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Effects of Aqueous Leaf Extract of *Mimosa pudica* on Insect Repellent–Induced Injury in the Trachea of Adult Wistar Rats

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Author for Correspondence*: mabel.ehi-omosun@uniben.edu/ folasademaryam@gmail.com /+234-704-994-8914/ORCID ID: 009-0000-5612-8466. <https://dx.doi.org/10.4314/sokjmls.v10i1.5>**Abstract**

The use of insect repellent in occupational settings can lead to insecticide poisoning, a serious health hazard that can have severe consequences, including morbidity and mortality, if left untreated. The aim of this study was to investigate the effects of aqueous leaf extract of *Mimosa pudica* on insect repellent-induced injury in the adult Wistar rats. Thirty (30) adult Wistar rats (male and female) weighing between 236 g and 266 g were divided into five (5) groups of six (6) rats each. Group A rats were administered feed and water only. Group B rats were exposed to 2 puffs of insect repellent (10 ml/puff) at a concentration of 100 mg/m³, administered at 8 am by inhalation via fume distributor glass-chamber of dimensions 32.5 cm³ × 32.5 cm³ × 16.5 cm³ for 1 hour daily. Group C rats received 500mg/kg body weight per day (BWT/D) of *Mimosa pudica*. Groups D and E rats were exposed to 2 puffs of insect repellent (10 ml/puff) at a concentration of 100 mg/m³, administered at 8 am by inhalation via fume distributor glass-chamber of dimensions 32.5 cm³ × 32.5 cm³ × 16.5 cm³ for 1 hour daily and received 250mg/kg BWT/D (low dose) and 500mg/kg BWT/D (high dose) of *Mimosa pudica* respectively. The dosages of the extract were given for 30 consecutive days via an orogastric tube. The weights of the animals in each group were taken and recorded weekly and the difference between them and previous weights were noted. At the end of 30th day of exposure, the Wistar rats were weighed and euthanized under chloroform anesthesia. Blood samples were taken through cardiac puncture

into plain specimen bottles for biochemical analysis. The trachea was harvested and processed for histological examination. The data obtained were analyzed using the one-way Analysis of Variance, with level of significance set at P<0.05. Histopathological examination confirmed normal tracheal architecture in the control group. The tracheal tissue of rats in Group C, which received 500mg of extract only, appeared normal, with intact mucosa, sub-epithelium, and cartilage. There were observable histological variations in the tracheal histoarchitecture of the exposed rats in Group B, D and E which include patchy mucosal ulceration, sub epithelial infiltrates of inflammatory cells, focal sub-epithelial oedema, focal mucosal erosion, focal sub-epithelial infiltrates of inflammatory cells, focal congestion and vasodilatation (evidence of tracheitis). This study revealed that exposure to insect repellent has detrimental effects on rat health, including significant weight reduction (p<0.05), biochemical alterations indicative of renal impairment and acidosis. Additionally, our results showed that *Mimosa pudica* did not exert a protective effect against tracheitis induced by insect repellent in Wistar rats. Given similar biological responses, these effects of insect repellent on rats can be reasonably extrapolated to humans, highlighting the importance of protective measures for individuals occupationally exposed to insect repellent.

Keywords: Insect repellent; Tracheitis; *Mimosa pudica*; Histoarchitecture; Exterminators

Introduction

Insect repellents are substances or products designed to prevent insects, such as mosquitoes, ticks, flies, and other biting or stinging insects, from coming into contact with humans or animals (Ibrahim *et al.*, 2017). These products typically contain active ingredients that deter insects through various mechanisms (Mark *et al.*, 2016). There are three (3) major types of insect repellents namely chemical-based repellents, natural or plant-based repellents and insecticide-treated clothing and gear (Akhere, 2015). Chemical-based repellents contain synthetic active ingredients, such as N,N-Diethyl-meta-toluamide (DEET), picaridin, and oil of lemon eucalyptus, which provide long-lasting protection against insects. Natural or plant-based repellents are derived from plants, such as citronella, lemongrass, and geraniol, offering a more environmentally friendly and gentle alternative to chemical-based repellents while insecticide-treated clothing and gear involve fabrics and materials treated with insecticides, like permethrin, which repel and kill insects, providing protection for extended periods without the need for reapplication (Cheesbrough, 2016; Mark *et al.*, 2016).

Insect repellents work by different mechanisms. For instance, chemical-based repellents, such as DEET and picaridin, work by creating a vapor barrier on the skin's surface that prevents insects from detecting human scent. These repellents bind to the skin and clothing, releasing a vapor that confuses insects and prevents them from landing and biting (Mark *et al.*, 2016). Natural or plant-based repellents, such as citronella and lemongrass, work by releasing a strong, pungent scent that masks human odor and repels insects. These repellents often contain volatile oils that are released into the air, creating a barrier that prevents insects from detecting human scent. Insecticide-treated clothing and gear, such as permethrin-treated fabrics, work by killing or repelling insects that come into contact with the treated surface. Permethrin, a synthetic insecticide, is absorbed into the fabric and released slowly over time, creating a toxic environment for insects that land on the treated surface (Mark *et al.*, 2016). This mechanism of action provides long-lasting protection against insects, even after repeated washing and wear.

Pest eradicators, exterminators and pest control technicians are often exposed to insect repellent daily for hours during the course of their work. Breathing the fumes of insect repellent can occur during fumigation, while working directly with insect repellent or using equipment sprayed with insect repellent (Harrison, 2014; Mark *et al.*, 2016). Insect repellent contains an organic chemical, N,N-diethyl-meta-toluamide, the active ingredient which is toxic to humans with significant morbidity and mortality if left untreated. Inhalation of these N, N-diethyl-meta-toluamide fumes can cause tracheitis and other respiratory problems (Mark *et al.*, 2016). Moreover, insect repellent can trigger inflammation in the respiratory tract, lungs, and other tissues, exacerbating conditions like asthma and chronic obstructive pulmonary disease (Harrison, 2014).

Previous studies have shown that exposure to insect repellent fumes cause respiratory allergies and shortness of breath in experimental animals (Gupta *et al.*, 2016). Insect repellent, a severe respiratory and skin irritant exerts its toxicity by inhibiting acetylcholinesterase, leading to acetylcholine accumulation, hyperstimulation, and disruption of neural function, causing neurotoxicity, respiratory failure, and metabolic perturbations (Mark *et al.*, 2016). This results in a range of symptoms, from coughing, sore throat, difficulty swallowing, airway obstruction sneezing, tachypnea, chest pain, and muscle weakness to paralysis and respiratory distress which can be fatal (Mark *et al.*, 2016).

The trachea is a key part of the respiratory system that leads from the larynx to the bronchi (Drake *et al.*, 2015). It is located anteriorly in the neck and dives posteriorly in the mediastinum as it travels towards the carina (Harrison, 2014). The trachea is a long, U-shaped structure that is composed of hyaline cartilage on the anterior and lateral walls, with the trachealis smooth muscle forming the posterior border of the trachea (Moore and Dalley, 2014). It is made up of 16-20 tracheal cartilages anterolaterally and a fibromuscular wall posteriorly, which help to keep it open and prevent it from collapsing (Mark *et al.*, 2016). The entire tracheal lumen is lined with a mucous membrane, which produces mucus to help trap dirt and dust

particles (Moore and Dalley, 2014). The main function of the trachea is to allow passage of inspired and expired air into and out of the lung (Mark *et al.*, 2016). Any injury to the trachea causes distortion of the smooth functioning of the respiratory system.

Tracheitis is a disease that causes inflammation of the trachea (Mark *et al.*, 2016). This inflammation generally involves the mucous membrane, lumen, muscularis layer and sub-epithelial areolar connective tissue of the trachea and lowers the trachea's ability to provide a safe sturdy passageway for inspired and expired air into and out of the lungs (Harrison, 2014). A severely inflamed trachea can block the respiratory tract, causing severe breathing difficulties and discomfort. Tracheitis may occur when an irritating substance causes destruction and inflammation of the tracheal lumen which then makes passage of inspired and expired air into and out of the lung difficult (Mark *et al.*, 2016). Signs and symptoms of insect repellent related tracheitis include high fever, general malaise, wheezing, dyspnoea, cyanosis, tracheal stenosis, haemoptysis and dry hacking cough (Mark *et al.*, 2016).

Mimosa pudica, also known as the "Sensitive Plant" or "Touch-Me-Not," is a unique and intriguing plant species native to South and Central America (Alumiasunya *et al.*, 2017). Its remarkable leaf structure and behavior have captivated botanists, naturalists, and the general public alike. The leaves of *Mimosa pudica* are compound, consisting of 10-20 pairs of small, oval-shaped leaflets. Each leaflet is about 1-2 cm long and has a delicate, almost translucent texture (Elgailani and Alsakka, 2016). The leaflets are arranged oppositely on the stem, with a small, curved stalk (petiole) attaching each leaflet to the central stem.

One of the most distinctive features of *Mimosa pudica* is its ability to exhibit seismonastic movements. When the plant is touched, shaken, or exposed to other forms of mechanical stress, the leaflets fold up quickly, and the compound leaves droop downward. This movement is often referred to as "sleep" or "nyctropism." The seismonastic movement is made possible by the presence of

specialized cells called "pulvini" at the base of each leaflet (Abramson and Samuel, 2017). These cells contain large amounts of water and are extremely sensitive to mechanical stimuli. When the plant is disturbed, the pulvini cells rapidly lose water, causing the leaflets to fold up and the leaves to droop (Nosayaba *et al.*, 2019).

In some parts of the world, for example, South and Central America, *Mimosa pudica* is used in traditional medicine to treat various ailments, including cough, catarrh, fever, chest pain, abdominal cramps and dysentery (Burrett, 2016; Kanoba *et al.*, 2019). *Mimosa pudica* contains a variety of phytochemicals, including alkaloids, flavonoids, glycosides, phenolic acids, saponins, steroids, tannins, and terpenoids (Burrett, 2016). These phytochemicals contribute to the plant's medicinal properties, including anti-inflammatory, antioxidant, and antimicrobial activities. *Mimosa pudica* was chosen as a potential therapeutic agent for insect repellent induced tracheitis due to its well-documented antioxidant and anti-inflammatory properties, which align with the need to counteract the oxidative stress and inflammation caused by insect repellent toxicity. Hence, the objective of this paper was to evaluate the effects of aqueous leaf extract of *Mimosa pudica* on insect repellent-induced tracheitis in Wistar rats.

Materials and Methods

Plant Materials: The *Mimosa pudica* leaves used in this study were collected from the University of Benin Farm Project, located in Benin City, Nigeria. The plant material was authenticated by Botanists at the Department of Plant Biology and Biotechnology, Faculty of Sciences, University of Benin, Benin City, Edo State, Nigeria. A voucher specimen (voucher number: UB/MPI/002) was lodged at the department's herbarium for future reference.

Extract Preparation: *Mimosa pudica* leaves were oven-dried at 40°C after air-drying for 7 days. The dried leaves were then grounded using a 2018 model mechanical grinder (Dozenmann, U.S.A). The cold maceration method was used to extract the powdered material by soaking 500g of the powdered *Mimosa pudica* leaf in 1litre of water for 24 hours at room temperature (Nosayaba *et al.*,

2019). The cold macerated method was selected for extracting active compounds from *Mimosa pudica* leaves due to its ability to yield high-quality extracts with minimal degradation of phytochemicals. This technique enables the extraction of a wide range of bioactive compounds, including flavonoids, saponins, and terpenoids, which are known to contribute to the medicinal properties of *Mimosa pudica*. Additionally, cold maceration is a simple and scalable method that can be easily replicated in a laboratory setting, making it an attractive choice for our research. The soaked *Mimosa pudica* was filtered with the aid of cotton wool. Using evaporating dishes, the filtrate was concentrated over a hot water bath to obtain 20g concentrated jellylike extract of *Mimosa pudica* leaf which was then transferred into plain specimen bottles for storage in a refrigerator at 4°C. Acute oral toxicity of the extract was evaluated.

Experimental animals: Thirty (30) adult Wistar rats (both genders) of 236g-266g in weight were purchased from the Animal House, Department of Anatomy, University of Benin, Nigeria and were utilized for this experiment. The rats were housed in cages with 6 per cage, wood shavings bedding, a 12-hour light/dark cycle, 50-60% humidity, and an ambient temperature of 25-28°C. The housing conditions were designed to minimize stress and ensure the comfort and well-being of the animals. The animals were left to acclimatize for 2 weeks before commencement of the experiment. During this period, they were allowed access to standard animal feed manufactured by Bendel Flour Mill, Ewu, and clean water *ad libitum*. The supplier, Bendel Flour Mill, situated in Ewu, Edo State, Nigeria, delivered Standard Animal Feed in pellet Form. The relevant details are: batch number: BMI/EWCB/004 and expiration date March 2024. We ensured that the feed's nutritional value remained uncompromised by using it within the recommended time frame.

Ethical Consideration: Ethical approval was obtained from the Research Ethics Committee of the College of Medical Sciences, University of Benin, Nigeria (The approval number obtained is CMS/REC/2014/508). Each animal procedure was carried out in accordance with approved protocols and in compliance with the recommendations for the proper management and utilization of laboratory animals used for research (Buzek and Chastel, 2010).

Induction of Tracheitis:

Tracheitis was induced in the test animals by exposure to insect repellent at 100mg/m³, delivered as 2 puffs (10 m/puff) via a handheld sprayer in a fume distributor glass-chamber FDGC), with daily 1-hour exposure for 30 days (Burrett, 2016). The insect repellent was manufactured by DZT Chemicals located at 208 Industrial Estate, Sango-Ota, Ogun State, Nigeria. The product details are: Product Name –: Insect repellent, Product Code - DVN-029, Batch number - DCN/8907/006CN/024, Manufacturing date - September 2023, and Expiration date - October 2025. We ensured that the concentration and dose of insect repellent used in this study were accurate and reproducible, and we provide these details to facilitate replication by other researchers. A pilot study was done on the 28th day of the experiment which confirmed tracheitis.

Experimental design: In this study, 30 animals were divided into 5 groups comprising of 6 rats per-group. Animals in group A which served as control received standard feed and clean water *ad libitum*. Animals in group B was exposed to *insect repellent* only via inhalation. Animals in group C received 500mg/kg body weight per day (BWT/D) of *Mimosa pudica*. Animals in group D were exposed to *insect repellent* via inhalation and received 250mg/kg BWT/D of *Mimosa pudica*. Animals in group E were exposed to *insect repellent* via inhalation and received 500mg/kg BWT/D of *Mimosa pudica*. We selected the dosages of *Mimosa pudica* for groups D and E based on previous studies that have demonstrated the safety and efficacy of this plant extract in animal models. Our selection of these dosages was also guided by the results of our pilot study. The dosages were given for 30 consecutive days via an orogastric tube. The weights of the animals in each group were taken and recorded weekly and the difference between them and previous weights were noted.

On the 30th day of exposure to *insect repellent*, the animals were weighed, euthanized under chloroform anaesthesia and blood samples were taken through cardiac puncture into plain specimen bottles for biochemical analysis. A midline incision was made through the ventral wall of the thoracic neck of the rats to access the

trachea. The harvested organs were immediately fixed in 10% formal saline for 24 hours to prevent tissue degradation and autolysis before the histological procedures. The tissues were sectioned into about 5µ (5 micrometer) thick sections and processed according to the method of Drury and Wallington (1980). The thin tissue sections were histologically processed using the methods of fixation, embedding and tissue staining for microscopy. Histological sections were examined under a Leica DM750 light microscope with digital camera (Leica ICC50) attached. Photomicrographs of the tissue sections were taken at magnification of x40.

Statistical analysis:

Data were analyzed with a Statistical Software Package and, Microsoft Excel, 2010. The results were presented as Mean ± Standard error of mean (SEM). The data were subjected to using the student's- t-test. The level of significant was set at p 0.05.

Results

Body Weight Findings:

Changes in body weights of the animals in all the experimental groups are presented in Table 1.

Body weight analysis revealed a significant increase (p<0.05) in all the groups. The weight gain was significantly reduced (p <0.05) in the insect repellent exposed groups compared to the control group, suggesting insect repellent's inhibitory effect on weight gain.

Biochemical findings:

Table 2 presents the biochemical test results for all experimental groups. A comparative analysis of serum bicarbonate, urea, and creatinine levels revealed significant increases in urea and creatinine, and a decrease in bicarbonate, in exposed groups (B, D, and E) compared to the control group (A).

Histological Findings:

The histological findings for all experimental groups are presented in Figures 1-5. Normal tracheal morphology was observed in the control group (A) and Group C, which received 500mg/kg body weight extract only. However, histological examination of Groups B, D, and E revealed tracheal damage, characterized by mucosal ulceration, inflammation, oedema, and vascular changes, consistent with tracheitis.

Table 1: Changes in body weights of the rats in all the experimental groups:

Groups	Initial body weight (Grams)	Final body weight (Grams)	Weight Difference	P-value
Group A (Control)	5.60 ± 0.67	7.74 ± 0.59	0.94 ± 3.33	0.000
Group B (Insect repellent Exposure only)	0.60 ± 0.18*	0.18 ± 0.08*	0.80 ± 0.13	0.000
Group C (Extract only)	3.42 ± 0.15*	5.36 ± 0.09*	0.68 ± 2.19	0.000
Group D (Low dose of extract + Insect repellent)	0.38 ± 0.15*	0.04 ± 0.17*	0.58 ± 0.19	0.000
Group E (High dose of extract + Insect repellent)	0.28 ± 0.15*	0.02 ± 0.115*	0.50 ± 0.03	0.000

n=6; Values are Mean ± S.E.M; The P-value speaks to the difference between the initial and final weight.

Table 2: Serum Bicarbonate (HCO₃⁻), Urea, and Creatinine Profiles in Experimental Groups

Parameter	Group A	Group B	Group C	Group D	Group E	P-Value
Bicarbonate (Mmol/L)	20.11 ± 3.23	10.56 ± 1.31*	18.87 ± 3.41*	6.13 ± 12.44*	8.13 ± 12.44*	P<0.001
Urea (mg/dl)	24.11 ± 3.23	84.11 ± 3.23*	26.87 ± 3.41*	94.10 ± 0.41*	98.10 ± 0.41*	P<0.001
Creatinine (mg/dL)	0.60 ± 12.34	1.24 ± 0.28*	0.80 ± 12.24*	1.80 ± 12.24*	1.80 ± 12.24*	P<0.001

Values are Mean ± S.E.M; * =p<0.05 compared with control

Shown below are photomicrographs of H&E sections of the trachea of the experimental animals at the end of the study.

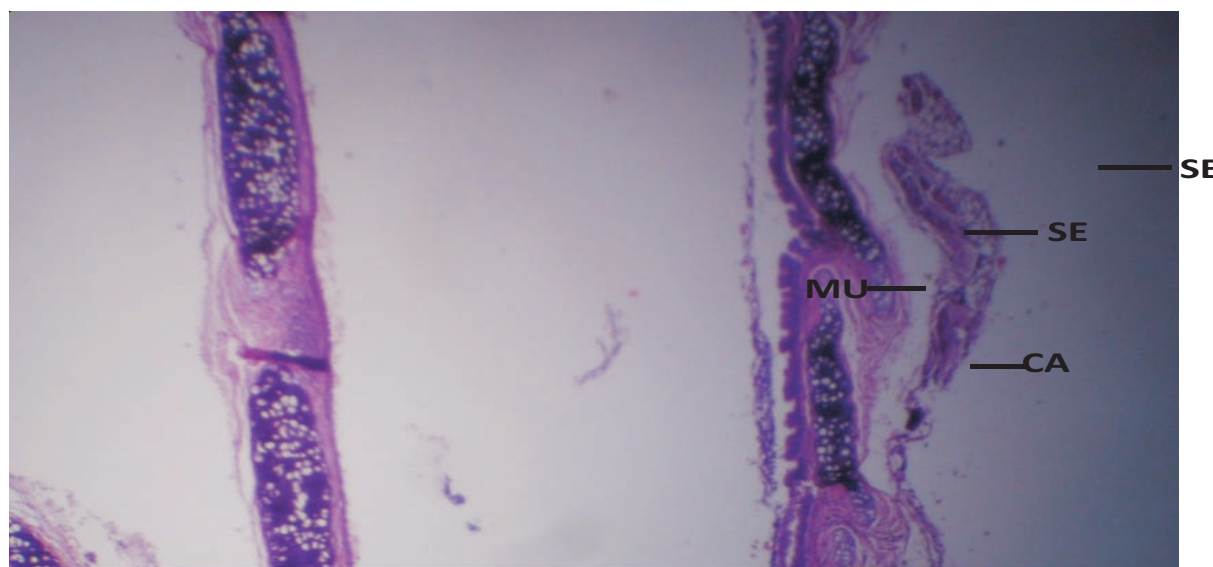


Figure 1: Rat trachea, control, show: normal architecture: mucosa (MU), subepithelial connective tissue (SE), cartilage (CA): H&E 40 X.

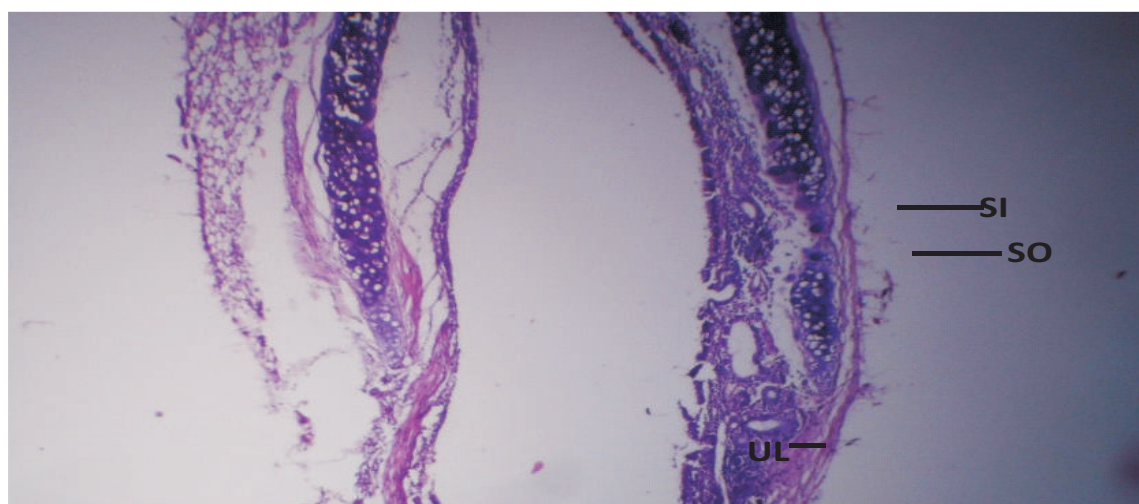


Figure 2: Rat trachea exposed to mosquito repellent only show: patchy mucosal ulceration (MU), subepithelial infiltrates of inflammatory cells (SI) and subepithelial oedema (SO): H&E 40 X.

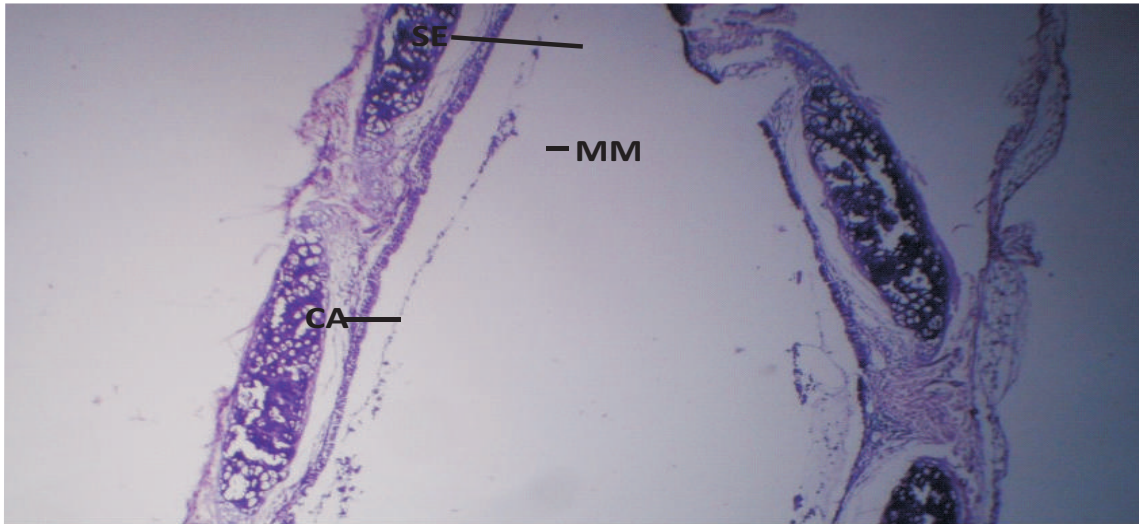


Figure 3: Trachea given 500mg extract only show: mucosal membrane (MM), subepithelium (SE) and cartilage (CA), all normal: H&E 40 X.

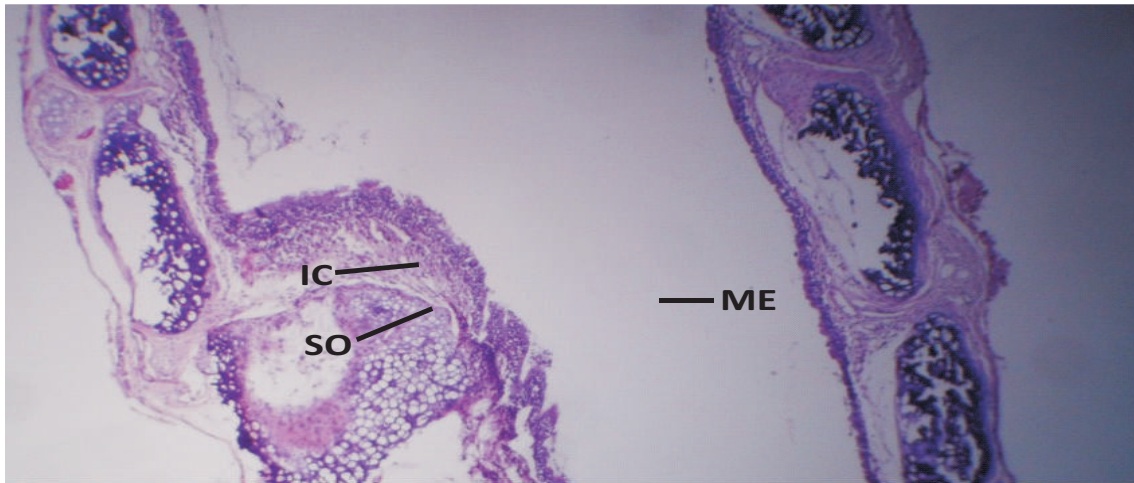


Figure 4: Rat trachea given 250mg extract + insect repellent show: focal mucosal erosion (ME), focal subepithelial infiltrates of inflammatory cells (IC) and congestion, focal subepithelial oedema (SO): H&E 40 X.

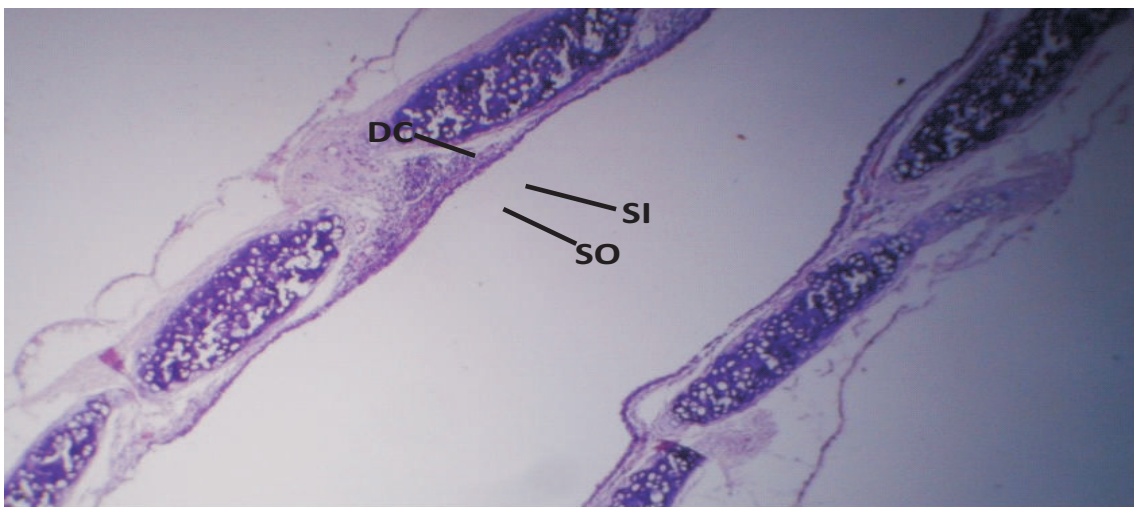


Figure 5: Rat trachea given 500mg extract + insect repellent showing subepithelial congestion and vasodilatation (DC), subepithelial oedema (SO): subepithelial infiltrates of inflammatory cells (SI): H&E 40 X.

Discussion

Insect repellent exposure has been reported to cause tracheal damage through inflammation and oxidative stress. *Mimosa pudica* is rich in antioxidants and bioactive compounds known for their protective effects. Literature reports that *Mimosa pudica* has anti-tussive, anti-inflammatory and anti-histamine properties (Burrett, 2016). Against this background, this study was conducted to evaluate the effects of aqueous leaf extract of *Mimosa pudica* on insect repellent-induced tracheal damage in Wistar rats.

Body weight analysis showed a significant increase in all groups, but the magnitude of weight gain was significantly lower ($p < 0.05$) in the insect repellent-exposed groups compared to the control group, suggesting a negative impact of insect repellent on weight gain.

Findings of the study for biochemical analysis revealed significant elevation in urea, and creatinine levels of the rats in groups B, D, and E compared to the control group (A), and group C, which was given only the extract whereas bicarbonate levels exhibited a significant decline in groups B, D, and E relative to the control and C groups. These findings suggest that insect repellent exposure can lead to severe health complications, including respiratory problems and renal failure. Given the similar biological responses, these effects of insect repellent on rats can be reasonably extrapolated to humans, highlighting the importance of protective measures for individuals occupationally exposed to insect repellent.

Observations based on photomicrography showed that exposure to insect repellent induced inflammation of the trachea (tracheitis) comprising, mucosal ulceration, sub-epithelial infiltrates of inflammatory cells, vascular dilatation, mural congestion, mural oedema and focal mucosal erosion. Administration of graded concentrations of *Mimosa pudica* aqueous leaf extract, followed by exposure to insect repellent, however, did not protect the trachea from insect repellent-induced inflammation. Thus, the presence of the signs of tracheal inflammation: infiltrates of inflammatory cells, oedema, vascular dilatation and congestion across the

tracheal wall lingered. Our histomorphological findings indicate that *Mimosa pudica* lacks the necessary anti-inflammatory compounds to protect the trachea from inflammation induced by insect repellent exposure.

Conclusion

This study concludes that insect repellent exposure has adverse health consequences in rats, including weight reduction, renal impairment, and acidosis. Moreover, *Mimosa pudica* failed to provide protection against insect repellent-induced tracheitis in Wistar rats.

Recommendation

Given the limitations of *Mimosa pudica* in protecting against tracheal inflammation, we suggest exploring alternative natural products or pharmacological agents with anti-inflammatory effects to safeguard against insect repellent-induced tracheitis.

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