



EFFECTS OF AQUEOUS LEAF EXTRACT OF MIMOSA PUDICA ON INSECT REPELLENT-INDUCED INJURY IN THE LUNGS OF ADULT WISTAR RATS

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ABSTRACT

The aim of this study was to investigate the effects of aqueous leaf extract of *Mimosa pudica* in the lungs of adult Wistar rats exposed to insect-repellent mist. 30 adult Wistar rats were randomly assigned into 5 groups; group A-E comprising of 6 rats per group. Group A rats were placed on rat food and water only. Group B rats were exposed to insect repellent only via inhalation. Group C rats were given *Mimosa pudica* extract orally at 500mg/Kg body weight per day (BWT/D) and Group D rats were exposed to Mosquito repellent and received low dose of the plant extract at (250mg/kg BWT/D) and Group E rats were exposed to mosquito repellent and received (500mg/kg BWT/D) of extract. The dosages were given for 30 consecutive days via orogastric method. There was significant increase in body weight in all treated groups. The haematological outcome showed that insect repellent caused some derangements in haematological parameters especially haematocrit, plateletcrit and mean corpuscular hemoglobin concentration. Histologically, Group D showed an expanded lumen of the bronchioles and shrunken activated immune system. Group E showed a more activated immune system and contracted bronchial artery. Mosquito repellent induced vascular ulceration, bronchiolar mucosal ulceration and activated lymphoid tissue in Group B. These effects were reversed by *Mimosa pudica* leaf extract. In conclusion, *Mimosa pudica* had ameliorative effect against mosquito repellent induced injury in the lungs.

Keywords: *Mimosa pudica*, Mosquito repellent, Lungs, Insecticides

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INTRODUCTION

Mimosa pudica also called sensitive plant, touch-me-not or shame plant is a creeping annual or perennial flowering plant of the pea/legume family *Fabaceae*. *Mimosa pudica* was formally described by Carl Linnaeus in species plantarum in 1753 (Centre for Plants Biodiversity Research). The stem is erect in young plants, but becoming creeping or trailer with age.

The leaves are bi pinnately compound with one or two pinnae pairs, and 10-26 leaflets per pinna. The flowers are insect and wind pollinated (US Forest Service, 2008). *Mimosa pudica* is well known for its rapid plant movement. It undergoes changes in leaf orientation termed "sleep" or nyctinastic

movement, the foliage closes during darkness and reopens in light (Raven *et al.*, 2005).

Mimosa pudica (*M. pudica*) contains the toxic alkaloid mimosine, which has been found to also have anti-proliferative and apoptotic effects (Restivo *et al.*, 2005). *Mimosa pudica* contains various compounds, including "alkaloids, flavonoids C-glycosides, sterols, terpenoids, tannins, saponin and fatty acids" (Genest, 2008). The roots of the plant have been shown to contain up to 10% tannin. A substance similar to adrenaline has been found within the plant's leaves. *Mimosa pudica*'s seeds produce mucilage made up of D-glucuronic acid and D-xylene.

Additionally, extracts of *M. pudica* have been shown to contain crocetin dimethylester, tubulin and green-yellow fatty oils. A new class of phytohormone turgorines, which are derivatives of galli acid, have been discovered within the plant (Azmi, 2011).

The leaves of *M. pudica* also contain a wide range of carbon to mineral content as well as a large variation in (Carbon-13) values. The roots contain sac-like structures that release organic and organo-sulphur compounds including sulphur dioxide (SO₂), methylsulfinic acid, pyruvic acid, lactic acid, ethanesulphic acid, pyruvic, lactic acid, ethanesulfinic acid, propanesulphonic acid and thioformaldehyde (Volkov *et al.*, 2010).

Insecticides are substances used to kill insects (IUPAC, 2006). Insecticides contain two types of chemicals; first is the synthetic insecticides assigned to groups based on the mode of toxic action, such as groups of organochlorines, organophosphate, carbamates, and pyrethroids insecticides; the second is natural insecticides such as Azadirachtin, rotenone, spinosad and abamectin. It causes adverse health effects to humans and ecosystems. Previous studies show that synthetic insecticides such as melathion, methomyl, chlorpyrifos, pirimiphos-methyl, dimethoate, and beta-cyfluthrin caused oxidative stress and liver

and kidney damage in experimental animals (Witt *et al.*, 2014).

Sniper insecticide, a synthetic organophosphorus, which belongs to the DDVP chemical family (2, 2-dichlorovinyl dimethyl phosphate compound), is indiscriminately being used by many Nigerians as indoor insecticide, which is not the original purpose. Natural insecticides contains chemical, mineral, and biological materials and some products are available commercially e.g. pyrethrum, neem, spinosad, rotenone, abamectin, *Bacillus thuringiensis* (Bt), garlic, cinnamon, pepper and essential oil products (Robinson *et al.*, 1990).

Respiratory diseases are pathological conditions affecting the organs and tissues that makes gas exchange difficult in air-breathing animals. Respiratory disease range from mild and self-limiting, such as the common cold, influenza and pharyngitis to life threatening disease such as bacterial pneumonia, pulmonary embolism, tuberculosis, acute asthma, lung cancer (Iyawe *et al.*, 2007).

MATERIALS AND METHODS

The leaves of *Mimosa pudica* that were used for this research work were collected at the back of nursing building, Medical Complex, University of Benin and identified as *Mimosa pudica* at the department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State, Nigeria.

The leaves were air-dried for two weeks. The air-dried leaves were grounded using the British milling Machine into fine powder and weighed. 500g of the powdered leaves were soaked with 1.5litres of distilled water for 24hours with constant shaking and stirring with a stirring rod. After 24 hours, it was

filtered using filter paper. The residues were discarded, then the filtrate was concentrated to paste level using crucible and water bath at 45°C to actualize the final crude extract.

Phytochemical constituents of *Mimosa pudica* include alkaloids e.g the toxic alkaloid 'mimosine'. It also contains a wide variety of compounds like flavonoids, C-glycosides, sterols, terpenoids, tannis, saponin and fatty acids"(Genest, 2008). Acute oral toxicity of the extract was evaluated and appropriate doses of the extract were made by diluting with distilled water into 250mg/kg body

weight and 500mg/kg body weight which were administered to the rats.

Experimental animals: 30 adult Wistar rats weighing between 180g and 280g were purchased from the animal house, Department of Anatomy, University of Benin, Benin City, Edo State Nigeria and were utilized for this experimental research. The rats were given a period of two weeks to adapt to their new environment before commencement of the experiment. During this period, the animals were allowed free access to standard animal feed (Vital grower's feed, manufactured by Bendel Flour Mill, Ewu) and clean water *ad libitum*.

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). Lung injury was induced by exposing the rats to sniper insect repellent daily for 30 days.

Experimental design: Thirty (30) experimental adult male Wistar rats were randomly assigned into Five (5) groups; group A-E comprising of six (6) rats per group. Animals in control group were designated as **Group A** and they received grower's mash and water *ad libitum* throughout the experimental period. Animals assigned to **Group B** were exposed to 15ml of insect repellent daily via inhalation. Animals assigned to **Group C** were given *Mimosa pudica* extract orally at 500mg/Kg body weight per day (BWT/D). Animals assigned to **Group D** were exposed to 15ml of insect repellent via inhalation and received low dose of *Mimosa pudica* plant extract 250mg/kg body weight per day (BWT/D) and **Group E** rats were also exposed to 15ml of

insect repellent via inhalation and received high dose of *Mimosa pudica* plant extract 500mg/kg body weight per day (BWT/D). The experimental period lasted for 30days. Administration of plant extract was done orally via the use of an orogastric tube for 30 consecutive days.

Method of sacrifice and sample collection: After the 30th day, the animals were sacrificed under chloroform anesthesia. Blood samples were collected with plain specimen bottles for biochemical analysis and the lungs of each rat were harvested and immediately fixed in 10% formal saline for 24 hours before histological analysis.

The harvested tissues were histologically assessed using the following methods: fixation, embedding and tissue staining for microscopy. The tissue sections were examined under Leica DM750 research microscope with digital camera (Leica ICC50) attached. Digital photomicrographs of the tissue sections were taken at H & E x40 and x100 magnifications.

White blood cells (WBCs), lymphocytes, monocytes, granulocytes, red blood cells (RBCs), haemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelets, mean platelet volume were analyzed using an auto-analyzer (2006 model, manufactured by Hoddler and Stoughton Group of company, London with a recognized biochemical kit (2010 model, Diagnostical Merck, London). The weights of the experimental animals were taken after 30 days and the difference between them and previous weights were noted. The data were subjected to statistical analysis and P values calculated using the students' t-test.

RESULTS

The results were presented as mean and standard error of mean (tables 1 and 2).

Results based on statistical analysis showed that there was significant increase in body

weight across all groups when the initial weight was compared to the final weight. This could be as a result of growth of the rats after 30 days, whereas there was no significant change in the organ weight. There was a statistically significant increase in the haematocrit value of rats administered *Mimosa pudica* only (**Group B**) indicating a condition known as polycythemia. *Mimosa pudica* that was administered at low dose (**Group D**) prevented this significant increase.

There was a statistically significant decrease in mean corpuscular haemoglobin concentration of rats in the group (**Group E**) exposed to insect repellent and 500mg/kg body weight of *Mimosa pudica* plant extract. There was a statistically significant increase in plateletcrit of rats in the group exposed to insect repellent and 500mg/kg body weight of *Mimosa pudica* plant extract (Group E). This is a condition where the bone marrows are caused to produce too many platelets. When it occurs due to an infection, it is referred to as secondary thrombocytosis.

Table 1: Comparison of Haematological Parameters in All Experimental Groups

	Control	Mosquito repellent only	M. pudica only	Mosquito repellent + M. pudica (low dose)	Mosquito repellent + M. pudica (high dose)	P-value
White blood cells (10³/μL)	16.62±2.07	13.66±1.19	13.65±1.74	17.65±1.75	16.67±1.78	0.357
Lymphocytes (10³/μL)	15.06±2.01	12.18±1.04	12.23±1.79	15.98±1.73	14.28±1.29	0.377
Monocytes (10³/μL)	1.14±0.14	1.06±0.17	1.12±0.07	1.15±0.13	1.75±0.64	0.550
Granulocytes (10³/μL)	0.38±0.07	0.44±0.11	0.33±0.07	0.53±0.20	0.45±0.12	0.766
Red blood cells (10⁶/μL)	6.22±0.20	6.51±0.14	6.24±0.24	6.68±0.20	6.18±0.30	0.457
Haemoglobin (g/dL)	14.06±0.29	14.66±0.22	13.90±0.38	14.35±0.34	13.42±0.52	0.228
Haematocrit (%)	37.86±0.95	40.50±0.49*	38.03±0.79	39.45±0.65	38.38±0.95	0.157
Mean corpuscular volume (μm³)	61.06±1.96	62.32±1.43	61.18±1.35	59.25±1.68	62.68±2.61	0.717
Mean corpuscular haemoglobin (pg)	22.68±0.62	22.54±0.34	22.33±0.40	21.53±0.51	21.80±0.43	0.381
Mean corpuscular haemoglobin concentration (g/dL)	37.14±0.34	36.18±0.53	36.53±0.39	36.35±0.44	34.93±0.77*	0.083
Platelets (10³/μL)	653.40±55.61	634.40±37.14	712.00±12.00	662.17±35.35	804.00±85.40	0.174
Meanplatelet volume (μm³)	7.64±0.19	8.06±0.27	7.70±0.25	8.17±0.26	8.03±0.21	0.446
Plateletcrit (%)	0.50±0.04	0.51±0.03	0.55±0.02	0.54±0.02	0.64±0.06	0.122

*Significantly different from the control group

Table 2: Changes In Weights of Rats In All The Experimental Groups

Groups	Initial body weight	Final Body weight	P-value
Control	164.40±9.76	177.80±11.41*	0.017
Mosquito repellent only	145.83±6.49	162.00±8.47*	0.02
M. Pudica only	149.50±3.24	168.00±4.97*	0.006
Mosquito repellent+m. Pudica (low dose)	151.67±11.47	170.17±8.46*	0.009
Mosquito repellent+m. Pudica (high dose)	148.50±4.55	161.50±3.33*	0.004

n=6, Mean value±SEM, **P<0.01

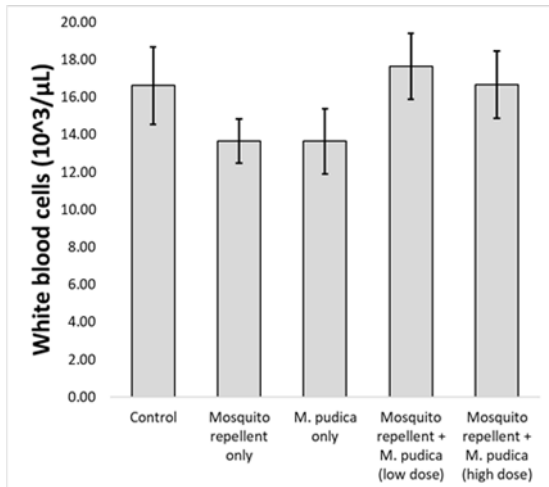


Figure 1. Chart showing white blood cells. There were no statistically significant differences (P>0.05) in the number of white blood cells across the groups

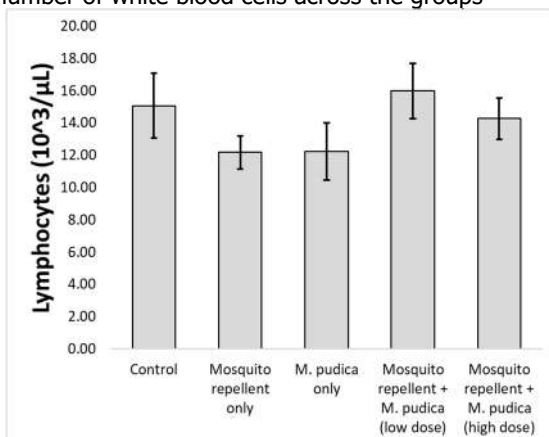


Figure 2. Chart showing lymphocytes. There were no statistically significant differences (P>0.05) in the number of lymphocytes across the groups

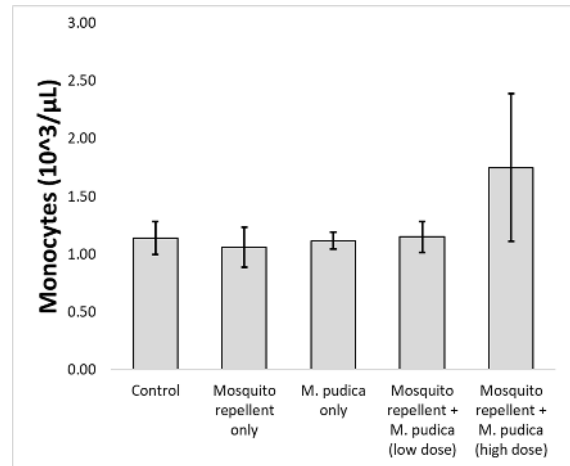


Figure 3. Chart showing monocytes. There were no statistically significant differences (P>0.05) in the number of monocytes across the groups

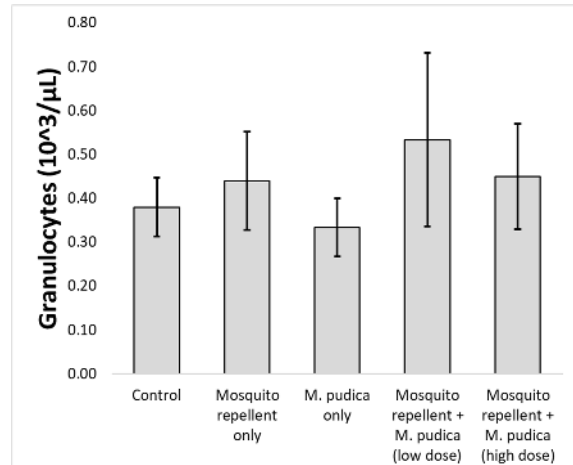


Figure 4. Chart showing granulocytes. There were no statistically significant differences (P>0.05) in the number of granulocytes across the groups

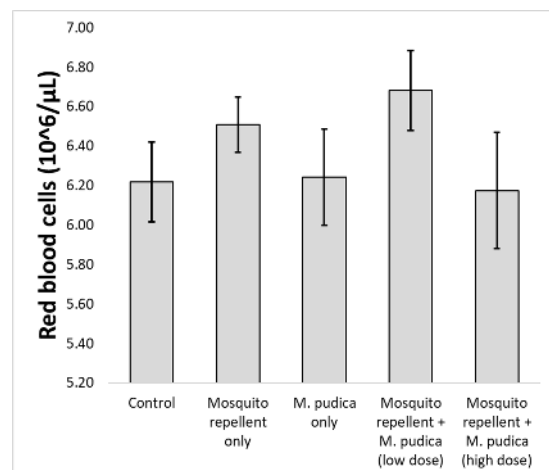


Figure 5. Chart showing red blood cells. There were no statistically significant differences (P>0.05) in the number of red blood cells across the groups.

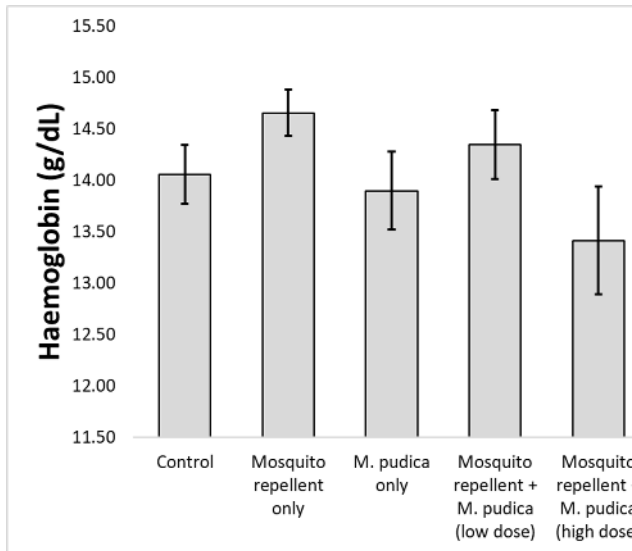


Figure 6. Chart showing levels of haemoglobin. There were no statistically significant differences ($P>0.05$) in the levels of haemoglobin across the groups

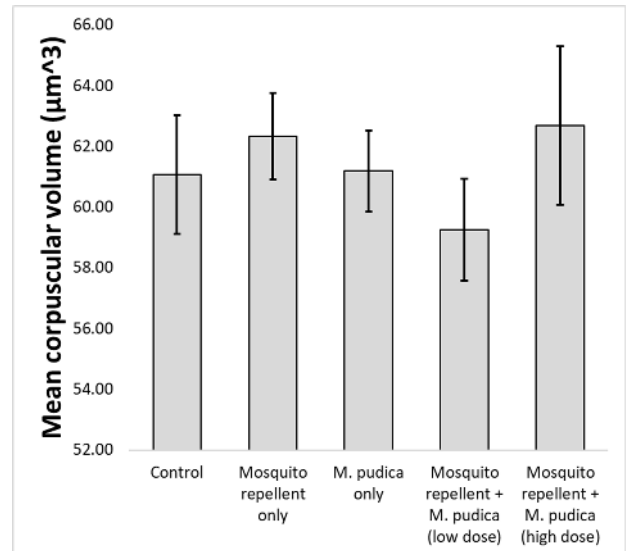


Figure 8. Chart showing Mean corpuscular volume. There were no statistically significant differences ($P>0.05$) in Mean corpuscular volume across the groups

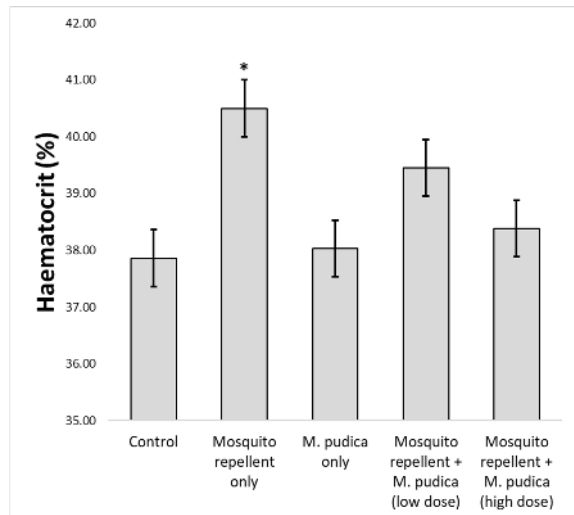


Figure 7. Chart showing haematocrit value *Significantly different from the control group. There was a statistically significant increase ($P<0.05$) in the haematocrit value in Group B, when compared to the control group.

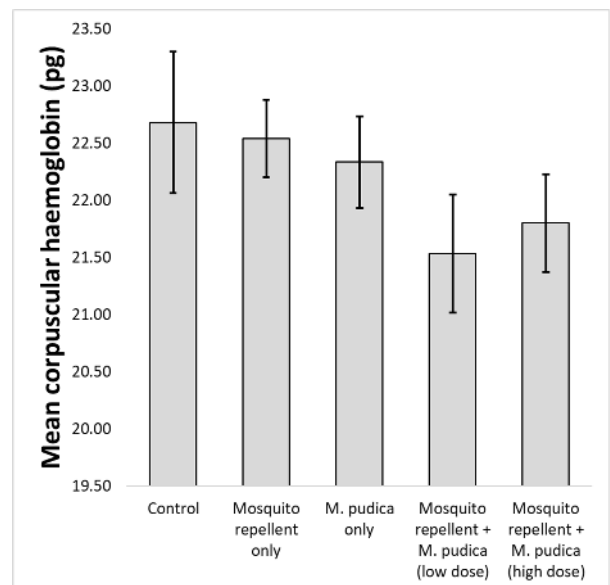


Figure 9. Chart showing Mean corpuscular haemoglobin. There were no statistically significant differences ($P>0.05$) in Mean corpuscular haemoglobin across the groups

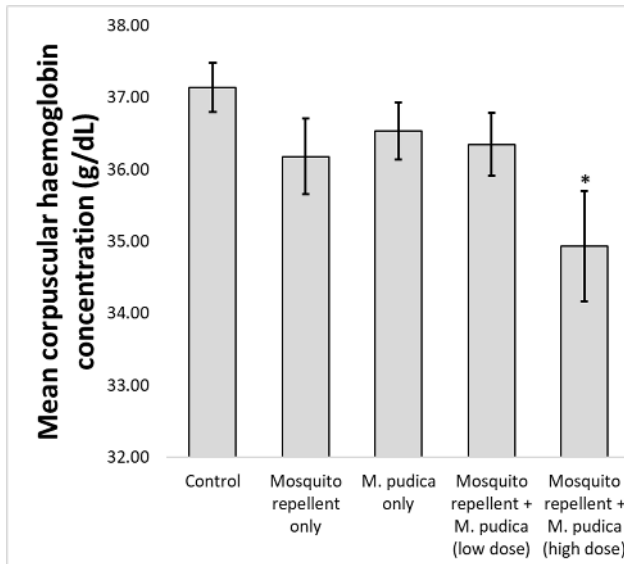


Figure 10. Chart showing Mean corpuscular haemoglobin concentration. *Significantly different from the control group. There was a statistically significant decrease ($P < 0.05$) of Mean corpuscular haemoglobin concentration in in Group E, when compared to the control group.

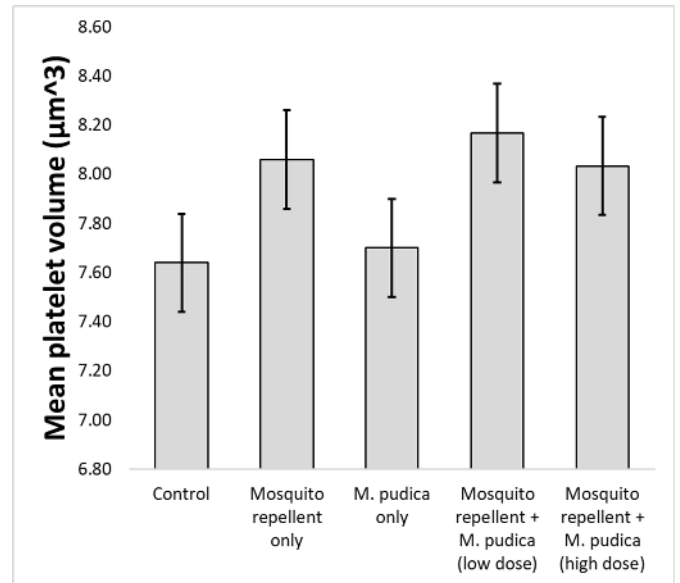


Figure 12. Chart showing mean platelet volume. There were no statistically significant differences ($P > 0.05$) of mean platelet volume across the groups

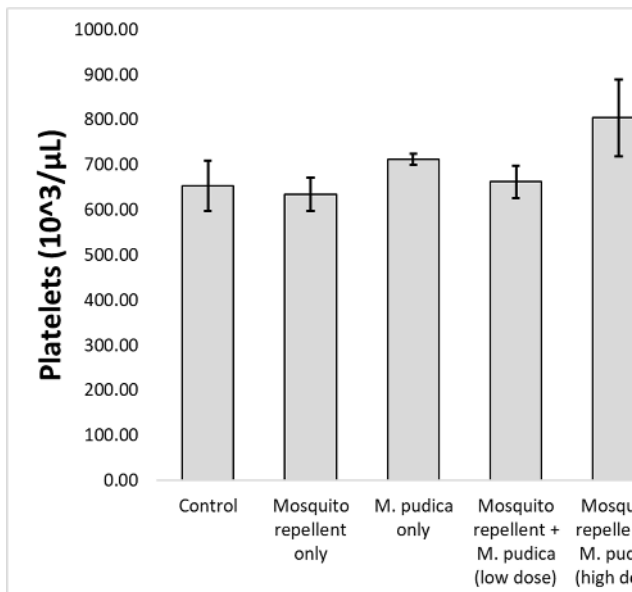


Figure 11. Chart showing platelets. There were no statistically significant differences ($P > 0.05$) in the number of platelets across the groups

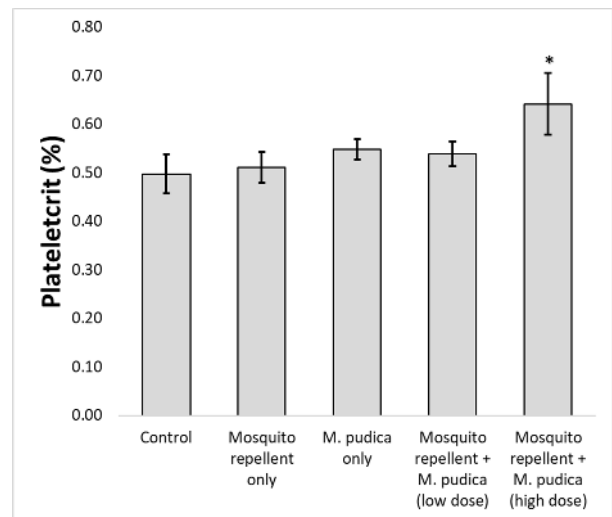


Figure 13. Chart showing platelet crit. *Significantly different from the control group. There was a statistically significant increase ($P < 0.05$) of plateletcrit in Group E, when compared to the control group.

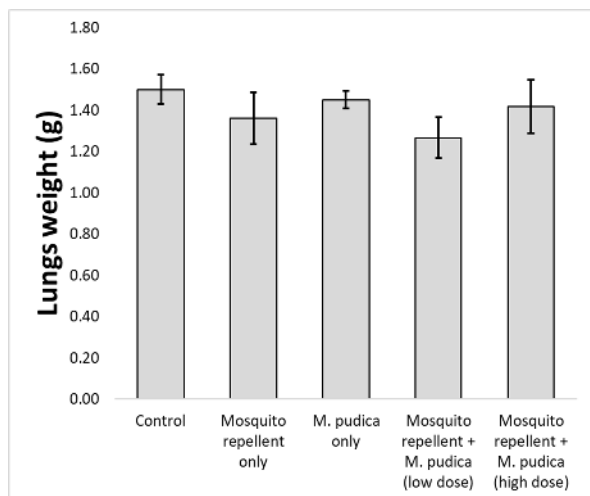


Figure 14. Chart showing lung weights. There were no statistically significant differences ($P > 0.05$) of mean platelet volume across the groups

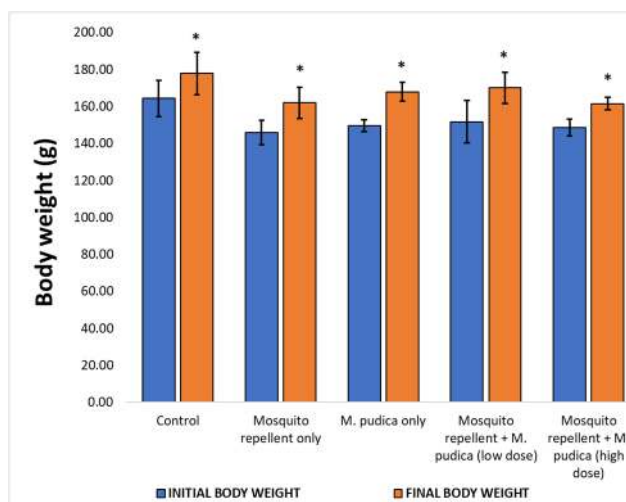


Figure 15. Chart showing the initial body weight in comparison with the final body weight. *Significantly different from the initial body weight. There were statistically significant increases ($P < 0.05$) of body weights in all the groups when the initial body weights were compared to the final body weights.

Legends for photomicrograph

Figures 1 and 2 are photomicrographs of a section of rat lungs in the control group (Group A) at H&E $\times 40$ and $\times 100$ magnifications respectively showing **A.** Alveolar spaces **B.** interstitial space, **C.** terminal bronchiole and **D.** bronchial artery
 Figures 3 & 4 are photomicrographs of a section of rat lungs given mosquito repellent only (Group B) at H&E $\times 40$ and $\times 100$ magnification respectively showing **A.** vascular ulceration **B.** bronchiolar mucosal ulceration **C.** activation of lymphoid tissue and **D.** bronchiolar dilation

Figures 5 & 6 are photomicrographs of a section of rat lungs given 500mg/kg body weight of plant extract only (Group C) at H&E $\times 40$ and $\times 100$ magnifications respectively showing **A.** normal alveoli, **B.** active interstitial congestion and **C.** normal bronchiole

Figures 7 & 8 are photomicrographs of a section of rat lungs given insect repellent + low dose of *Mimosa pudica* leaf extract (250mg/kg) (Group D) at H&E $\times 40$ and $\times 100$ magnifications respectively showing normal architecture of **A.** alveoli, **B.** bronchiole and **C.** vascular microstructure

Figures 9 & 10 are photomicrographs of a section of rat lungs given mosquito repellent + High dose of *Mimosa pudica* leaf extract (500 mg/kg) (Group D) at H&E $\times 40$ and $\times 100$ magnification respectively showing **A.** normal alveolar, **B.** bronchiolar microstructure and **C.** florid activation of bronchiolo-alveolarlymphoid aggregate and **D.** active vascular congestion and dilatation.

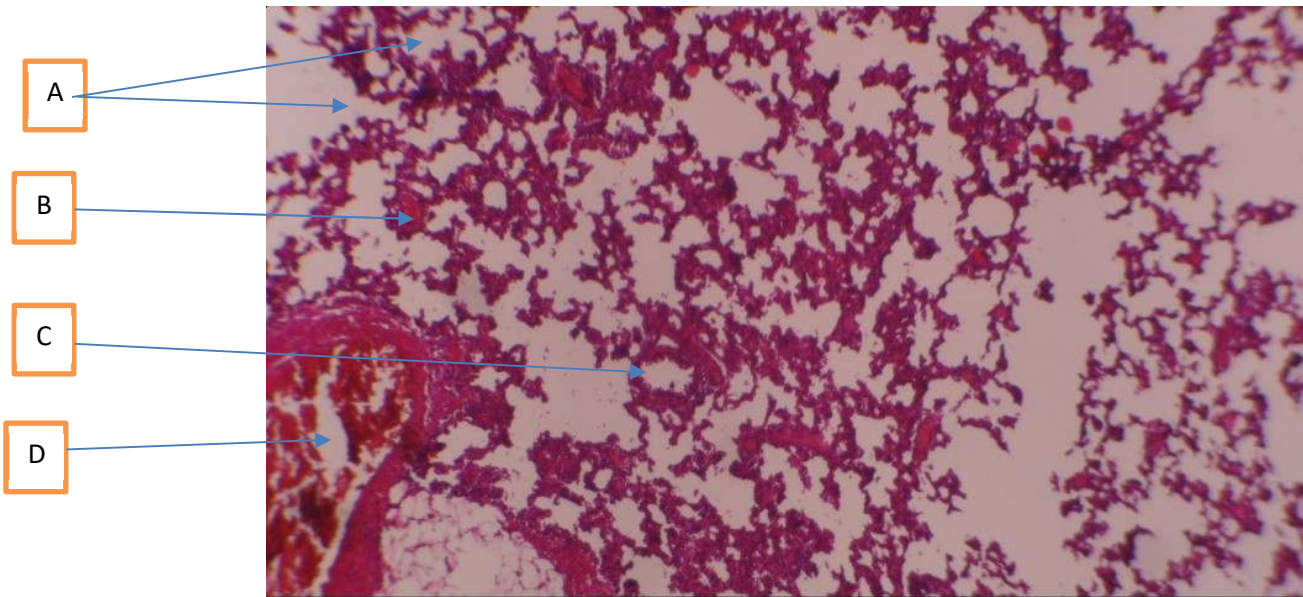


Figure 1: Lungs (Control). Composed of **A:** Alveolar spaces **B.** interstitial space **C.** terminal bronchiole and **D.** bronchial artery (H&E x 40)

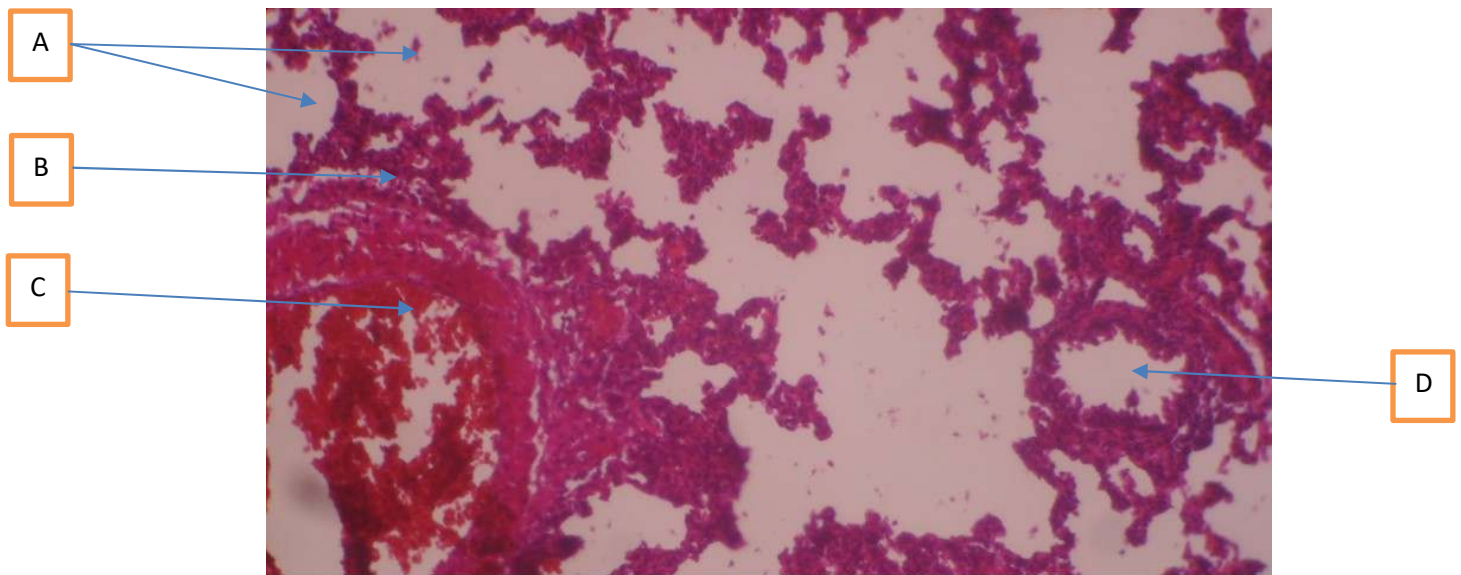


Figure 2: Higher magnification of the above: **A. B. C. D** (H&E x 100)

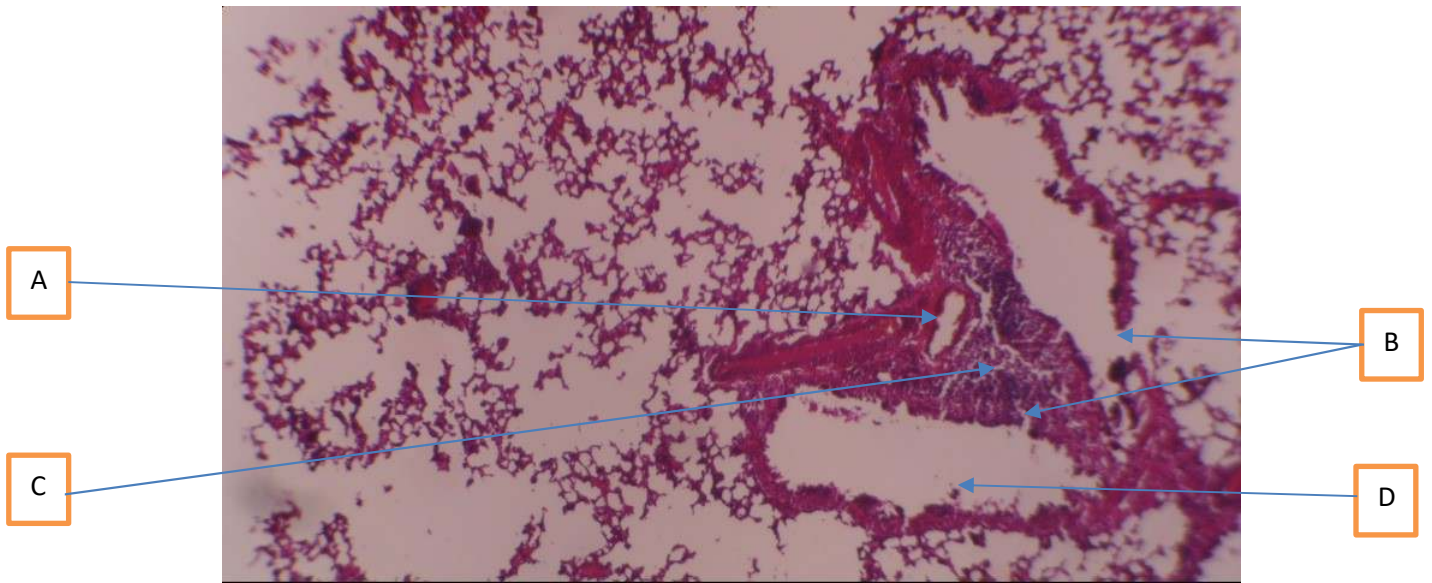


Figure 3: Rat given mosquito repellent: **A.** vascular ulceration **B.** bronchiolar mucosal ulceration **C.** activation of lymphoid tissue and **D.** bronchiolar dilation (H&E x 40)

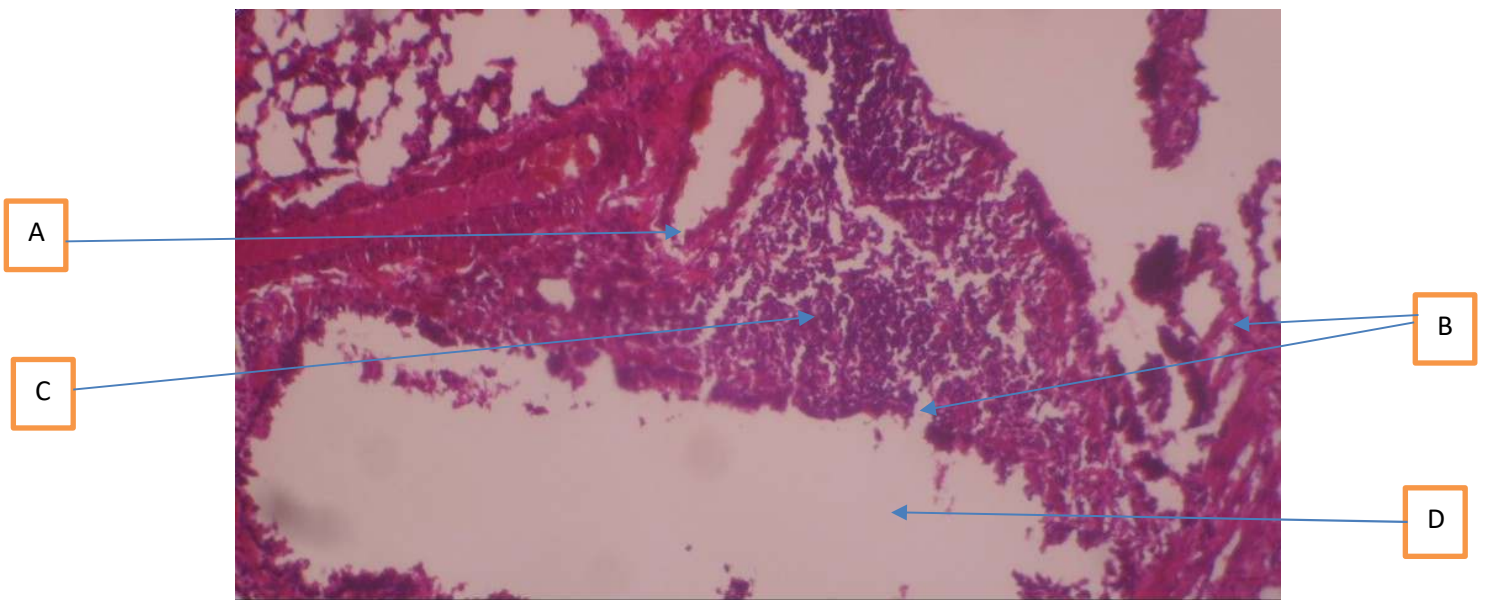


Figure 4: Higher magnification of the above: **A. B. C. D** (H&E x 100)

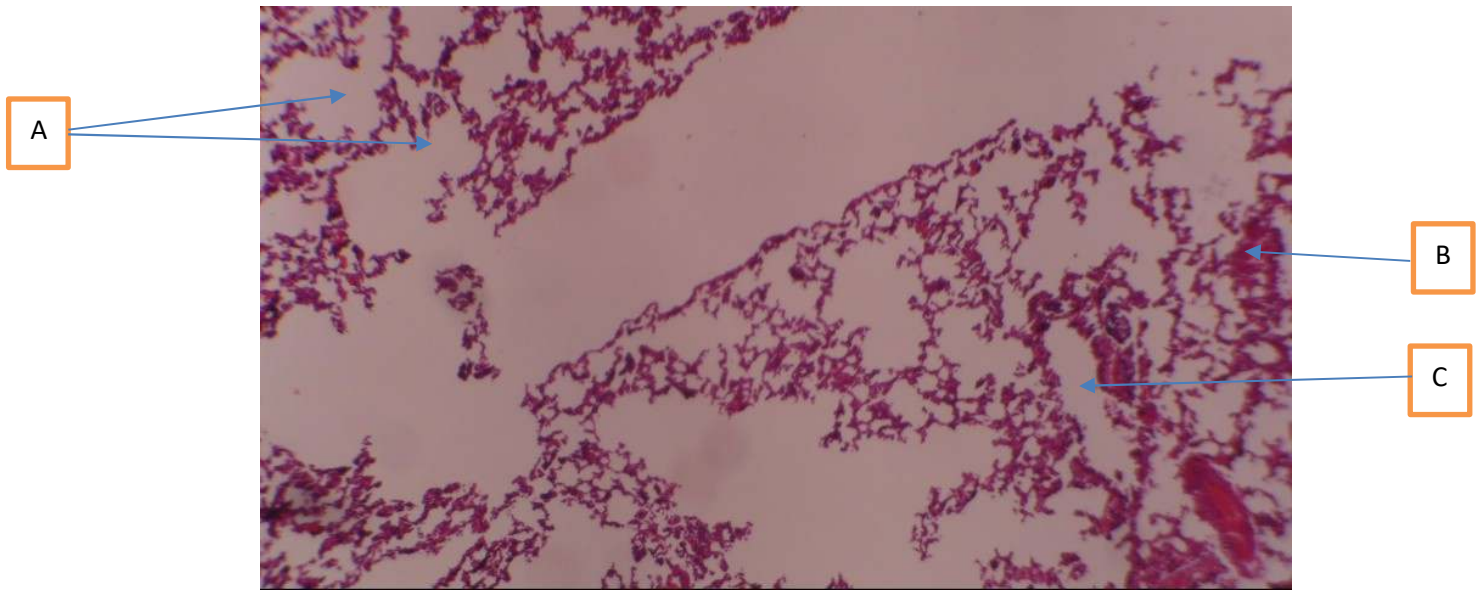


Figure 5: Rat given Extract only: **A.** normal alveoli **B.** active interstitial congestion and **C.** normal bronchiole (H&E x 40)

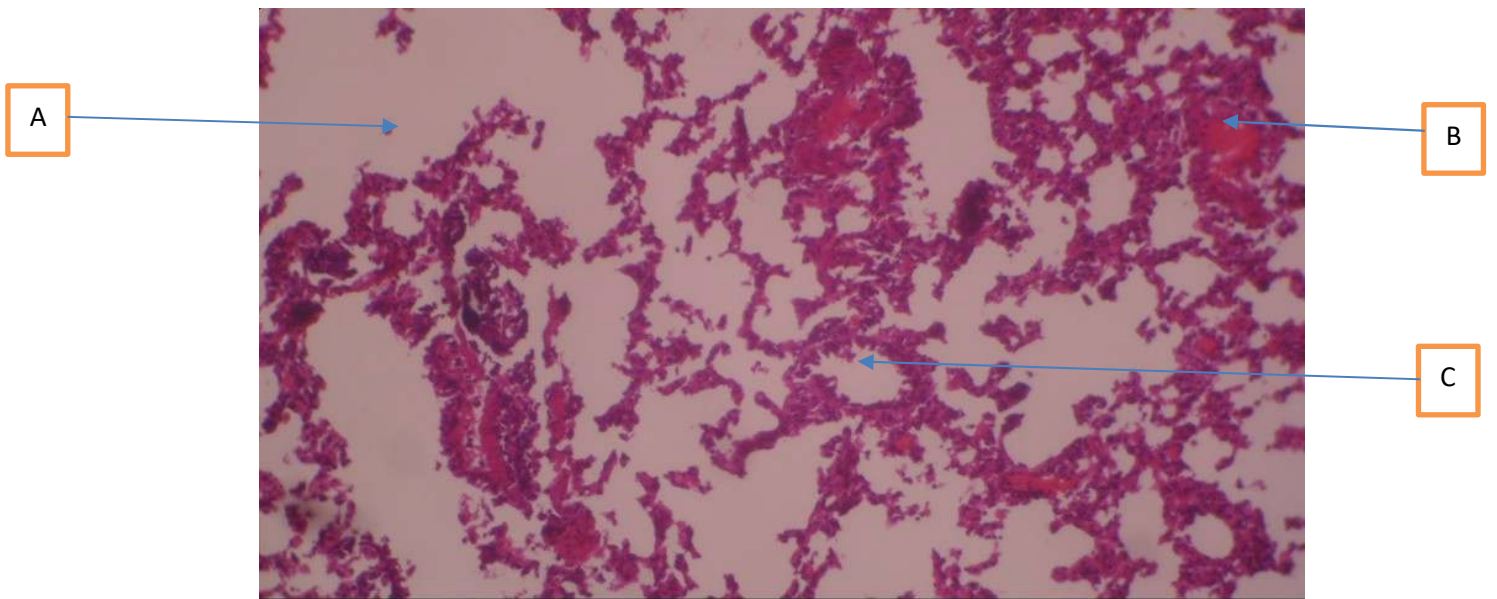


Figure 6: Higher magnification of the above: **A.** **B** and **C** (H&E x 100)

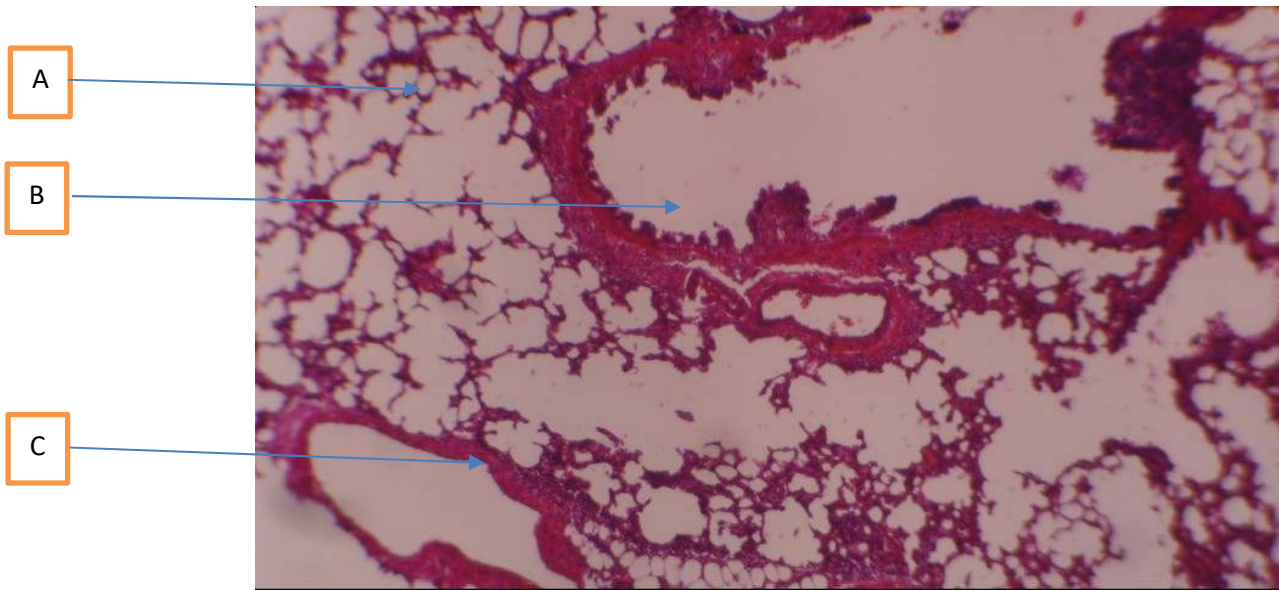


Figure 7: Rat given Toxicant + low dose extract: **A.** normal alveoli **B.** normal bronchiole and **C.** normal vascular microstructure (H&E x 40)

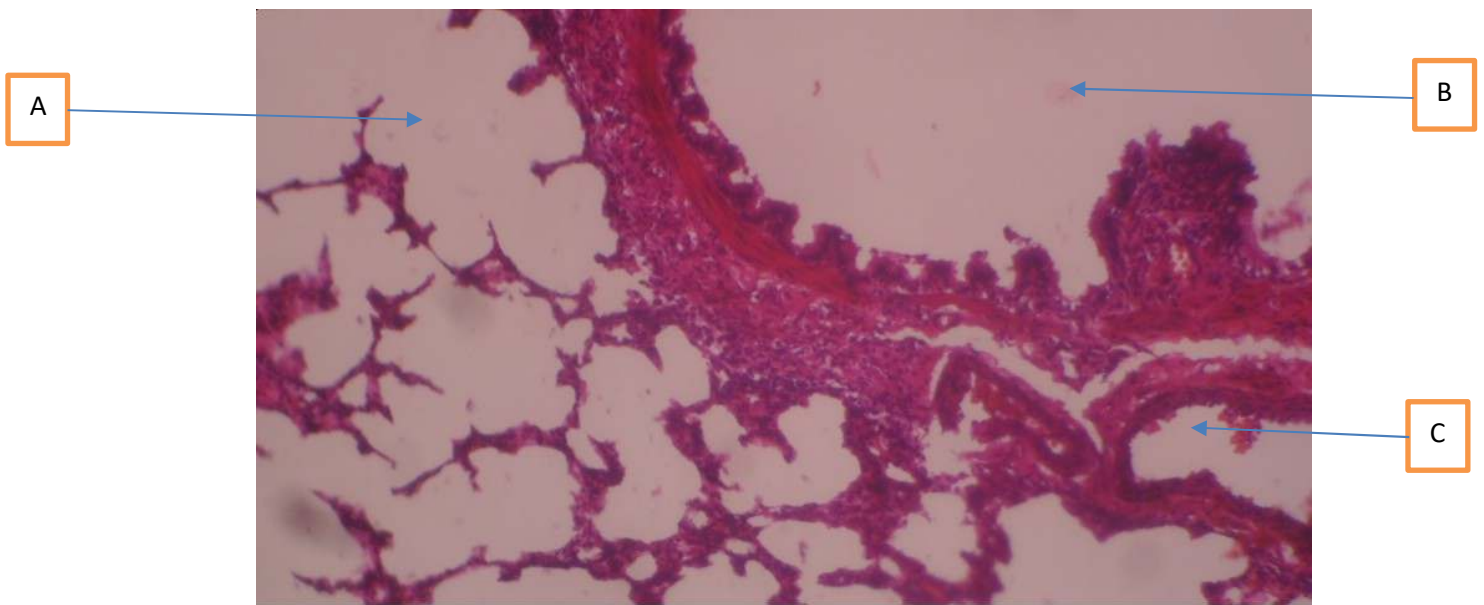


Figure 8: Higher magnification of the above: **A. B. & C** (H&E x 100)

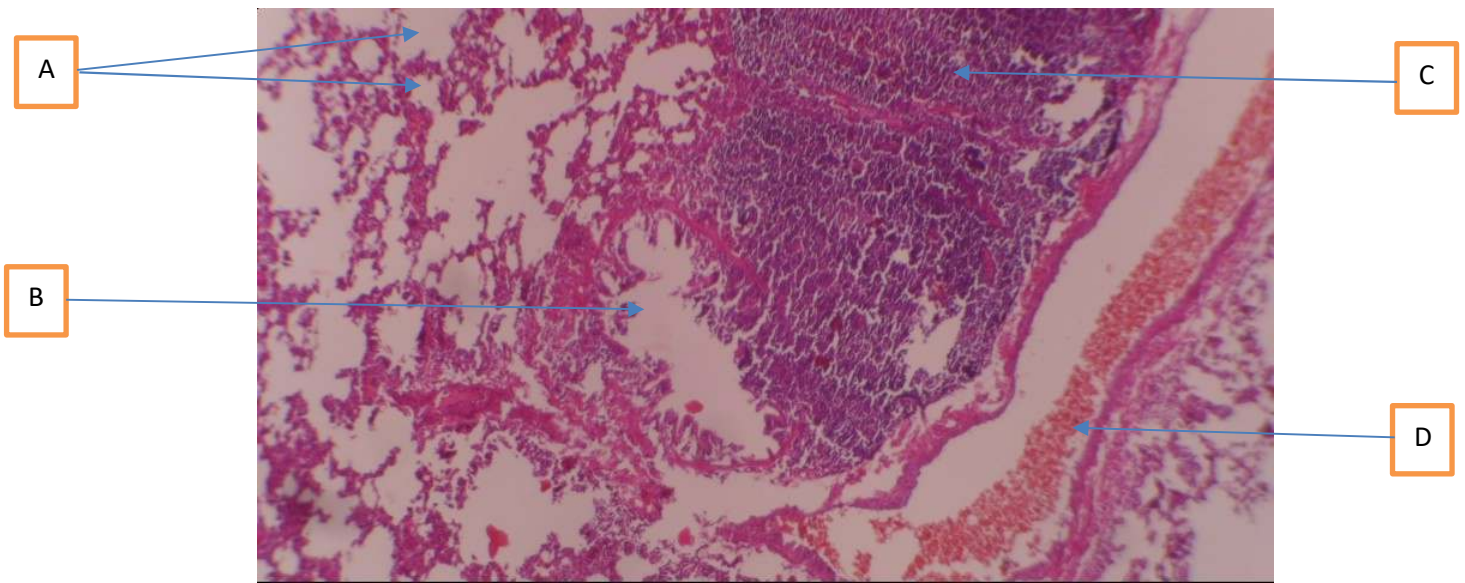


Figure 9: Rat given Toxicant + high dose extract: **A.** normal alveolar **B.** bronchiolar microstructure and **C.** florid activation of bronchiolo-alveolar lymphoid aggregate and **D.** active vascular congestion and dilatation (H&E x 40)

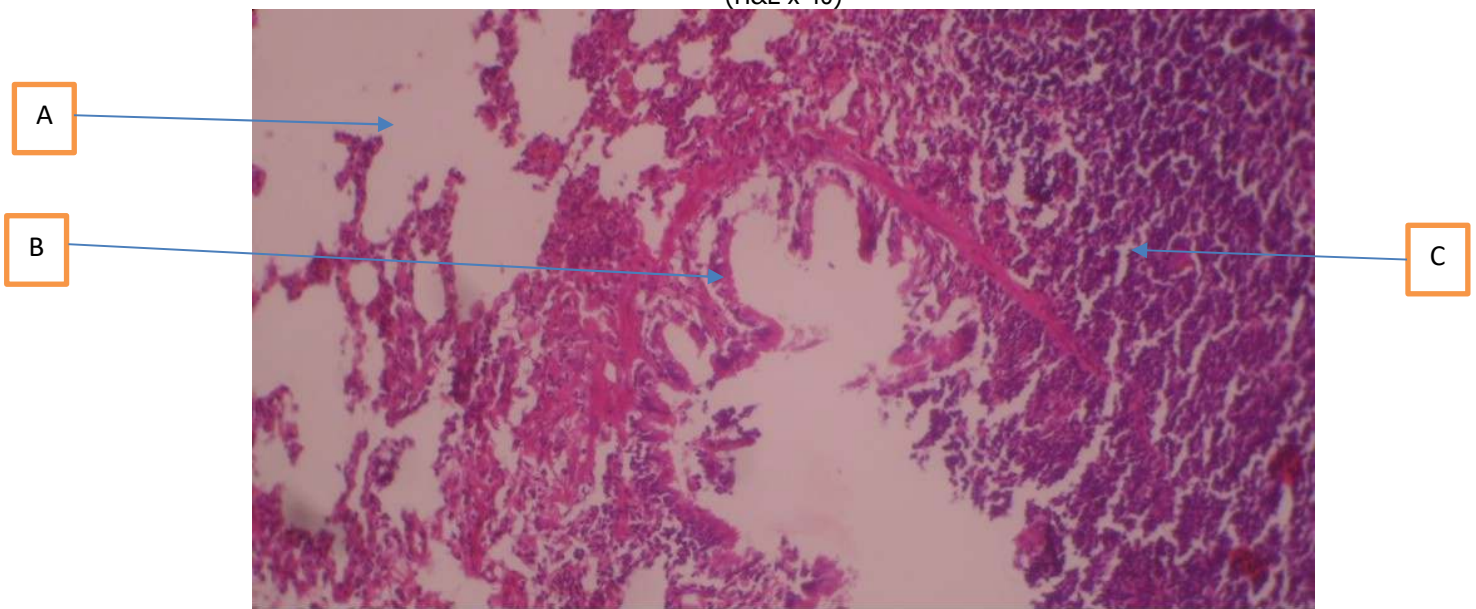


Figure 10: Higher magnification of the above: **A. B. & C** (H&E x 100)

DISCUSSION

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practice since prehistoric times. This study was designed to evaluate the effects of aqueous leaf extract of *Mimosa pudica* in the lungs of adult Wistar rats exposed to insect repellent via inhalation.

The findings of this research for the body weight showed a significant increase in the body weight of the rats in all the groups. This can be attributed to normal growth during the period of the study.

The findings from this research showed that there was a statistically significant increase

in the hematocrit value of the rats treated with mosquito repellent only (Group B). This condition is known as polycythemia or erythrocytosis. It is associated with excessive production of red blood cells in the body. There was also a statistically significant decrease in mean corpuscular haemoglobin in the blood of rats exposed to insect repellent and treated with 500mg/kg body weight of *Mimosa pudica* plant extract (Group E), a condition that follows a reduced haemoglobin concentration in the blood. It may result from iron deficiency leading to anemia.

There was a significant increase in plateletcrit in the blood of rats exposed to mosquito repellent and treated with 500mg/kg body weight of *Mimosa pudica* plant extract (Group E). This usually arises from excessive production of platelets by the bone marrow in response to mucosal ulceration.

Histomorphological studies of the lungs of rats exposed to mosquito repellent only (Group B) shows that there was vascular ulceration, bronchiolar mucosal ulceration, activation of lymphoid tissue and bronchiolar dilatation (Figures 3 and 4). *Mimosa pudica* caused active interstitial congestion (Figures 5 and 6) as seen in the lungs of rats that received plant extract of *Mimosa pudica* only at 500mg/kg body weight (Group C). Conclusively, *Mimosa pudica* plant extract

administered at 250mg/kg body weight after exposure to mosquito repellent was able to reverse the damage caused as seen in the photomicrographs of lung sections of rats in Group D (Figures 7 and 8). Photomicrographs taken from lung sections of rats that received 500mg/kg body weight of *Mimosa pudica* after exposure to mosquito repellent (Group E) shows that there was a less ameliorative effect at this dose. There was also a florid activation of bronchiolo-alveolar lymphoid aggregates and active vascular congestion and dilatation (Figures 9 and 10).

CONCLUSION

In conclusion, *Mimosa pudica* had ameliorative effects against mosquito repellent-induced vascular ulceration, bronchiolar mucosal ulceration and activation of bronchiolo-alveolar lymphoid tissue. These effects were seen to be inversely proportional to the dosage as the results were more desirable at lower doses and could be compared to the control group. It is therefore valuable in combating lung injuries.

RECOMMENDATION

Advanced studies should be carried out to develop this plant (*Mimosa pudica*) into a proper drug to combat lung diseases.

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