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INVESTIGATION OF SELECTED INFLAMMATORY BIOMARKERS AND LUNGS HISTOMORPHOLOGY IN *Rattus norvegicus* (Norway rats) EXPOSED TO MOSQUITO COIL SMOKE

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Abstract: Mosquitoes are vectors that are responsible for malaria, thus many families have indulged in using mosquito coil in order to control its spread. This study aimed on investigation of selected inflammatory biomarkers and lungs histomorphology of *Rattus norvegicus* (Norway rats) exposed to Kill-Fast mosquito coil smoke. One milliliter of blood specimen was withdrawn from five groups of four male rats per group with mean weight 0.330 ± 0.05 kg exposed to approximately15 mg concentration of Kill-Fast mosquito coil smoke for 30, 60, 90 and 120 days (experimental groups) respectively with the exception of the control group. This was followed by the measurement of inflammatory biomarkers: C-reactive protein (latex turbidimetry) method and interleukin-6 (ELISA) method as well as lungs histomorphology assessment of the rats after anaesthesizing with chloroform technique and excised. The data were analyzed using SPSS version 23.0 (SPSS Inc., Chicago, IL, USA; Version 23.0) and analysis of variance (ANOVA) was used for the statistical analysis. The results revealed significant mean values elevation of C-reactive protein ($p<0.01$) and interleukin-6 ($p<0.01$) in the rats exposed to the smoke of this coil for 30, 60, 90 and 120 days respectively as compared to control. These significant mean values elevations corroborated with the lungs histomorphological assessment of the *Rattus norvegicus* (Norway rats). In conclusion, inflammatory disorder as well as histomorphological alterations of the lungs, were established in the experimental *Rattus norvegicus* (Norway rats). It is therefore recommended that the biochemical aspect of this study should be carried out in users of Kill-Fast mosquito coil so as to ascertain if the same findings will be obtained

Keywords: Investigation, Inflammatory biomarkers, lungs histomorphology, *Rattus norvegicus,* mosquito coil

1. Introduction

Mosquitoes are tiny flies made up of about 3,600 species which are primarily vectors of human and animal illnesses such as West Nile virus, malaria, and dengue [1]. This has influenced the usage of various techniques by many individuals to curb the spread of these diseases, among such techniques are: the use of aerosols, mosquito coils, liquid vapourizers, and vapourizing. However, among these various techniques, mosquito coils are mostly used [2].

This coil which gives rise to many pollutants with their respective effects on human when burnt was invented in the 1890s by a Japanese business man named Eilchiro Ueyama. However, the main pollutants that are of health concerns are:

(i) Particulate matter which is a complex combination of very minute particles and liquid droplets which has the ability to easily move deeper into the lungs as well as bloodstreams unfiltered, thus associated with increased mortality. Due to their tiny size, particles of 10 micrometres or fewer may enter the deepest parts of the lungs such as the bronchioles and alveoli, thus causing cough and bronchitis. Once these compounds enter the lungs, they stay there for months and cause chemical changes as well as structural damage which in turn lead to decrease in the lungs function, allergy and lungs inflammation [3] as shown in Figures 1, lung cancer as shown in Figure 2 and 3 respectively

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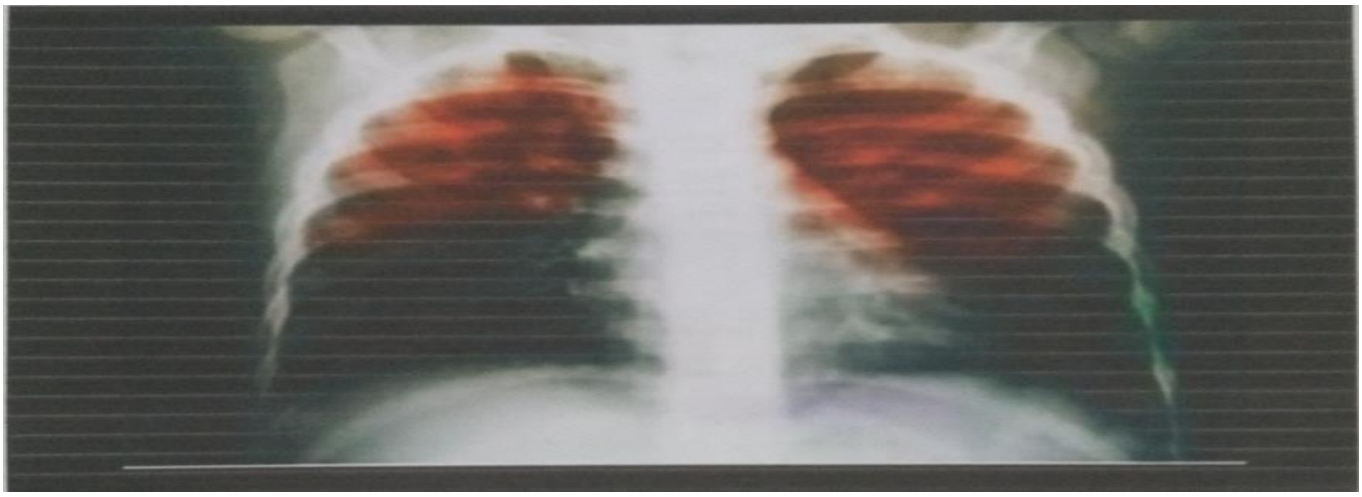

Figure 1: Structure of an inflamed lung (Source: https://googleweblight.com)

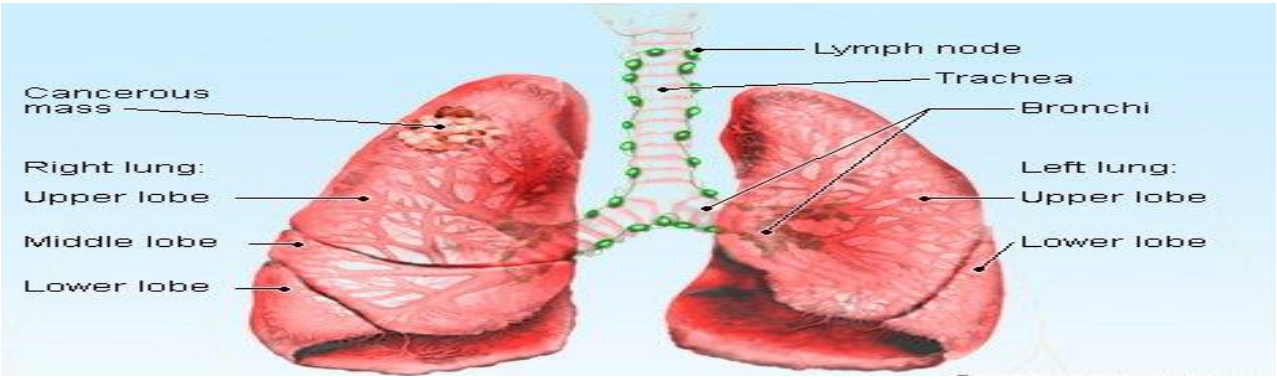

Figure 2: Structure of a lung showing a cancerous mass (Source: https://googleweblight.com)

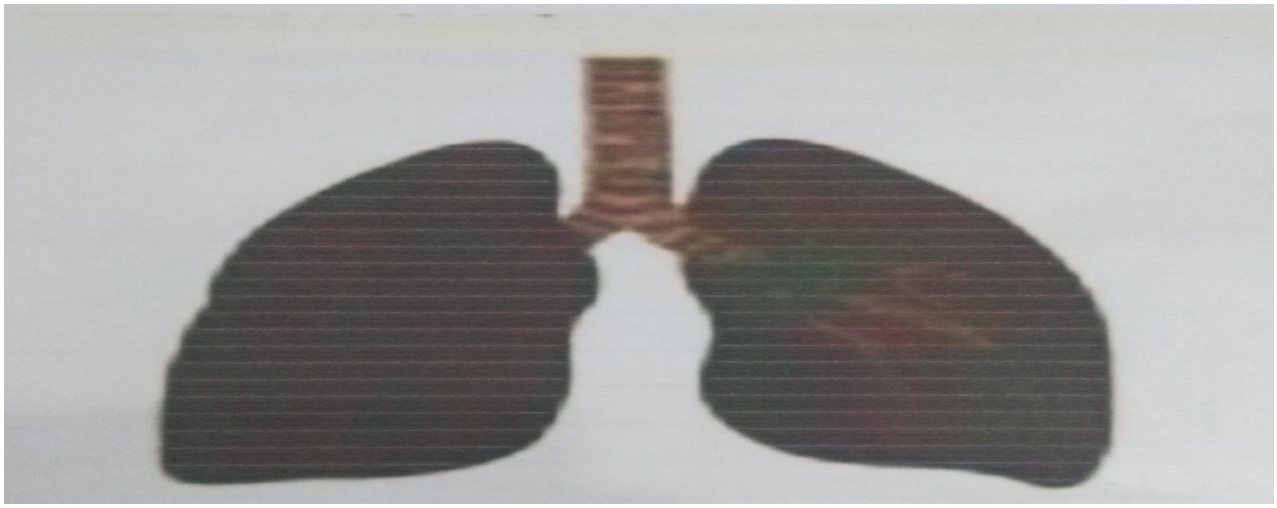

Figure 3: Structure of a lung cancer (Source: https://googleweblight.com)

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(ii) Carbon monoxide, which is a toxic gas that has no colour or smell. It prevents vital organs like the heart and brain from receiving enough oxygen from the bloodstream, which in turn reduces their capacity to function properly. When carbon monoxide reacts with haemoglobin in the blood, carboxyhaemoglobin is formed. This toxic gas can cause brain oxygen starvation and death if it is inhaled for an extended period of time [4]. Besides, it affects a wide range of systems such as the respiratory, cardiovascular, and neurological systems. The heart has to work harder to pump what it wrongly interprets as oxygenated blood from the lungs to the rest of the body when carbon monoxide levels increase. As a result of the ensuing swelling of the airways, the lungs get even less air. Long-term exposure to irritants damages the lungs, causing cardiovascular problems and lung disease [5].

(iii) Formaldehyde, few investigations have indicated that mosquito coil smoke includes carbonyl compounds, such as the gaseous, odourless, combustible, and toxic chemical formaldehyde. Burning one mosquito coil has been reported to release particulate matter as well as emission of formaldehyde that is equivalent to burning a significant number of cigarettes [6].

(iv) Polycyclic aromatic hydrocarbons, one of the polycyclic aromatic hydrocarbons (PAHs) emitted when mosquito coils are burned is benzopyrene. This chemical, which is created when a benzene ring fuses with pyrene, is hazardous to human health because it may cause cancer. Animal studies have connected exposure to this substance to cardiovascular disorders, poor foetal development, lung disease, skin disease, bladder disease, liver disease and stomach malignancies [7].

Mosquito coils are insect repellents/insecticides which burn slowly for as long as 8 hours or more with the subsequent emission of smoke that protects humans against mosquitoes. This in turn prevents malaria which is a public health problem reported to be responsible for the main cause of death in many countries within the tropical region, especially in sub-Saharan Africa. In 2016, 445, 000 deaths were accounted for globally due to malaria, out of which 90% occurred in African region [8] (WHO, 2017). The scourge of this endemic parasitic infection accounts for 60% of outpatients visit to clinics in Nigeria. This situation has made many people in communities that are of low income to embark on the routine and chronic use of mosquito coils in order to reduce the population of mosquitoes despite the fact that it is not one of the preventive measures recommended by World Health Organization (WHO) to curb mosquitoes [9].

However, only a limited number of scientists have ascertained the potential dangers of inhaling the smoke produced by burning mosquito coils. They found that small particles (1 µm), metal fumes, and vapour in the smoke can reach the alveolar region of the lungs, where they can cause respiratory symptoms like coughing and sneezing, tracheal inflammation, renal damage, and weight loss [10].

Toxic heavy metals, particulates, carbon monoxide and other pollutants from mosquito coils have been linked to a wide range of health issues in earlier research such as respiratory disorder, liver disorder, lung cancer, cardiovascular disorder, renal disorder, etc [11]. Nonetheless, several researches have stated that the toxicity of mosquito coils and their impact on human health are still unknown [12]. In addition, [13] found that nighttime exposure to smoke created by a burning mosquito coil made in Indonesia is probably not connected with health hazards. Some researchers have indicated that the use of mosquito coils is safe for both people and animals, while others have expressed worry over their use in enclosed spaces [14]. However, both studies determined that mosquito coils provide less of a risk to human health when precautions are taken during its use.

Despite all of these results from earlier research about the health risks of inhaled smoke from mosquito coils, in addition to its increased usage globally, coupled with the paucity of literature on this research title, it is essential to conduct this study which is aimed on a study on selected inflammatory biomarkers and lungs histomorphology in *Rattus norvegicus* (Norway rats) exposed to mosquito coil smoke in order to assess inflammatory disorder that may be linked to the lungs which are the primary target organs of smoke. This study which is considered extremely relevant is with a view that the findings would help increase knowledge as well as proffer suitable guidelines for the proper use of mosquito coil.

2. Materials and Methods

2.1 Study Area

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This study was conducted in the Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

2.2 Ethical Approval

This study which was carried out in accordance with the National Guidelines for Animal Usage in Research got ethical permission from the College Health Research Ethics Committee, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

2.3 Mosquito Coil Used

The mosquito coil used for this study is Kill-Fast mosquito coil and was purchased at Favour Supermarket Swali market, Bayelsa State, Nigeria

2.4 Experimental Design

This study involved the use of animal model only.

2.4.1 Selection Criteria

2.4.2 Inclusion and Exclusion Criteria

The *Rattus norvegicus* (Norway rats) used for this study were within the age range of 5-7 months. These rats which were all males were apparently healthy with a weight range of 0.330 ± 0.05 kg. Those that were suspected of having ill health were excluded.

2.4.3 Animal Study

The animal study consisted of forty-one (41) male *Rattus norvegicus* (Norway rats) of 5-7 months old and approximately having a weight range of 0.330 ± 0.05 kg. These rats were purchased from the Pharmacology Department at Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria and taken to the Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria where they were acclimatised for two weeks in well ventilated iron standard rat cages and fed with pre-mix rat feed and water *ad-libitum* 3 times daily prior to the commencement of the study.

2.4.4 Pilot Study

A pilot study was conducted in order to find the minimum concentration of smoke generated from Kill-Fast mosquito coil that would cause 100% (LC_{100}) death in the experimental male *Rattus norvegicus* (Norway rats). A total of 16 rats of 5-7 months old weighing approximately 0.330 ± 0.05 kg were used for this study. These rats were grouped into four, with four rats/group and were designated as A, B, C and D respectively. The rats in group A were exposed to approximately 30 mg / 8 hour of smoke generated from Kill Fast mosquito coil, while those in groups B, C and D were exposed to approximately 45 mg /8 hour, approximately 60 mg / 8 hour and approximately 75mg/8 hour of smoke generated from Kill Fast mosquito coil respectively. Thereafter the rats were monitored for 24 hours for any signs or symptoms of smoke toxicity including death. The rats were considered dead when they fail to respond to agitation. The following signs and symptoms such as convulsion (+), respiratory distress (++), coma (+++) and death (+++) occurred in all the rats in group C (LC_{100}).

2.5 Sample Size Determination

The Resource Equation approach in accordance to [15] was used to estimate the sample size for this study.

This approach measures a parameter called E, which represents the degree of freedom of an analysis of variance (ANOVA). The degree of freedom (E) value should range from 10 to 20. Additional animals will need to be added if the degree of freedom (E) is less than 10, but if it is greater than 20, adding more animals will reduce the likelihood of producing meaningful findings. Any sample size that maintains the degree of freedom (E) in the range of 10 to 20 should be regarded as sufficient. The following formula is used to calculate the degree of freedom (E):

Degree of freedom (E) = Total number of animals – Total number of groups

Sample size = 25 – 5 = 20

2.6 Sample Collection and Processing

One milliliter of blood specimen was collected from the cardiac of each *Rattus norvegicus* (Norway rats) into lithium heparin anti-coagulated bottles. The specimens were mixed homogenously in order to prevent clotting and spun at 2,500 rpm for 10 minutes with the aid of a Gulfex macro-centrifuge 800D.

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The obtained plasma was used for the quantitative measurement of C-reactive protein and interleukin-6 using the specific methods as shown below:

2.7 Measurement of Plasma C- reactive Protein Level

This was measured in accordance with the latex turbidimetry method as described by Spin-react Diagnostic manual, Spain and modified by [16].

2.8 Measurement of Plasma Interleukin-6 Level

This was measured in accordance with the description of ELISA method with catalogue number E.EE-HO.102 as modified by [17].

2.9 Statistical Analysis

SPSS version 23.0 (SPSS Inc., Chicago, IL, USA; Version 23.0) was used for all statistical analysis. The data were grouped into unexposed (control group), 30 days, 60 days, 90 days and 120 days exposed (experimental group). The Kolmogorv-Smirnov Z was used to study the differences in the various parameters between the tests and control group. Student "t" test and ANOVA were used for comparing values of the measured biochemical parameters between the control and experimental groups. Data were expressed as means and standard deviation. All post hoc testing were done using Turkey HSD and Games-Howell methods as applicable. Differences in means between groups were considered significant at $p < 0.05$.

3. Results

3.1 Determination of LC_{50} in *Rattus norvegicus* (Norway rats) exposed via whole-body inhalation to Kill-Fast mosquito coil smoke

A total of 16 male *Rattus norvegicus* (Norway rats) of 5-7 months old weighing 0.330 ± 0.05 kg were used for this study. These rats were randomly divided into four groups with four rats per group designated as A, B, C and D and were subsequently exposed via whole body to inhalation of approximately 15 mg / 0.330 ± 0.05 kg, approximately 30 mg / 0.330 ± 0.05 kg, approximately 45 mg / 0.330 ± 0.05 kg and approximately 60 mg / 0.330 ± 0.05 kg concentrations of smoke generated from Kill-Fast mosquito coil in a room measuring 8 feet by 8 feet for a period of 8 hours for one day respectively.

After the above exposure, the rats were monitored for a period of 24 hours for any signs and symptoms as well as death that may result from the toxicity of the smoke generated from the Kill-Fast mosquito coil. The rats were considered dead upon failure to agitation response. The following signs and symptoms such as convulsion (+), respiratory distress (++), coma (+++) and death (+++) occurred in 50% (LC_{50}) of the *Rattus norvegicus* (Norway rats) in group C that were exposed to 45mg/0.330 ± 0.05kg concentration of smoke generated from Kill-Fast mosquito coil on a single day.

The LC_{50} was calculated using the method described by [18] as shown:

Calculation:

$$LD50 = \frac{(Do+Dn)}{2}$$

Do = Dose at which no death occurred

Dn = Dose at which 50% death occurred

$$\frac{15\text{ mg} + 45\text{ mg}}{2}$$

$$= \frac{60\text{ mg}}{2}$$

$$= 30\text{ mg}$$

3.2 Sub-Chronic Toxicity Study

In this study the experimental *Rattus norvegicus* (Norway rats) were randomly grouped into four. Each group consisted of four male *Rattus norvegicus* (Norway rats) within the age range of 5-7 months old, weighing 0.330 ± 0.05 kg. These groups were designated as experimental groups 1, 2, 3 and 4 with the exception of the control group as shown:

3.2.1 Control Group

This group comprised of four male *Rattus norvegicus* (Norway rats) that were not exposed to any quantities of smoke from Kill-Fast mosquito coil or any other source via whole-body inhalation or any other pattern of exposure.

3.2.2 Experimental Group One

This group consisted of four male *Rattus norvegicus* (Norway rats) that were subjected to approximately 15 mg concentration of smoke from a Kill-Fast mosquito coil by whole-body inhalation for eight hours per day for 30 days.

3.2.3 Experimental Group Two

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This group consisted of four male *Rattus norvegicus* (Norway rats) that were subjected to approximately 15 mg concentration of smoke from a Kill-Fast mosquito coil by whole-body inhalation for eight hours per day for 60 days.

3.2.4 Experimental Group Three

This group consisted of four male *Rattus norvegicus* (Norway rats) that were subjected to approximately 15 mg concentration of smoke from a Kill-Fast mosquito coil by whole-body inhalation for eight hours per day for 90 days.

3.2.5 Experimental Group Four

This group consisted of four male *Rattus norvegicus* (Norway rats) exposed via whole body to inhalation of approximately 15 mg concentration of smoke generated from Kill-Fast mosquito coil for a duration of eight hours daily for a period of 120 days.

At the conclusion of this experiment, the rats were slaughtered after being anaesthetized with chloroform. Thereafter the blood sample from each rat was collected through cardiac puncture for the biochemical evaluation of C-reactive protein and interleukin-6, while the lungs were removed and preserved in 10% formalin for histomorphological investigation.

3.3 Measurement of Kill Fast Mosquito Coil Smoke

Leonardo da Vinci extensively discussed the challenge of evaluating smoke as early as the 15th century. He went on to differentiate between white smoke and black smoke (carbonised particles) [19]. In this work, the In-line capture method was used to assess the smoke produced by one Ride-On mosquito coil as described by [20].

Analytical balance (OHAUS) model CP213 S/N B143092087, OHAUS Corporation, No. 471, Guiping Road, Shanghai, China, was used to weigh a Whatman filter paper 1 (110mm), catalogue number 1001-1110, both before and after it was exposed to smoke from a Kill-Fast mosquito coil. The difference between the two measurements, which is equivalent to the smoke's mass, was estimated as illustrated:

Calculation:

Weight of filter paper before exposure to smoke = 0.842 mg

Weight of filter paper after exposure to smoke = 15.732 mg

Difference: 15.732 mg - 0.842 mg = 14.890 mg

Approximately 15 mg

The results of inflammatory biomarkers: C-reactive protein and interleukin-6 in plasma of *Rattus norvegicus* (Norway rats) exposed to approximately15 mg concentration of Kill-Fast mosquito coil smoke for 30 days (experimental group one), 60 days (experimental group two), 90 days (experimental group three) and 120 days (experimental group four) as compared with that of the *Rattus norvegicus* (Norway rats) not exposed to Kill-Fast mosquito coil smoke or any other source of smoke (control group) are as shown in Table 1.

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Table 1. Results of mean values of inflammatory biomarkers in *Rattus norvegicus* (Norway rats) exposed to Kill-Fast mosquito coil (experimental groups) compared with the non-exposed (control group)

Group	C-reactive protein (mg/L)	Interleukin-6 (pg/ml)
Control (n=4)	2.34 ±0.54$^{\alpha}$	0.14 ± 0.06$^{\gamma}$
30 days (n=4)	7.80 ± 1.30$^{\gamma}$	6.60 ± 1.14$^{\beta}$
60 days (n=4)	13.00 ± 4.30$^{\beta}$	11.00 ± 2.00$^{\delta}$
90 days (n=4)	25.20 ± 3.11$^{\delta}$	24.00 ± 4.59$^{\varepsilon}$
120 days (n=4)	32.00 ± 3.16$^{\varepsilon}$	35.00 ± 4.83$^{\alpha}$
F-value	96.85	93.92
P-value	0.00**	0.00**

KEY: n= Number of rats

Significant difference observed, $p < .01$. ANOVA was used to test differences across groups. All post hoc testing were done using Turkey HSD or Games-Howell methods, where applicable.

C-reactive protein: The results showed $^{\alpha}$ significant differences in the mean values of C-reactive protein between control and each of 30, 90, and 120 days exposed groups, p=.00, respectively.

$^{\beta}$ Significant difference was observed between 60 days exposed group and control, $p = .02$.

$^{\gamma}$ Significant differences were observed between 30 days exposed group and each of 90 and 120 days exposed groups, $p = .00$, respectively.

$^{\delta}$ Significant difference was observed between 90 and 60 days exposed groups, $p = .01$.

$^{\varepsilon}$ Significant difference was observed between 120 and 60 days exposed groups, $p = .00$.

Interleukin-6: The results showed $^{\gamma}$ significant differences in the mean values of interleukin-6 between control and each of 30, 90, and 120 days exposed groups, p=.00, respectively.

$^{\delta}$ Significant difference was observed between 60 days exposed group and control, $p = .02$.

$^{\beta}$ Significant differences were observed between 30 days exposed group and each of 90 and 120 days exposed groups, $p = .00$, respectively.

$^{\varepsilon}$ Significant difference was observed between 90 and 60 days exposed groups, $p = .01$.

$^{\alpha}$ Significant difference was observed between 120 and 60 days exposed groups, $p = .00$.

Histomorphological assessment of lung tissue exposed to Kill-Fast mosquito coil

The histomorphology of the excised lungs from both the control and experimental *Rattus norvegicus* were further studied. Before the commencement of this histomorphology study, the organs were respectively placed in labeled bottles containing 10% formal saline fixative and subsequently sliced and dehydrated with different concentrations of alcohol: 50%, 70%, 80%, 95% and 100%. After which they were cleared of the dehydrating agent with xylene and embedded in molten paraffin wax with the resulting tissue blocks trimmed and sectioned in accordance with the method as described by [21] at a thickness of 4mm. The tissue slides were then stained using Haematoxylin and Eosin staining technique and carefully examined for histomorphological lesion with the aid of Olympus binocular light research microscope (XSZ-107BN, No 071771). Photomicrographs were then taken using a Kodak Digital Camera (Kodak Easyshare C183) for subsequent histomorphological examination. This aspect of the study which is pertinent was embarked on in order to ascertain the true picture of the lungs' histomorphology whether it would align with the biochemical findings following the chronic exposure to smoke generated Kill Fast mosquito coil.

The histomorphological revelations on lungs excised from the *Rattus norvegicus* (Norway rats) exposed to approximately 15 mg concentration of smoke generated from Kill-Fast mosquito coil on daily basis for a period of 30 days (experimental group one), 60 days

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(experimental group two), 90 days (experimental group three) and 120 days (experimental group four) as compared with that of the *Rattus norvegicus* (Norway rats) not exposed to smoke generated from Kill-Fast mosquito coil or any other source of smoke (control group) are shown in plates 1-4 respectively.

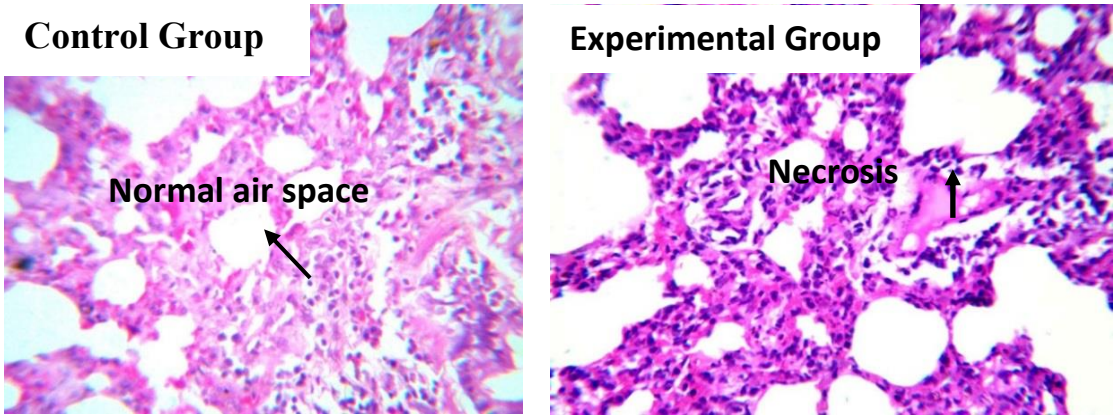

PLATE 1: Photomicrographs of lung tissue stained with haematoxylin and eosin x 40 magnification
Necrosis as indicated by the arrow was observed in the lung tissue of the experimental group of the *Rattus norvegicus* (Norway rats) exposed to approximately 15 mg concentration of Kill-Fast mosquito coil smoke for a period of 30 days as compared to that of the control group which showed a normal status. This histomorphological finding is in conformity with the significant elevation of the measured inflammatory biomarker: C-reactive protein and interleukin-6

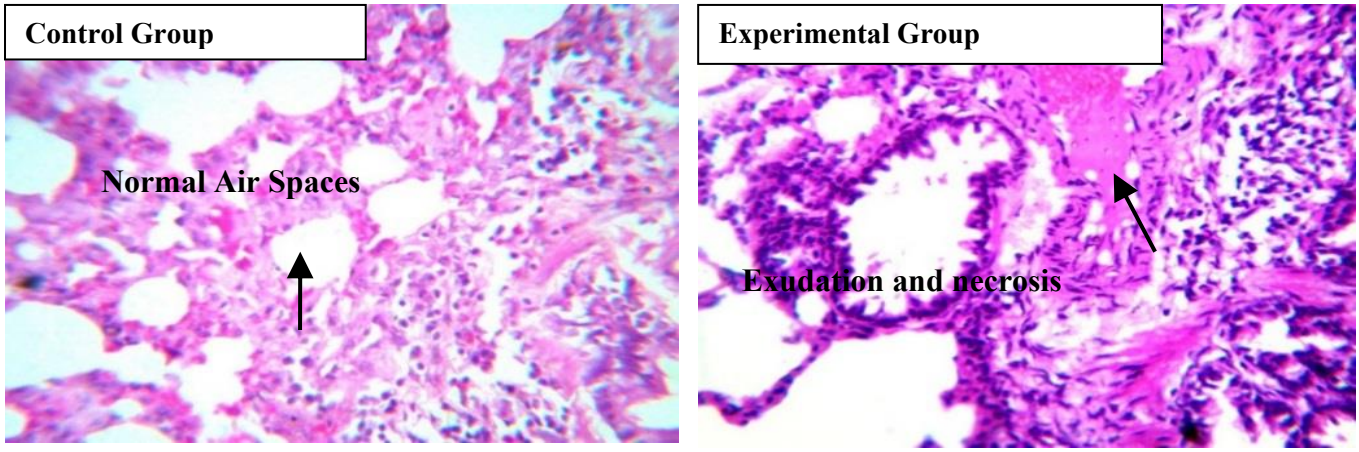

PLATE 2: Photomicrographs of lung tissue stained with haematoxylin and eosin x 40 magnification
Exudation and necrosis as indicated by the arrow were observed in the lung tissue of the experimental group of the *Rattus norvegicus* (Norway rats) exposed to approximately 15 mg concentration of Kill-Fast mosquito coil smoke for a period of 60 days as compared to that of the control group which showed a normal status. This histomorphological finding is in conformity with the significant elevation of the measured inflammatory biomarker: C-reactive protein and interleukin-6

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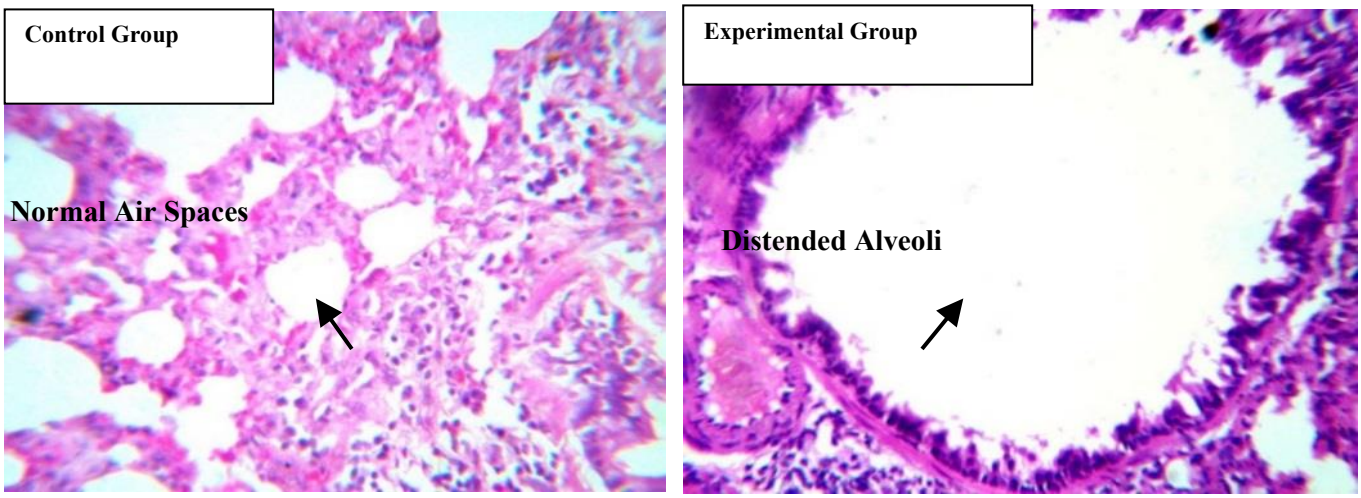

PLATE 3: Photomicrographs of lung tissue stained with haematoxylin and eosin x 40 magnification
Distended alveoli of the lung tissue as indicated by the arrow was observed in the experimental group of the *Rattus norvegicus* (Norway rats) exposed to approximately 15 mg concentration of Kill-Fast mosquito coil smoke for a period of 90 days as compared to that of the control group which showed a normal status. This histomorphological finding is in conformity with the significant elevation of the measured inflammatory biomarker: C-reactive protein and interleukin-6

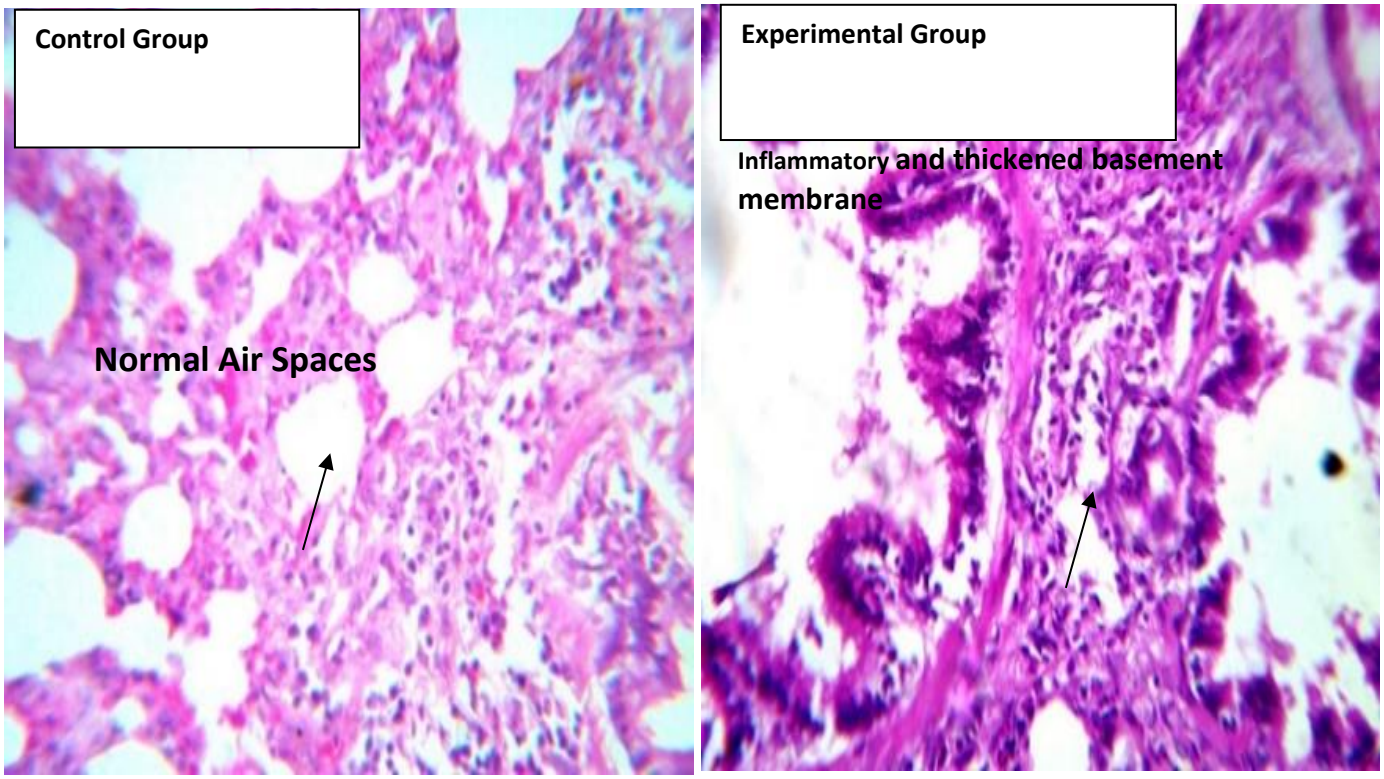

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PLATE 4: Photomicrographs of lung tissue stained with haematoxylin and eosin x 40 magnification
Inflammatory and thickened basement membrane as indicated by the arrow was observed in the lung tissue of the experimental group of the Rattus norvegicus (Norway rats) exposed to approximately 15 mg concentration of Kill-Fast mosquito coil smoke for a period of 120 days as compared to that of the control group which showed a normal status. This histomorphological finding is in conformity with the significant elevation of the measured inflammatory biomarker: C-reactive protein and interleukin-6

4. DISCUSSION

In underdeveloped nations, malaria is recognised as a public health problem that is responsible for an increased number of infections and deaths. Humans have determined to employ various control technologies (aerosols, mosquito coils, mosquito nets, etc.) that prevent mosquito bites in an effort to lessen or prevent malaria [22].
When mosquito coils are burned, they emit smoke that includes several harmful compounds, but relatively few research have been conducted to explore these toxins [23]. There is a need for more research in this area in order to determine what chemicals manufacturers put in mosquito coils that may contribute to health and environmental dangers when burned. This is due to the fact that only *d-cis-transallethrin* at a normal concentration of 0.25% is labelled as the active component, while the additional chemicals are often categorised as "others." Nonetheless, [24] and [25] found that the particulate matter of mosquito coil contains both heavy metals and organic compounds of concern. Even at low quantities, heavy metals are harmful to humans due to their relative density [26].
The lungs are the principal organs of the respiratory system in humans, with the purpose of transferring oxygen from the environment into the bloodstream and releasing carbon dioxide from the circulation into the atmosphere via a process of gas exchange [27].
In this study, C-reactive protein and interleukin-6 biomarkers were measured to determine the inflammatory response of the lungs in *Rattus norvegicus* (Norway rats) exposed to approximately 15 mg concentration of Kill-Fast mosquito coil smoke for 30, 60, 90, and 120 days as shown in Table 1
The mean values of plasma C-reactive protein in the exposed *Rattus norvegicus* (Norway rats) for periods of 30 days versus the control *Rattus norvegicus* (Norway rats), 60 days exposed *Rattus norvegicus* (Norway rats) versus the control *Rattus norvegicus* (Norway rats), 90 days exposed *Rattus norvegicus* (Norway rats) versus the control *Rattus norvegicus* (Norway rats) and 120 days exposed *Rattus norvegicus* (Norway rats) versus the control *Rattus norvegicus* (Norway rats) were significantly elevated ($p<0.01$) as shown in Table 1.
As shown further in Table 1, the mean values of plasma interleukin-6 in the exposed *Rattus norvegicus* (Norway rats) for periods of 30 days versus the control *Rattus norvegicus* (Norway rats), 60 days exposed *Rattus norvegicus* (Norway rats) versus the control *Rattus norvegicus* (Norway rats), 90 days exposed *Rattus norvegicus* (Norway rats) versus the control *Rattus norvegicus* (Norway rats) and 120 days exposed *Rattus norvegicus* (Norway rats) versus the control *Rattus norvegicus* (Norway rats) were significantly elevated ($p<0.01$)
These biochemical findings, which are consistent with the prior work of [28] for C-reactive protein and [29] for interleukin-6, may be suggestive of an inflammatory response of the lungs following inhalation of toxic chemicals such as carbon monoxide, cadmium, lead, etc. generated from the burnt Kill-Fast mosquito coil, which may have triggered the increase synthesis/release of these inflammatory biomarkers.
These biochemical results are consistent with the histomorphological investigation of the lungs which indicated necrosis in the rats exposed for 30 days, as contrasted to the control rats, whose lungs exhibited normal tissue condition, as seen in Plate 1 while plate 2 depicts exudation and necrosis in the lungs of the 60 days exposed rats in comparison to the normal tissue state of the control rats, plate 3 displayed enlarged alveoli in the lungs of the exposed rats for 90 days, as compared to the normal tissue state of control rats, plate 4 depicts the inflamed and thickened basement membrane in the lungs of 120 days exposed rats as opposed to the control group, which exhibited normal tissue state. These histomorphological findings are consistent with [30] previous research.

5. Conclusion

In conclusion, this study has revealed the following:

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Prolonged exposure to approximately15 mg concentration of Kill-Fast mosquito coil smoke on daily basis for 30 days, 60 days, 90 days and 120 days respectively triggers elevation of C-reactive protein and interleukin-6 biochemical parameters (inflammatory biomarkers) as seeing in Table 1 as well as inflammatory disorder of the lungs in *Rattus norvegicus* (Norway rats) as seeing in plates 1-4.

It is pertinent to note that this study has gone a step further to unveil the lungs as a susceptible organ to smoke generated from Kill-Fast mosquito coil in *Rattus norvegicus* (Norway rats)

Recommendations

It is therefore recommended as follow:

(i) The inflammatory biochemical markers: C-reactive protein and interleukin-6 assessment should be reproduced in *Rattus norvegicus* with a larger sample size followed by the same assessment in human in a well control manner to ascertain if such findings could be the same

(ii) Usage of mosquito coils should be limited to outdoor or well ventilated indoor areas so as to curtail its adverse effect on humans.

(iii) More awareness campaign and education about the risks and safety use of mosquito coils are needed for both users and healthcare professionals.

(iv) Mosquito coil manufacturers should be enlightened to specify details of the ingredients that are used for the manufacturing of coils which should be accompanied with scientific references.

(v) Only mosquito coils that are certified safe for use by the relevant authorities should be sold in the market.

Conflict of Interest: None

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Contribution to Knowledge

The findings from this present study would contribute to knowledge as follows:

(i) Bridge the major gap in knowledge regarding the use of mosquito coils such as frequency and duration of usage indoors, ventilation rates in homes where they are used as well as the general cleaning habit of its users

(ii) Enlighten the public, healthcare providers and academic world at large on the need to adhere strictly to the necessary precautions, if mosquito coils are to be used in preventing spread of mosquitoes.

(iii) Mosquito coils is a reduced cost method for mitigating malaria and other mosquito borne diseases, it may pose additional health hazards such as inflammatory disorder of the lungs to humans if used indiscriminately taking into consideration the manifestation of this inflammatory disorders in the experimental *Rattus norvegicus* (Norway rats).

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