EVALUATION OF TOXIC EFFECT OF D-ALLETRIN BASED MOSQUITO'S COIL ON THE LUNGS, AND SELECTED HAEMATOLOGICAL PARAMETERS OF ADULT WISTAR RATS.

ABSTRACT

The effect of inhaling mosquito coil smoke on the haematology and histology of rat's lungs was studied. Mosquito coils while slowly burning emit smoke containing one or more insecticides, each coil burns for several hours and are used in close proximity to persons requiring protection against mosquitoes in order to prevent malaria, a disease of wide distribution. This study is aimed at determining the effects of mosquito coil on lungs of adult wistar *rats*. A total of 16 adult wistar rats were used in this study weighing 100g – 200g. They were randomly divided in four groups of four rats each. Rats in group 1 served as control and received only food and distilled water; while the experimental groups II – IV were exposed to mosquito coil smoke for 1 hour, 2 hours and 3 hours respectively for a period of 28 days. At the end of the experimental period, blood was collected from each rat through ocular puncture for blood analysis. The rats were sacrificed with the lungs harvested and fixed in 10% formal saline for routine histological analysis. The body weight of the exposed groups observed showed insignificant decrease when compared with the control group. The organ weight of lungs showed significant increase when compared to the control group. Haematological analysis for blood obtained revealed a significant ($p<0.05$) increase in Hb, PCV, RBC and WBC counts in all exposure groups when compared with the control group. Histological analysis of the lungs showed severe hemorrhage severe inflammation of the lungs. Results from this study demonstrates that mosquito coil smoke not only challenges the immune system but also affects the blood oxygen regulation level through damage in the lungs.

Keywords: D-alletrin, haematological parameters, packed cell volume, mosquito coil.

BACKGROUND OF THE STUDY

Mosquito coils are smoldering insecticides used in close proximity to persons requiring protection against mosquitoes in order to prevent malaria, a disease of wide distribution. This is paramount because the burden caused by malaria is considerable amounting to 300- 400 million clinical cases per year- 80% of which occur in Africa and are responsible for almost one million deaths per year [1]. The scourge of malaria had led parents and their children in low-income communities to adopt the use of mosquito coils to control the mosquito populations around residential areas [2].

The availability and the relative cheapness of this insecticide have made mosquito coils very popular in Asia, South America and Africa including Nigeria. The annual worldwide consumption of the four major types of residential insecticides products – aerosols, mosquito coils, liquid vaporizers and vaporizing units are in the billions of units [3].

Mosquito coils are often used overnight in sleeping quarters where elevated exposure may occur and children and their parents are often exposed to chemically complex mosquito coil smoke containing small particles (1μm), metal fumes and vapors [4] that may reach the alveolar region of the lung [5]. Researchers have also found that the gas phase of mosquito coil smoke contain carbonyl compounds (formaldehyde and acetaldehyde) with properties that can produce strong irritating effects on the upper respiratory tract [6].

Mosquito coils consist of an insecticide/repellent, organic fillers capable of burning with smoldering binder and additives such as synergists, dyes and fungicides. The most common active ingredients in mosquito coils are various pyrethroids that are effective against many genera of mosquitoes including Aedes, Anopheles and Mansonia [3].

Around the world, burning mosquito coils indoors is a common and efficient method for keeping mosquitoes away (WHO, 2005). Every year, approximately 50 billion mosquito coils are burned with two billion people being exposed to the smoke generated. Mosquito coil burning, which involves incomplete biomass combustion, is a significant source of indoor air pollution. Toxic smoke comprising gases and particles is produced during the combustion process. The gaseous phase contains carbon monoxide, volatile organic compounds (compounds with boiling points lesser or equals 250ºC and derived from burning of all types of fossil fuels. Examples includes carbon disulfide, butanal etc., allethrin, and carbonyl compounds [8, 4]. Particles generated are mainly of size smaller than 1 μm (including nanoparticles) and are the typical respirable dusts [8, 4].

Epidemiologic studies have also shown that long term exposure to mosquito coil smoke can induce asthma and persistent wheeze in children [7, 9, 10]. Toxicological studies using mosquito coils in rats showed focal deciliation of the tracheal epithelium, metaplasia of epithelial cells and morphologic alteration of the alveolar macrophages.

Liu *et al.* [4] found that essential oil based mosquito repellent products can generate ultrafine particles and ozone. Not only are these particles more likely to reach and accumulate in human lung alveoli tissue, they also contain a higher concentration of particulates constituting a larger surface area to which harmful substances, such as heavy metals (Cd, Zn and Pb), aldehyde, allethrin, and polycyclic aromatic hydrocarbons (PAHs) can attach [4, 8]. Harmful health effects may be related to the high concentration of particulates and chemicals attached to the particles.
The key to recognizing cases of lung cancer that are a result of occupational or environmental exposures is clinical investigation and consideration of all possible causes for the disease that are present. The histological type of occupational-induced lung cancers is usually different to that lung cancers caused by another factor due to the difference in the etiology of diseases. Between 2008 and 2012, three patients were diagnosed with SCLC at the Shanghai Pulmonary Hospital. These patients, whose cases have been reported in the present study, were aged around 40 years old, which is younger than the general age of patients with lung cancer. Notably, all patients had been employed in the manufacture of mosquito coils in Xinghua, Jiangsu, for a mean of 9.1 years. The mean overall survival time after presentation was 10.7 months. The primary routes of occupational exposure to mosquito coil toxins are inhalation and dermal contact. Based upon the clinical and pathological findings, it was hypothesized that occupational exposure to mosquito coils was a major factor involved in the pathogenesis of the lung cancer and some other related diseases.

Therefore, this work is aimed at investigating the effects of mosquito's coil inhalation on lungs of adult wistar rats

MATERIALS AND METHOD

Location of Study

This study was conducted in the Animal House of the Department of Anatomy, Faculty of Basic Medical Sciences, College of Health sciences, Nnewi campus, Nnamdi Azikiwe University, Anambra state.

Test Article

Mosquito coils were purchased from various retail outlets located within Nnewi Anambra State, Nigeria. The brand commercially purchased for the experiment contain pyrethroids (d- allethrin) 0.2% w/w and inert ingredient 99.8% w/w. The mosquito coil used measured 12cm diameter, 85cm length and 175g weight.

Experimental Animals

A total of 16 Male Wistar Rats weighing 100-200g were used in this study and they were housed in the animal house, College of Health Sciences, Nnewi Campus, Nnamdi Azikiwe University, Anambra State. Animals were kept in standard cages at a room temperature of 27±2°C. The animals were maintained with normal laboratory chow (Grower feed) and water *ad libitium*. They were acclimatized for a period of two weeks and before the administration of Mosquito coil which lasted for a period of 28 days. The animals were kept on 12hours light and dark cycles.

Experimental Design

16 Male Wistar Rats weighing between 100-200g were used for this study. The animals were allowed to acclimatize for a period of two weeks, after which they were randomly selected into 4 groups of 4 animal each.

Groups	**Treatment/Exposure duration**
Group 1 (control)	No exposure (Animals received only distilled water and laboratory chow).
Group 2	Inhale mosquito coil for 1 hour (Acute inhalation study).
Group 3	Inhale mosquito coil for 2 hours (Subchronic inhalation study).
Group 4	Inhale mosquito coil for 3 hours (Subchronic inhalation Study).

The exposure of the mosquito coil last for a period of 28 days. The mosquito coil exposure took place in the inhalation room starting with animals in group 2, 3, and 4. After the expiration of the 28 days, the animals were sacrificed. Blood was collected for test on haematological parameters, lungs harvested for histopathological (Macroscopic examination) findings.

Estimation of Haematological Indices

- **Total White Blood Count**

(The total white blood cells (WBCs) were enumerated by the method of Robert.)[Robert, 1993]. Blood was drawn exactly to the 0.5 mark in a white blood cell diluting pipette. Immediately diluting fluid (Glacial acetic acid – 2 cc, gentian violet – 0.025 g, distilled water made up to - l00ml) was also drawn to the 11 mark. Mix the contents of the pipette for 3-5 min, load a drop at the junction of the cover glass and the Neubauer counting chamber. Count the white cells in the four large corner squares contain sixteen smaller secondary squares, each with an area of 0.04 mm^2.

$$\text{WBC per cu mm} = \frac{\text{Number of WBC X dilution (20)}}{\text{Volume (0.4)}}$$

Estimation of Hemoglobin

Hemoglobin was determined by the method followed by [Robert, 1993]
Five milliliters of buffered cyanide/ ferri cyanide reagent was pipetted into clean and dry test tubes; 0.02 ml of blood was added to the same tubes. The pipette was thoroughly flushed with reagent. The solution was mixed well and incubated at 30°C. Absorbance A of sample was read against distilled water after 3 min at 546 nm. The concentration 'C' of hemoglobin was calculated as follows; $C = 36.77 \times A$ (g/100 ml).

Estimation of Packed Cell Volume

Pack cell volume (PCV) was determined by the method followed by [Robert, 1993]

Fill the capillary tube two-thirds to three- quarters full of well-mixed oxalated venous blood and seal one end of the tube with plasticine, place the filled tube in the micro-hematocrit centrifuge, with the plugged end away from the center of the centrifuge. Centrifuge at a preset speed of 10000 to 12000 rmp for 5 minutes. Lastly place the spine tube in the micro-hematocrit reader and take the value.

Estimation of Red Blood Cell

Red blood cell was determined by the method followed by [Robert, 1993]
Draw the blood from the rat using cardiac puncture and put in a RBC pipette up to 0.5 mark. Take RBC diluting fluid up to 101 mark in single mark pipette or RBC pipette. Rotate the pipette equally in your hands to mix the solution well by swirling. Take the haemocytometer and place it on the flat surface of the work bench. Place the cover slip on the counting chamber. Allow a small drop of diluted blood, hanging from the pipette, to sweep into the counting chamber by capillary action. Make sure that there is no air bubble and there is no overfilling beyond the ruled area. Leave the counting chamber on the bench for 3 minutes to allow the cells to settle. Observe the cells by placing the counting chamber on the mechanical stage of the microscope. Focus on the center room of the chamber and start counting the cells from upper left corner of the room. It is advisable to complete all counts of the four squares and then move to the center square, which is the fifth square to be counted.

Tissue Processing

Tissue sections will be produced by normal histochemical methods of fixation, dehydration, clearing, impregnation, embedding, sectioning, mounting, and staining. The micrographs of relevant stained sections were subsequently taken with a photomicroscope.
Fixation: After weighing the organ, a small part was cut from it and immediately fixed in 10% formal saline in order to preserve the various constituents of the cells in their normal micro anatomical position and to prevent autolysis and putrefaction.

Fixation is a very important step in tissue processing as it does not only protect the tissue from autolysis and putrefaction but also hardens the tissue to withstand other chemicals applied in subsequent treatments and for easy handling.

Dehydration: After fixation, the tissue were transferred and dehydrated in ascending grades of alcohol (50%, 70%, 90%, 95% and 100% or absolute alcohol once for 2hours each but twice in absolute alcohol).

The tissue was placed in ascending grades of alcohol to prevent distortion and distortion to the cell structure would have happened if directly placed in absolute alcohol. However, sufficient time was allowed in absolute alcohol to enable complete dehydration.

Clearing: The tissue was cleared twice in Xylene for 1 to 2 hours each time. This is to avoid over exposure in the clearing agent, which will make them brittle. Xylene was used as the clearing agent as it does not only remove alcohol but is equally miscible with paraffin used in embedding.

Impregnation: The tissues were placed in molten paraffin wax at a constant temperature of 56ºC (3ºC above the melting point of paraffin wax used) in an oven and were passed through two changes of paraffin wax in the oven, 4 hours each. This was done to replace the clearing agent or antemedium with molten paraffin wax and can also be referred to as *Infiltration.* The tissue were subsequently removed from the oven and embedded in paraffin wax.

Embedding: Embedding is a process of burying a tissue in molten paraffin wax. The paraffin becomes a solid firm structure when it is cold. This forms a support medium for the tissue during microtomy.

The tissues were then immersed in molten paraffin wax at a constant temperature of 36ºC to 60ºC in an oven of paraffin bath changing it twice for 2-4 hours each time. They were left to cool and solidify in metallic embedding moulds. The tissue blocks obtained were casted on to the wooden blocks for sectioning.

Sectioning: This was done using a Rotatory Microtome. The tissue blocks were mounted on wooden blocks. With the microtome knife and blocks positioned accurately, sections were made at 5 microns each. The ribbons of sections were floated in warm water bath (37ºC) to straighten them. The best ribbons were picked with forceps and placed on albuminised slides. The slides were labeled using diamond pencils and transferred to a slide rack. They were then placed in an oven to keep the specimens warm before staining.

Staining: The tissues were stained using Ehrlich's Haematoxylin and Eosin stains. The staining procedure is as follows:

- The slides treated with paraffin wax were cleared in xylene for 3 minutes, and were rehydrated in descending grades off alcohol; absolute alcohol for 2 minutes to remove xylene, 90% alcohol for 2 minutes, 70% alcohol for 2 minutes, 30% alcohol for 2 minutes, and then rinsed in water for 1 minute each.
- The tissues were stained by immersing them in aqueous solution of haematoxylin for 30 minutes, and then rinsed in water to remove excessive stains. The tissues were subsequently differentiated in 1% acid alcohol for 1minute. This process called *Bluing* gave the tissues their characteristic blue background.
- The tissues were then stained in aqueous eosin for 10 minutes.
- The tissues were now immersed in ascending grades of alcohol as follows; 50%, 70%, 90% and absolute alcohol for I minute each and then cleared in xylene for 1 minute.

Staining gives contrasting colors to different elements of the cells or tissue thus making them conspicuous and easy to study.

Mounting: The slides were removed from the rack through their edges with the aid of forceps and placed on the filter papers. Blotting was done in one direction on the filter papers using the index finger and few drops of xylene were placed on the slides to make them wet. A drop of Dibuty Phthalate
Xylene (DPX) mountant was placed on the slide which was laid in the middle to minimize the likelihood of trapping air bubbles. The slides were quickly inverted over cover slip and then brought down horizontally until the mountant made contact.

Statisical Analysis

Data will be analyzed using Statistical Package for Social Sciences (SPSS Version 23). The results will be expressed as mean ± S.E.M. Data for Hematological Indices (Red Blood cell, White Blood Cell, Hemoglobin, and Pack cell volume) and Relative Organ weight will be analyzed using One-way ANOVA, followed by Post hoc LSD. While body weight will be analyzed using Student dependent T-test. Values were considered significant at $P<0.05$

RESULT

Physical and Behavioral Changes

- Feeding was normal throughout the study
- Cough and sneezing was observed during administration hours
- Watery feaces was seen in some of the rats in group III

Table 3.1: Show the Effect of mosquito coil on Body weight.

	Group	Mean ± SEM	*P-value*	*T-value*
Group A	Initial	150.00 ± 4.08	0.058	-3.000
	Final	165.00 ± 6.48		
Group B	Initial	135.00 ± 17.08	0.393	-0.920
	Final	157.50 ± 17.50		
Group C	Initial	127.50 ± 12.50	0.284	-1.177
	Final	142.50 ± 2.50		
Group D	Initial	147.50 ± 4.79	0.233	-0.502
	Final	140.00 ± 14.14		

Data were analyzed using Student dependent T-test and values were considered significant at $P<0.05$. $*P<0.05$ means significant, $P>0.05$ means not significant.
Result from the table above showed that there was no significant increase in the body weight in group A when comparing the Initial weight (150.00±4.08) to the Final weight (165.00±6.48). From group B, there was an insignificant increase in the body weight when comparing the Initial weight (135.00±17.08) to the Final weight (157.50±17.50). For group C, there was an insignificant increase in the body weight when comparing the Initial weight (127.50±12.50) to the Final weight (142.50±2.50). For group D, there was an insignificant decrease in the body weight when comparing the Initial weight (147.50±4.79) to the Final weight (140±14.14).

Table 3.2: Comparative Analysis of Packed Cell Volume (%) between the Experimental Groups of Animals Investigated.

PCV (%)	Mean ± SEM	*P-value*	*F-value*
Group A	36.50 ± 0.50		
Group B	38.50 ± 0.50	0.0000	100.00
Group C	44.50 ± 0.50	0.0120	
Group D	50.50 ± 0.50	0.0129	

Data were analyzed using One way ANOVA followed by Post HOC Fisher's LSD multiple comparism, and data were considered significant at $P<0.05$. *$P<0.05$ means significant and $P>0.05$ means not significant.

The result of comparative analysis of packed cell volume (PCV) between the various groups of animals is shown in table 4.2 above. The highest packed cell volume was found in Group 4 (50.50 ± 0.50%), followed by Group 1 (46.50 ± 0.50%), and then Group 3 (44.50 ± 0.50%). The least packed cell volume was found in Group 2 (38.50 ± 0.50%). ANOVA result showed that there was statistically significant difference in mean packed cell volume (PCV) between the various experimental groups studied ($F = 100.00$, $p < 0.05$).

Table 3.3: Comparison of Hemoglobin **level (g/dL) between the Experimental Groups Studied.**

Hemoglobin **(g/dL)**	Group	*P-value*	*F-value*
Group A	8.10 ± 7.10		
Group B	12.70 ± 1.05	0.449	1.090
Group C	14.95 ± 0.05	0.451	
Group D	16.75 ± 0.05	0.460	

Data were analyzed using One way ANOVA followed by Post HOC Fisher's LSD multiple comparism, and data were considered significant at $P<0.05$. *$P<0.05$ means significant and $P>0.05$ means not significant.

The result of comparison of hemoglobin level between the various groups of animals is presented in table 4.3 above. As observed in the table, there was a steady increase in hemoglobin level from

Group 1 down to Group 4. The highest level of hemoglobin was seen in Group 4 (16.75 ± 0.05g/dL), followed by Group 3 (14.95 ± 0.05g/dL), and then Group 2 (14.05 ± 1.05g/dL). The least level of hemoglobin was seen in Group 1 (8.10 ± 7.10g/dL). ANOVA result showed that there was no statistically significant difference in mean level of hemoglobin between the various experimental groups ($F = 1.090$, $p > 0.05$).

Table 3.4: Comparative Analysis of Red Blood Cell Count ($x10^6$ cells/µL) between the Various Groups of Animals Studies.

RBC ($x10^6$/µL)	Group	*P-value*	*F-value*
Group 1	5.65 ± 0.05		122.387
Group 2	5.50 ± 0.10	0.000	
Group 3	6.38 ± 0.03	0.008	
Group 4	7.15 ± 0.05	0.013	

Data were analyzed using One way ANOVA followed by Post HOC Fisher's LSD multiple comparism, and data were considered significant at $P<0.05$. *$P<0.05$ means significant and $P>0.05$ means not significant.
The result of comparative analysis of red blood cell count (RBC) between the various experimental groups of animals is presented in table 4.4 above. Red blood cell count was highest in Group 4 (7.15 x 10^6 cells/µL), followed by Group 1 (6.65 x 10^6 cells/µL), and then Group 3 (6.38 x 10^6 cells/µL). The least red blood cell count was seen in Group 2 (5.50 x 10^6 cells/µL). ANOVA result showed statistically significant difference in mean red blood cell count between all the groups of animals ($F = 122.387$, $p < 0.05$).

Table 3.5: Comparative Analysis of White Blood Cell Count (cells/µL) between the Experimental Groups of Animals Studied

WBC (cells/µL)	Group	*P-value*	*F-value*
Group 1	6100 ± 2.50		496700
Group 2	6700 ± 3.00	0.000	
Group 3	9000 ± 2.50	0.018	
Group 4	11200 ± 3.00	0.024	

Data were analyzed using One way ANOVA followed by Post HOC Fisher's LSD multiple comparison, and data were considered significant at $P<0.05$. *$P<0.05$ means significant and $P>0.05$ means not significant.
In table 4.5 above, the result of comparative analysis of white blood cell count (WBC) between the four experimental groups studied is shown. As observed in the table, white blood cell count increased from Group 2 down to Group 3. The highest count of white blood cell was seen in Group 4 (11200 ± 3.00 cells/µL), followed by Group 1 (9500 ± 2.50 cells/µL), and then Group 3 (9000 ±

2.50 cells/µL). The least count of red blood cell was seen in Group 2 (6700 ± 3.00 cells/µL). ANOVA result showed statistically significant difference in mean white blood cell count between the four groups of animals studied (F = 496700, p < 0.05).

Table 3.6: Comparison of Relative Weight of the Lung between the Various Groups of Animals Investigated.

Relative Lung Weight	Mean ± SEM	***P-value***	***F-value***
Group 1	0.0113 ± 0.00275		
Group 2	0.0119 ± 0.00100	0.211	2.348
Group 3	0.0135 ± 0.00017	0.214	
Group 4	0.0164 ± 0.00065	0.238	

Data were analyzed using One way ANOVA followed by Post HOC Fisher's LSD multiple comparism, and data were considered significant at P<0.05. *P<0.05 means significant and P>0.05 means not significant.

The result of comparative analysis of relative weight of the lung between the four groups of animals investigated is presented in table 4.7 above. The mean and standard error of mean (SEM) relative weight of the lung for each of the groups is as follows: Group 1 (0.0113 ± 0.00275), Group 2 (0.0122 ± 0.00015), Group 3 (0.0164 ± 0.00065), and Group 4 (0.0119 ± 0.00100). Statistically significant difference was not found in mean relative weight of the lung between the four groups of animals investigated (F = 2.348, p > 0.05).

HISTOPATHOLOGICAL FINDINGS

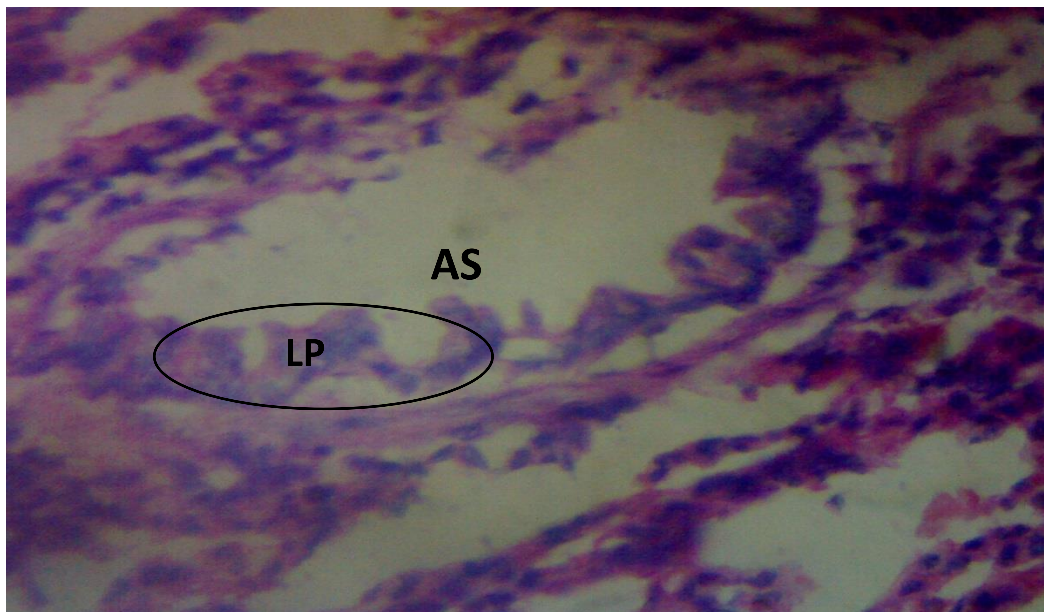

PLATE I (CONTROL). A Photomicrograph section of the Lungs showing the Lung Air Space (AS), Lung Parachenyma (LP) and Inter-alveolar septa are intact. The architecture of the lung is well preserved. Stained by H & E (X400).

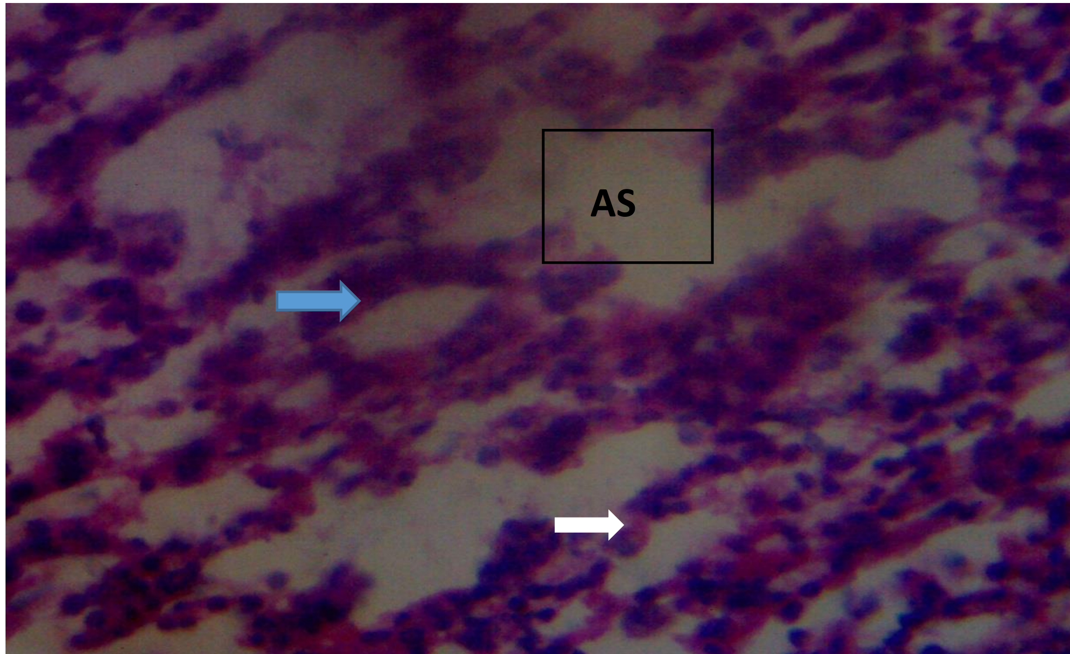

PLATE II (GROUP B) A Section of the Photomicrograph showing the Lung tissue, with vacuolation of the air space (AS), Thickened Interalveolar septum of the lungs (IS) indicated by blue arrow and mild hemorrhage (H) (white arrow). Stained by H & E (X 400).

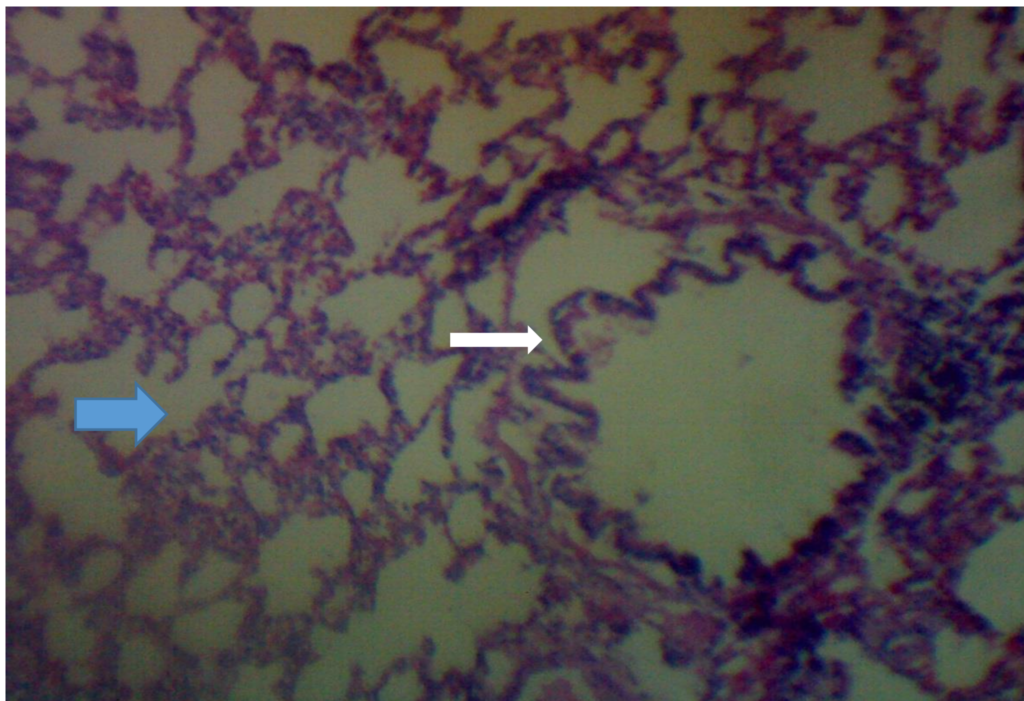

PLATE III (GROUP C) A Photomicrograph section of the lungs showing mild Inflammation of the lungs (MI) indicated by (blue arrow), with thin Alveolar septum (white arrow). Stained by H & E (X 400).

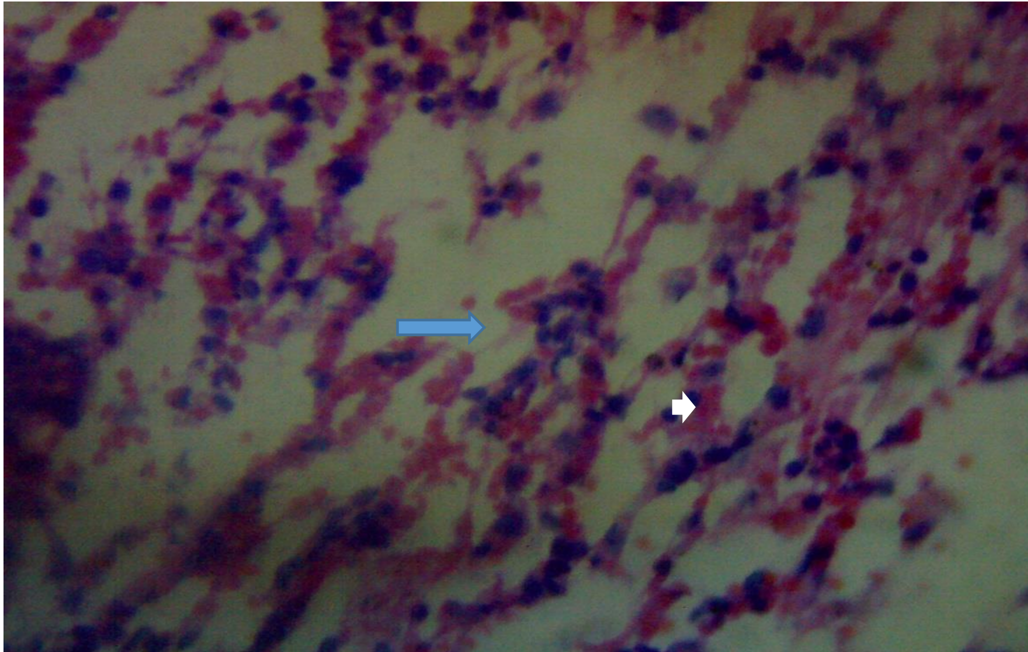

PLATE IV (GROUP D) A Section of the Photomicrograph cell of the lungs showing severe hemorrhage (white arrow) severe inflammation of the lungs air space (blue arrow). Stained by H & E (X400).

DISCUSSION

Mosquitoes are a major concern to human health because they serve as vectors to pass agents that cause diseases, such as malaria and the Zika virus etc which can have a multitude of harmful side effects, such as seizures, conjunctivitis, and death. The overuse of commercial mosquitocidal agents have caused mosquitoes to develop resistance. To prevent mosquitoes from biting humans, many methods are used to kill or repel mosquitoes. The major methods for killing mosquitoes are by using ovicides (for eggs), larvicides (for larvae), pupacides (for pupae), or mosquitocides (for adults). Repellents are used to repel the mosquitoes. However, these mosquitocides and repellents pose major health concerns to people as they have been found to be carcinogenic or toxic and negatively impacts the environment [11]. One of these harmful adulticides is permethrin. Permethrin is part of the pyrethroid family and adversely affects insects' nervous systems. It is more toxic to insects than to humans because insects can't break it down quickly, but it is still harmful to humans.

The present study which was designed to mimic the local and everyday use of this insecticide in residential areas using rats as models demonstra[illegible] potential health implications of mosquito coil smoke exposure. The physical and behavioral result revealed sneezing that resulted at the

initial stage of exposure. This could be as a result of irritants released in the coil smoke such as aldehydes, sulphates, and polycyclic aromatic hydrocarbons such as acenaphthene, penathrene, benzo(a)pyrene.
On the body weight, it was observed that the final body weight was higher than initial but was not statistically significant in all the experimental groups. Hence this agrees with other works of the same nature [12, 13]. The increase in body weight is probable the result of normal physiological growth because they are well fed during the course of the study and are within the growth range by weight for rats.
This study observed the comparable values of RBC, PCV, WBC and Hb concentration and related indices in pyrethroid-exposed and control rats. The significant increase in RBC and PCV agrees with other works of the same nature [14, 15]. This increase may be due to cyanide which is a by-product of mosquito coil smoke which is known to cause reduction in oxygen carrying capacity of RBC. The reduction of oxygen stimulates erythropoietin which in turn stimulates the bone marrow to produce RBC, however it contradicts the work of Saka *et al.,* [16] which demonstrated no significant increase in haematological parameters (WBC, RBC and PLT) when they exposed rats for one to three minutes; Hence the present study exposed the rats for 3 hours daily mimicking daily exposure of man to mosquito coil in normal setting. However marked increase in WBC and insignificant difference in Hemoglobin concentration recorded in rats exposed to the coil in this study confirm observation of Garba *et al.,* [14].
The lung tissue of rats exposed to mosquito coil smoke for 1 hour and 2 hours revealed acute and mild hemorrhage respectively when compared with the control group. The lung tissue of the rats exposed to the longest period (3 hours) revealed severe hemorrhage and severe inflammation when compared with the control. This inflammation could have been the result of irritation of various organs caused by toxic chemicals from smoke. Other pathological manifestation that has been associated with parathyroid mosquito coil but not observed in this study include exudative pneumonia, anthracosis, thrombosis and vasculitis, as observed by [17].
In recent times, natural extracts are being extensively studied as viable alternatives to the synthetic chemicals due to its rapid biodegradability and less hazardous to humans and non-targeted organisms [18]. Most are essential oils derived from different plant parts and unlike many chemical insecticides, consists of a mixture of bioactive compounds with multiple modes of action. Some of these alternative natural extracts are well studied by [19, 20].

CONCLUSION

The findings of this study have shown that exposure to mosquito coil do pose toxicity on lungs as well as the reviewed haematological variables.

DECLARATIONS

Competing interest

The authors declare that there is no conflict of interest.

Ethical Approval

Due ethical approval was obtained from the research ethics committee of the Faculty of Basic Medical Sciences, Nnamdi Azikiwe University.

Authors' contributions

Anyabolu A. E. designed the study and wrote the protocol.

Ezejindu D. N. managed the literature searches and wrote the first draft.
Obinwa B. N. handled the analyses of data, edited and wrote the final draft.
All authors participated in the experiment and tissue processing and also read and approved the final draft.

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