $\pm$ 0.4	7.8 $\pm$ 0.3
Group 3 (8 h/day)	9.1 $\pm$ 0.3	8.8 $\pm$ 0.2

Values are mean $\pm$ SD ($n = 4$). Statistical significance determined by one-way ANOVA ($P < 0.05$).

Histopathological Findings

Histological examination of lung tissues stained with hematoxylin and eosin (H&E) revealed significant pathological alterations in pyrethroid-exposed rabbits compared to controls.

Control Group (Fig. 2A): Normal alveolar structure with thin lamellar epithelium, intact connective tissue, and numerous blood vessels lined with ciliated columnar epithelium.

Group 1 (3 h/day; Fig. 2B): Thickened alveolar walls, congestion in bronchial lumen, proliferation of blood vessels, emphysema, and macrophage infiltration, indicative of acute lung injury.

Group 2 (5 h/day; Fig. 2C): Mildly congested bronchioles, inflammatory cell accumulation around bronchi and blood vessels, and pronounced emphysematous changes, suggesting active chronic lung injury.

Group 3 (8 h/day; Fig. 2D): Severe inflammatory infiltration, emphysema, and congested blood vessels within the stroma, confirming advanced chronic lung injury.

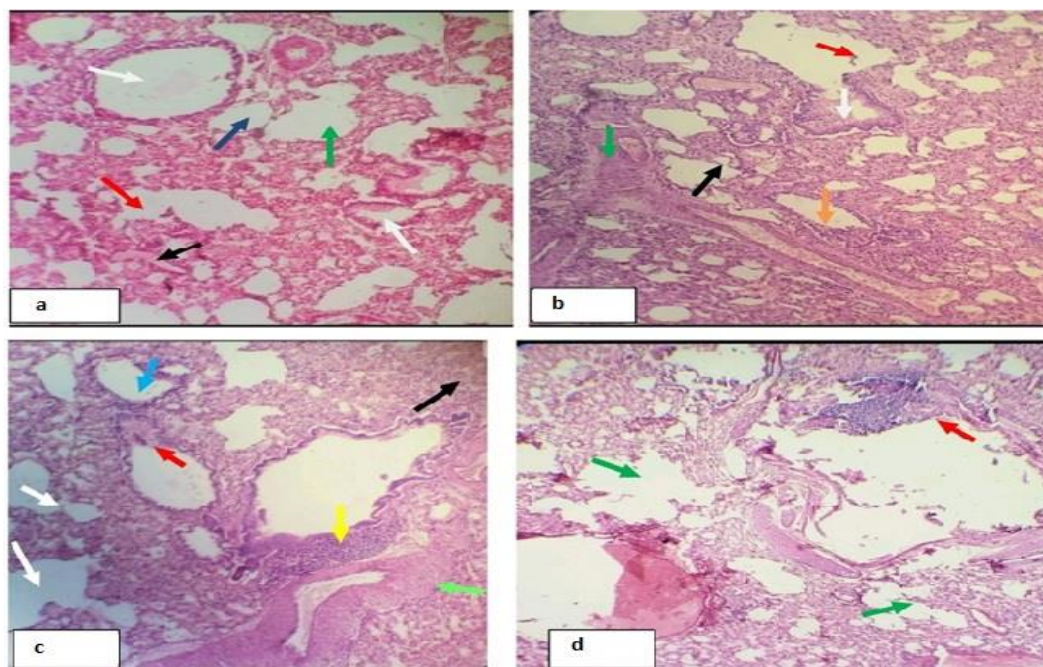


Fig 2. A. Control group lungs (normal morphology): white arrow = bronchus, blue arrow = alveolus, green arrow = alveolar duct, red arrow = alveolar sac, black arrow = blood vessel. **B.** Lungs exposed to coil smoke for 3 h/day for 30 days: green arrow = congested blood vessel, white arrow = congestion, black arrow = emphysema, orange arrow = thickened alveolar wall (H&E stain, 100×). **C.** Lungs exposed for 5 h/day for 30 days: red arrow = congestion, yellow arrow = inflammatory cell accumulation, green arrow = congested blood vessel, white arrow = emphysema, black arrow = edema fluid (H&E stain, 100×). **D.** Lungs exposed for 8 h/day for 30 days: green arrow = emphysema, red arrow = severe inflammatory infiltrate (H&E stain, 100×).

DISCUSSION

Pyrethroid containing mortein coils is a slow-blasting element that provides smoke. It may contain more than one insecticide; to protect humans against malaria and dengue mortein coil blaze for a specific time in a bound location. In many Asian countries such as; Malaysia, Thailand, China, India, etc., pyrethroid-containing mortein coils are generally used as a mosquito repellent (Nadia and Anum, 2014). The blazing of one mortein spiral might excrete a similar quantity of Post meridiem (PM) 2.5 mass estimated to ignite 75-137 gaspers (Liu et al., 2003). Although mosquito mortein coils create protection against malaria, on the other hand, it also exerts many poisonous actions on mammals; for example, it induces respiratory illness and resolve breathe heavily in the youngster when they are barely directed toward mortein coils smoke for long-lasting intervals of time (Azizi et al., 1991). The long-term exposure of animal studies to the smoke of mosquito coil indicated atypical expansion of vegetative cells, losing weight, and lung destruction (Nadia and Anum, 2014). The present research was designed to mimic the limited and steady use of pyrethroid insecticides in accommodation employing rabbits as a model to demonstrate the unrealized fitness

association of mortein spiral exhaust. Experimental rabbits become physically weak, urine becomes more concentrated, increases in food consumption, skin irritation, and dermal effects were observed in mosquito coil smoke treated rabbits in a dose-dependent manner, but despite not significant conforming with other works of the same category (Metker et al., 1978; Ishmael and Litchfield, 1988) who explained that no morphological abnormalities were observed in experimental animals due to the oral/dermal exposure of different concentrations of pyrethroid insecticides. Skin irritation has also been reported earlier (Handerson and Parkinson, 1981). After the first week of pyrethroid treatment, a slight reduction in the weight of animals in groups 1, 2, and 3 but in the control group, the animals gained weight over time. This decrease in weight in treatment groups could be due to stress on the pyrethroid. So, this result is in agreement with the work of the same nature (Garba et al., 2007; Nadia and Anum, 2014, and Lakkawar et al., 2004), who reported that the intended body weight of control group animals was slightly increased and reduced in all the experimental group animals that exposed to the smoke of mortein mosquito coil. The lung weight of treated animals with mortein coil smoke was also increased in male rabbits compared to the control group of male rabbits. The same results were reported by many researchers (Clapp et al., 1977; Ishmael and Litchfield, 1998; Butterworth and Hend, 1976; Hogan and Rinehart, 1977), who explained the increased liver, kidney, heart, and spleen weight due to oral exposure at the different concentrations of pyrethroid insecticides. The present study indicated that histopathological changes in the lungs of exposed rabbits like emphysema and congestion might be due to the cytotoxic effects of pyrethroid insecticides. The same results were reported by (Majeed et al., 2012), who explained the changes in the lungs of sheep exposed to the cypermethrin due to the stress on the respiration, which appears by the increase in the respiratory rate. Exposure to pyrethroid insecticides caused a slow deformation of the normal structure of alveoli in the lungs. Pyrethroid insecticides induce hyperplasia and rupture of cells among the alveolar cells. The inflammation in the lungs leads to pulmonary edema (Dorland, 1977). Pyrethroid containing mosquitocidal pesticides such as mortein coil began to condense the connective tissue of rabbit's alveolus. This stiffening was also distinguished in the lungs of 5 hours treated rabbits expressive of a drastic alveolus abrasion and intervening congestion of blood vessels was also seen with tenderness. Usually, these effects result from the activities of inflammatory and mast cells which manufacture cytokines and histamine and the activities of these cells may lead to lung edema. The same results were reported by (Chevalier et al., 1982). The epidemics data represent an enlargement in the number of cancer cases in persons, including agricultural production using pesticides (Sheikh et al., 2014). Pyrethroid convinces benign tumors in the respiratory system of mice (Ishmael and Litchfield, 1988). Lung cancer enlarges and a series of continuing disease alterations in the pulmonary epithelium (Vineis and Husgafvel, 2005). The pyrethroid may interconnect with DNA and destroy its structure. It is also known as genotoxic. They create chromosomal abnormalities and break a single strand of DNA (Sheikh et al., 2014). Molecular alterations occur due to pyrethroids, such as dropping heterozygosity, gene alteration, and abnormal gene booster methylation, potentially favorable molecular biomarkers of lung carcinogenesis (Vineis and Husgafvel, 2005). It has been approved that pyrethroid gives rise to oxidative pressure (Kale et al., 1999). This oxidative strain gives rise due to an imbalance of production of reactive oxygen, including free radicals and peroxide, which create destruction to the cell and collagen accumulation essential to reparatory fibrosis and edema (Erdogan et al., 2006; Evans and Cooke, 2004; Rahman and Macnee, 2000). So, we should ignore the use of mosquitocidal insecticides as they may harm our respiratory system.

CONCLUSION

The results of this study revealed that pyrethroid-containing mortein coil smoke induces histological alterations in the lungs (respiratory tract). However, the data cannot be interpolated directly from rabbits to human beings, but it may be terminated that the use of pyrethroid containing mortein coil smoke may cause dangerous action in diverse levels to the non-target model, such as alveolar walls and bronchi lesions, proliferation of blood vessels, emphysema and these all leads to chronic lungs injuries. It all demonstrated that we should avoid the use of such mosquito repellent insecticides that causes serious diseases and even genetic disorders at some extent in humans.

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