Biochemical Parameters and Histomorphological Assessments of Hepato-Renal Organs in Norway Rats (*Rattus norvegicus*) Exposed to Mosquito Coil Smoke

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Abstract

Mosquitoes are vectors responsible for malaria thus many families have indulged in using mosquito coil in order to control its spread. This study was aimed on biochemical parameters and histomorphological assessments of hepato-renal organs in Norway rats (Rattus norvegicus) exposed to mosquito coil smoke. Five milliliters of blood specimens were withdrawn from five groups consisting of five male rats per group with mean weight 0.103 ± 0.05 kg exposed to 15 mg concentration of mosquito coil smoke for 30, 60, 90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 alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, creatinine and urea using standard biochemical procedures. The data were analyzed using SPSS version 20.0 and analysis of variance (ANOVA) was used for the statistical analysis. The results revealed significant mean values elevations of alanine aminotransferase (16.00 ± 1.58) IU/L, aspartate aminotransferase (15.20 ± 1.30) IU/L and alkaline phosphatase (37.60 ± 2.30) IU/L in rats exposed for 120 days as compared to control alanine aminotransferase (7.80 ± 1.30) IU/L, aspartate aminotransferase (6.40 ± 1.67) IU/L and alkaline phosphatase (19.00 ± 1.22) IU/L respectively while significant elevation of creatinine (132.00 ± 5.24) µmol/L and urea (13.20 ± 2.77) mmol/L were revealed in rats exposed for 90 days as well as creatinine (148.20 ± 11.67) µmol/L and urea (16.60 ± 2.70) mmol/L for 120 days as compared to control creatinine (62.40 ± 4.77) µmol/L and urea (4.20 ± 1.41) mmol/L respectively. These significant mean values were reflected in the histomorphological assessment of the liver and kidney of the respective rats. In conclusion, this study revealed elevated liver biomarkers in the rats exposed to this mosquito coil smoke for 120 days as well as elevated kidney biomarkers in the rats exposed to smoke generated from this mosquito coil for 90 and 120 days respectively. These significant elevations correlated with the histomorphological alterations of the respective organs. It is therefore suggestive that prolonged usage of coil has adverse effects on hepato- renal organs of Norway rats (Rattus norvegicus)

Keywords: *Biochemical parameters, Histomorphological assessments, Hepato-renal organs, Norway rats, Mosquito coil smoke*

1. INTRODUCTION

Mosquitoes are little flies consisting of around 3,600 species that mostly act as vectors for diseases affecting humans and animals, such as West Nile virus, malaria, and dengue (Ralph, 2008). This has led individuals to use numerous measures to reduce the transmission of these diseases, including aerosols, mosquito coils, liquid vaporisers, and vaporising devices. Mosquito coils are frequently employed among these various methods (MamunaNazsalleh, 2019).

Mosquito coils were invented by Japanese entrepreneur Eilchiro Ueyama in the 1890s. These coils burn slowly for eight or more hours, releasing smoke that repels mosquitoes from biting humans, thus decreasing the prevalence of malaria, a major public health concern and a primary cause of death in many countries globally, especially in Africa and sub-Saharan Africa (WHO, 2017).

In Nigeria, sixty percent of outpatient clinic visits are linked to the prevalence of this endemic parasitic infection, leading many individuals, especially low-income earners, to frequently employ mosquito coils to reduce mosquito populations, thereby seeking to lessen the impact of malaria, despite the fact that this method is not recommended as a preventive measure by the World Health Organisation (WHO) (Anonymous, 2012)

Malaria is primarily treated with pharmaceutical methods, while the Anopheles mosquito vector is controlled with preventive measures such as insecticide-treated nets (ITN) and indoor residual spraying (IRS), as recommended by the World Health Organisation (Hogarh *et al.,* 2016).

Despite previous research indicating the health risks linked to inhaled smoke from mosquito coils, these coils are still widely used worldwide (Shi *et al.,* 2016). For example:

(i) A 1996 study by the World Health Organisation (WHO) projected the yearly worldwide usage of mosquito coils at almost 29 billion units (WHO, 1998).

(ii) In 2006, the worldwide consumer market for mosquito coils was appraised at 1 billion dollars, constituting 12% of the global pesticides market for that year (Ogona *et al.,* 2012).

(iii) According to the Environmental Protection Agency (EPA) (Yang *et al.*, 1997), more than 45 percent of residences in Taiwan employ mosquito coils.

(iv) Between 2006 and 2014, Suriname imported an average of 17 million boxes of mosquito coils annually, reflecting significant consumption in a country with a population of around 500,000 (John *et al.*, 2016).

(v) Approximately 40 percent of Ghana's population employs mosquito coils, with 43 percent of these individuals using them regularly (Hogarh *et al.*, 2016).

(vi) Mosquito coils are predominantly employed in rural areas marked by high incidences of risky behaviours (Chitra *et al.*, 2013).

(vii) In Northern Nigerian areas, mosquito coils are the primary insecticide employed. This region has employed it for several decades owing to its highest documented prevalence of malaria (Sani and Ibrahim, 2016).

If mosquito coils pose a risk, the public should be notified and their usage discouraged; however, if the risks are deemed acceptable, they may enhance existing malaria control measures, especially in disadvantaged areas (Sani and Ibrahim, 2016). Consequently, it is prudent to conduct this study concentrating on the biochemical parameters and histomorphological assessment of hepato-renal organs in Norway rats (*Rattus norvegicus*) exposed to mosquito coil smoke, so as to clarify the detrimental health effects linked to the extended use of mosquito coils

MATERIALS AND METHODS

2.1 Research Area

This study was performed 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

Following the acquisition of ethical approval from the College Health Research Ethics Committee at the College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, the study was executed in accordance with the National Guidelines for Animal Use in Research.

2.3 Experimental Framework

2.4 Animal Research

This study comprised forty-one (41) male *Rattus norvegicus*, aged 3-4 months, with an average weight of 0.103 ± 0.05 kg. The rats were obtained from the Pharmacology Department at Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria, and subsequently transferred to the Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. They were acclimatised for two weeks in well-ventilated iron standard rat cages and given pre-mixed rat feed and water *ad libitum* prior to the commencement of the study

2.5 Pilot Study

A pilot study was done to ascertain the concentration of smoke generated by the J-Kim mosquito coil that achieved 100% fatality (LC_{100}) in the male *Rattus norvegicus* employed in the experiment. This was determined to be 60 mg / 0.103 ± 0.05 kg.

2.6 Sample Size Determination

The Resource Equation method was utilised to ascertain the sample size for this study, adhering to the approach delineated by Wan and Wan (2017). This method quantifies a parameter termed E, which indicates the degree of freedom in an analysis of variance (ANOVA). The degree of freedom (E) must range from 10 to 20. If the degrees of freedom (E) are fewer than 10, additional animals must be added; conversely, if they exceed 20, adding more animals will reduce the likelihood of obtaining meaningful results.

Any sample size that maintains the degrees of freedom (E) within the range of 10 to 20 is deemed sufficient. The following formula is utilised to calculate the degree of freedom (E):

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

The sample size is 25 minus 5, yielding 20.

2.7 Collection and Analysis of Specimen

Five millilitres of blood specimens were collected from the cardiac area of each *Rattus norvegicus* into heparinised anticoagulant bottles. The specimens were uniformly mixed to prevent coagulation and centrifuged at 2500 revolutions per minute for 10 minutes using a Gulfex macro-centrifuge 800 D.

2.8 Measurement of Biochemical Parameters

The obtained plasma was employed for the quantitative evaluation of the following biochemical parameters: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase (liver enzyme biomarkers), creatinine, and urea (renal biomarkers), utilising the specific methodologies detailed below:

2.8.1 Aspartate Aminotransferase

The measurement was performed with the colorimetric method specified by Randox Laboratories, Limited, situated at 55 Diamond Road, Crumlin, County Antrim, BT29 4QY, United Kingdom, and modified according to Egoro *et al.,* 2023.

2.8.2 Alanine Aminotransferase

The measurement was performed utilising the colorimetric method specified by Randox Laboratories, Limited, situated at 55 Diamond Road, Crumlin, County Antrim, BT29 4QY, United Kingdom, and adapted by Egoro *et al.,* 2023.

2.8.3 Alkaline Phosphatase

The colorimetric endpoint method developed by Randox Laboratories, Limited, at 55 Diamond Road, Crumlin, County Antrim, BT29 4QY, United Kingdom, and later revised by Egoro *et al.*, 2023, was employed.

2.8.4 Creatinine

The Jaffe reaction method, as revised by Egoro *et al.* in 2023, was derived from Randox Laboratories, Limited, situated at 55 Diamond Road, Crumlin, County Antrim, BT29 4QY, United Kingdom.

2.8.5 Urea

The urease Berthelot method, modified by Egoro *et al.* in 2023, was derived from Randox Laboratories Limited, situated at 55 Diamond Road, Crumlin, County Antrim, BT29 4QY, United Kingdom.

2.9 Histomorphological Examination

This study examined the histomorphology of liver and kidney tissues extracted from both control and experimental male *Rattus norvegicus*. The organs were separately positioned in marked containers containing 10% formal saline fixative. The organs were subsequently sectioned and dehydrated with varying concentrations of alcohol: 50%, 70%, 80%, 95%, and 100%. Subsequently, they were dehydrated with xylene and embedded in molten paraffin wax, with the resulting tissue blocks cut and sectioned to a thickness of 4mm, in accordance with the methods described by Tijani *et al.* (2014). The tissue slides were prepared, stained using the Haematoxylin and Eosin method, and thoroughly examined for histomorphological lesions with an Olympus binocular light research microscope (XSZ-107BN, No 071771). Photomicrographs were subsequently obtained using a Kodak Digital Camera (Kodak Easyshare C183) for histomorphological study. This pertinent study was commenced to ascertain the true state and degree of damage to these vital organs.

2.10 Statistical Analysis

All statistical analyses were performed utilising SPSS version 20.0. The data were classified into an unexposed control group and experimental groups subjected to exposure for 30, 60, 90, and 120 days. The Kolmogorov-Smirnov test was utilised to evaluate normality. ANOVA was utilised to evaluate the disparities among the groups. Data were expressed as means and standard deviations. Variations in means among groups were considered significant at $p < 0.05$.

2.11 Evaluation of LC_{50} in *Rattus norvegicus* Exposed to Whole-body Inhalation of J-Kim Mosquito Coil Smoke

This study employed 16 male *Rattus norvegicus*, aged 3 to 4 months and weighing 0.103 ± 0.05 kg. The rats were randomly assigned to four groups, each consisting of four rats, designated A, B, C, and D. They were subsequently exposed to whole-body inhalation of smoke from J-Kim mosquito coils at concentrations of 15 mg / 0.103 ± 0.05 kg, 30

mg / 0.103 ± 0.05 kg, 45 mg / 0.103 ± 0.05 kg, and 60 mg / 0.103 ± 0.05 kg in an 8-foot by 8-foot room for a duration of 8 hours on a single day, respectively.

Following the previously indicated exposure, the rats were monitored for 24 hours for any signs of toxicity, including symptoms and mortality caused by the smoke emitted from the mosquito coil. The rats were considered dead in the absence of an agitation response.

Fifty percent (LC_{50}) of the *Rattus norvegicus* in group C displayed manifestations such as convulsions, respiratory distress, coughing, ocular irritation, dermal irritation, pharyngeal irritation, lethargy, coma, and mortality after exposure to a concentration of 45 mg / 0.103 ± 0.05 kg of smoke generated by the J-Kim mosquito coil.

The LC_{50} was calculated using the arithmetic method as described by Nwachukwu *et al.* (2015). This method entails calculating the sum of the concentration differences and mean dead which is divided by the number of rats per group and the resulting quotient subsequently subtracted from the LC_{100} i.e. the minimum concentration that caused 100% death.

Calculation:

$$LC_{50} = LC_{100-} \frac{\text{(sum of concentration differences} \times \text{mean dead)}}{\text{Number of rats per group}}$$

2.12 Sub-chronic Toxicity Evaluation of *Rattus norvegicus*

In this study, *Rattus norvegicus* were randomly allocated into five groups. Each group consisted of five male *Rattus norvegicus* aged 3 to 4 months, with an average weight of 0.103 ± 0.05 kg. The classifications of the groupings were as follows:

2.12.1 Experimental Group One

This consisted of male *Rattus norvegicus* subjected to a 15 mg concentration of smoke from J-Kim mosquito coils by whole-body inhalation for eight hours every day for thirty days.

2.12.2 Experimental Group Two

This consisted of male *Rattus norvegicus* subjected to a 15 mg concentration of smoke from J-Kim mosquito coils by whole-body inhalation for eight hours every day for a period of sixty days.

2.12.3 Experimental Group Three

This consisted of male *Rattus norvegicus* subjected to a 15 mg concentration of smoke from J-Kim mosquito coils by whole-body inhalation for eight hours every day for a period of ninety days.

2.12.4 Experimental Group Four

This consisted of male *Rattus norvegicus* exposed to whole-body inhalation of a 15 mg concentration of smoke generated by J-Kim mosquito coils for eight hours daily for a duration of one hundred and twenty days.

2.12.5 Control Group Five

This comprised male *Rattus norvegicus* that had not been subjected to mosquito coil smoke or any alternative smoke source.

At the experiment's conclusion, the rats were euthanised under chloroform anaesthesia. After obtaining blood samples from each rat through cardiac puncture to evaluate aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatinine, and urea biochemical parameters, the liver and kidney were excised and fixed in 10% formalin for histomorphological examination.

2.13 Measurement of Mosquito Coil Smoke Concentrations

Leonardo da Vinci meticulously analysed the challenges of evaluating smoke in the 15th century. He differentiated between white smoke and black smoke (carbonised particles) (Sorensen, 2016). This research utilised the In-line capture technique to assess the smoke produced by a mosquito coil, as described by Watson (2010).

An analytical balance (OHAUS) model CP213 S/N B143092087, produced by OHAUS Corporation at No. 471, Guiping Road, Shanghai, China, was employed to weigh a Whatman filter paper 1 (110mm), catalogue number 1001-1110, before and after its exposure to smoke from a mosquito coil. The difference between the two measurements, indicating the majority of the smoke, was evaluated as shown:

Calculation:
The mass of the filter paper before smoke exposure = 0.842 mg.

The mass of the filter paper after smoke exposure = 15.842 mg.

The difference is determined by subtracting 0.842mg from 15.842mg, yielding 15mg. This approach is most direct and likely the most accurate way for quantifying smoke concentration; nevertheless, it is only applicable in low-smoke conditions due to the swift obstruction of the filter.

3. RESULTS

The results of hepato-renal biochemical changes in plasma of *Rattus norvegicus* exposed to 15 mg concentration of the J-Kim mosquito coil smoke for 30 (thirty) days

(experimental group one), 60 days (experimental group two), 90 (ninety) days (experimental group three) and 120 (one hundred and twenty) days (experimental group four) as compared with that of the *Rattus norvegicus* not exposed to mosquito coil smoke or any other source of smoke (control group) are as shown in Table 1

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Creatinine (µmol/L)	Urea (mmol/L)
Control ((n=5)	7.80 ± 1.30	6.40 ± 1.67	19.00 ± 1.22	62.40 ± 4.77	4.20 ± 1.41
30 days (n=5)	8.00 ± 0.71	6.60 ± 1.34	19.20 ± 3.96	62.40 ± 6.23	4.22 ± 1.09
60 days (n=5)	8.20 ± 0.84	6.80 ± 0.84	19.40 ± 0.89	62.60 ± 2.79	4.26 ± 1.00
90 days (n=5)	8.20 ± 0.84	6.80 ± 1.48	19.40 ± 3.65	132.00 ± $5.24^{\lambda\pi}$	13.20 ± 2.77^{τ}
120 days (n=5)	16.00 ± 1.58^{ζ}	15.20 ± 1.30^{η}	37.60 ± 2.30^{θ}	148.20 ± 11.67^{μ}	16.60 ± 2.70^{φ}
F-value	51.92	39.80	46.02	197.44	46.41
P-value	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}

Table 1: Results of mean ± SD of hepato-renal biochemical parameters measured in *Ratttus norvegicus* exposed to J-Kim mosquito coil smoke (experimental group) compared with the non-exposed (control group)

KEYS: ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, ALP = Alkaline phosphatase, n = Number of rats

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

Hepatic enzymes biomarkers: The mean values of these hepatic enzymes in the exposed *Rattus norvegicus* for periods of 30 (thirty) days versus the control *Rattus norvegicus*: alanine aminotransferase (8.00 ± 0.71 versus 7.80 ± 1.30) IU/L, aspartate aminotransferase (6.60 ± 1.34 versus 6.40 ± 1.67) IU/L, alkaline phosphatase (19.20 ± 3.96 versus 19.00 ± 1.22) IU/L, 60 (sixty) days exposed *Rattus norvegicus* versus the control *Rattus norvegicus*: alanine aminotransferase (8.20 ± 0.84 versus 7.80 ± 1.30) IU/L, aspartate aminotransferase (6.80 ± 0.84 versus 6. 40 ± 1.67) IU/L, alkaline phosphatase (19.40 ± 0.89 versus 19.00 ± 1.22) IU/L and 90 (ninety) days exposed *Rattus norvegicus* versus the control *Rattus norvegicus*: alanine aminotransferase (8.20 ± 0.84 versus 7.80 ± 1.30) IU/L, aspartate aminotransferase (6.80 ± 1.48 versus 6.40 ± 1.67) IU/L, alkaline phosphatase (19.40 ± 3.65 versus 19.00 ± 1.22) IU/L revealed no statistical significant elevations ($p>0.05$) as shown in Table 1.

Alanine aminotransferase: Significant elevations observed between 120 (one hundred and twenty) days exposed group and each of 30 (thirty), 60 (sixty), 90 (ninety) days exposed groups and non-exposed control, $p = .00$ respectively.

Aspartate aminotransferase: Significant elevations observed between 120 (one hundred and twenty) days exposed group and each of 30, 60, 90 days exposed groups and non-exposed control, $p = .00$ respectively.

Alkaline phosphatse: Significant elevations observed between 120 (one hundred and twenty) days exposed group and each of 30 (thirty), 60 (sixty), 90 (ninety) days exposed groups and non-exposed control, $p = .00$ respectively.

As shown further in this Table 1, the mean values of these enzymes in the 120 (one hundred and twenty) days exposed *Rattus norvegicus* versus the control *Rattus norvegicus*: alanine aminotransferase (16.00 ± 1.58 versus 7.80 ± 1.30) IU/L, aspartate aminotransferase (15.20 ± 1.30 versus 6.40 ± 1.67) IU/L and alkaline phosphatase (37.60 ± 2.30 versus 19.00 ± 1.22) IU/L revealed significant elevation ($p<0.01$).

Renal biomarkers: As shown in Table 1, the mean values of plasma creatinine and urea in the exposed *Rattus norvegicus* for 30 (thirty) days versus the control *Rattus norvegicus*: creatinine (62.40 ± 6.23 versus 62.40 ± 4.77) µmol/L, urea (4.22 ± 1.09 versus 4.20 ± 1.41) mmol/L and 60 (sixty) days exposed *Rattus norvegicus* versus the control *Rattus norvegicus*: creatinine (62.60 ± 2.79 versus 62.40 ± 4.77) µmol/L, urea (4.26 ± 1.00 versus 4.20 ± 1.41) mmol/L respectively, revealed no significant difference ($p>0.05$).

However, there were significant elevations ($p<0.01$) in the mean values of both parameters in the exposed *Rattus norvegicus* for 90 (ninety) days compared with the control *Rattus norvegicus*: creatinine (132.00 ± 5.24 versus 62.40 ± 4.77) µmol/L, urea (13.20 ± 2.77 versus 4.20 ± 1.41)

mmol/L and 120 (one hundred and twenty) days exposed *Rattus norvegicus* compared with the control *Rattus norvegicus*: creatinine (148.20 ± 11.67 versus 62.40 ± 4.77) µmol/L, urea (16.60 ± 2.70 versus 4.20 ± 1.41) mmol/L respectively as shown in Table 1.

$^{\lambda}$Significant elevations were observed between 90 (ninety) days exposed group and each of control, 30 (thirty) and 60 (sixty) days exposed groups, p = .00 respectively.
$^{\mu}$Significant elevations were observed between 120 (one hundred and twenty) days exposed group and each of control, 30 (thirty) and 60 (sixty) days exposed groups, p = .00 respectively.
$^{\pi}$ Significant difference observed between 90 (ninety) and 120 (one hundred and twenty) days exposed groups, p = .01.

Urea: Significant differences were identified between the group exposed for 90 (ninety) days and the control, 30 (thirty) days and 60 (sixty) days groups, p =.00 for each. Significant differences were detected between the 120 (one hundred and twenty) days group and the control, 30 (thirty) days, and 60 (sixty) days groups, p =.00

Histomorphological Assessment of Liver Tissue Exposed to J-Kim Mosquito Coil

The histomorphological observation on liver excised from the *Rattus norvegicus* exposed to 15 mg concentration of smoke generated from J-Kim mosquito coil on daily basis for a period of 30 (thirty) days (experimental group one), 60 (sixty) days (experimental group two), 90 (ninety) days (experimental group three) and 120 (one hundred and twenty) days (experimental group four) as compared with that of the *Rattus norvegicus* not exposed to smoke generated from mosquito coil or any other source of smoke (control group) are as shown in plates 1-4 respectively.

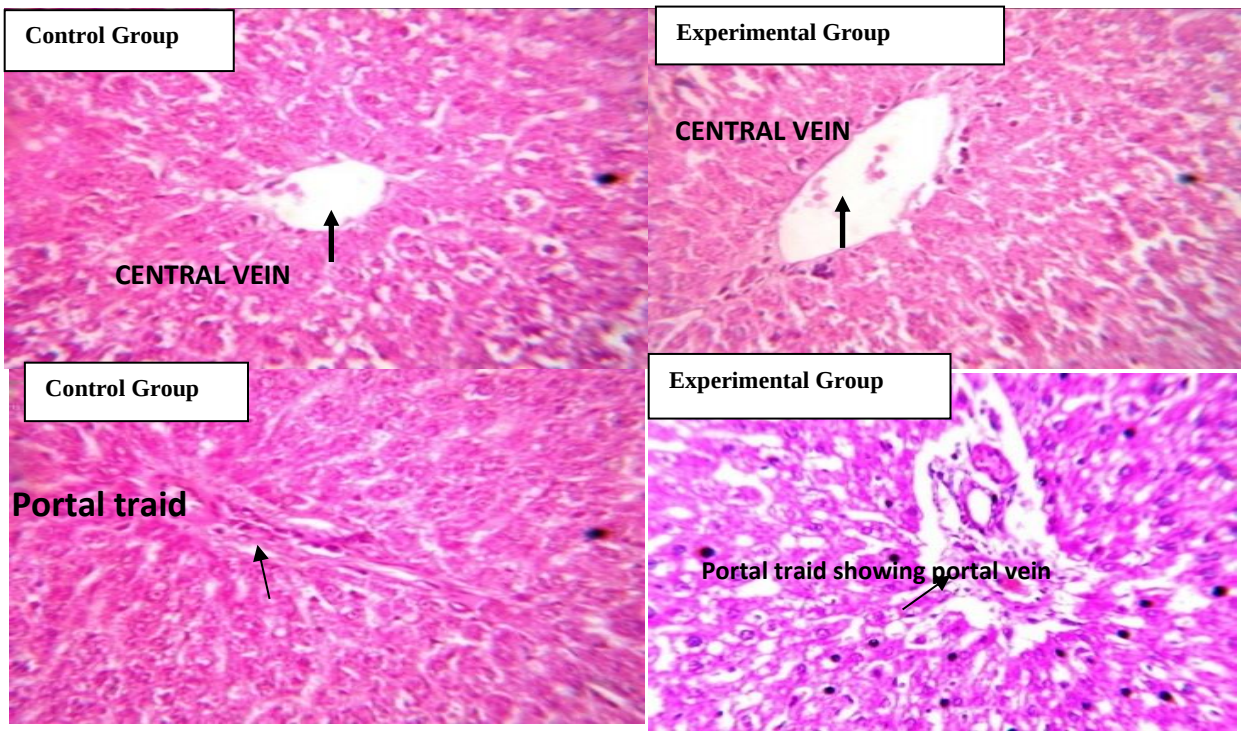

PLATE 1: Photomicographs of liver tissue stained with haematoxylin and eosin x 400 magnification

No toxicity was observed in the liver tissue of the experimental group of the *Rattus norvegicus* exposed to 15 mg concentration of J-Kim mosquito coil smoke for a period of 30 (thirty) days as compared to that of the control group which showed a normal status. This histological observation is in alignment with the measured biochemical marker which also revealed no statistically significant alteration as well

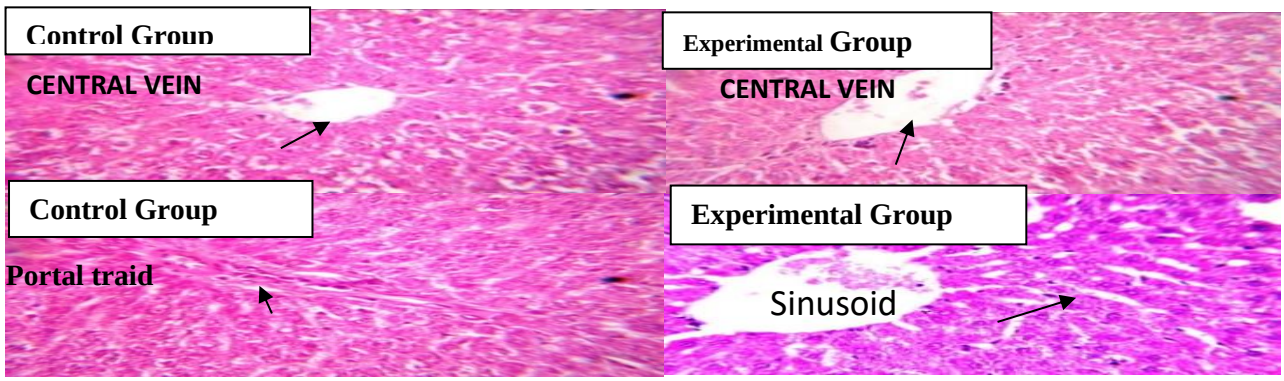

PLATE 2: Photomicrographs of liver tissue stained with haematoxylin and eosin x 400 magnification

No toxicity was observed in the liver tissue of the experimental group of the *Rattus norvegicus* exposed to 15 mg concentration of J-Kim mosquito coil smoke for a period of 60 (sixty) days as compared to that of the control group which showed a normal status. This histological observation is in alignment with the measured biochemical marker which also revealed no statistically significant alteration

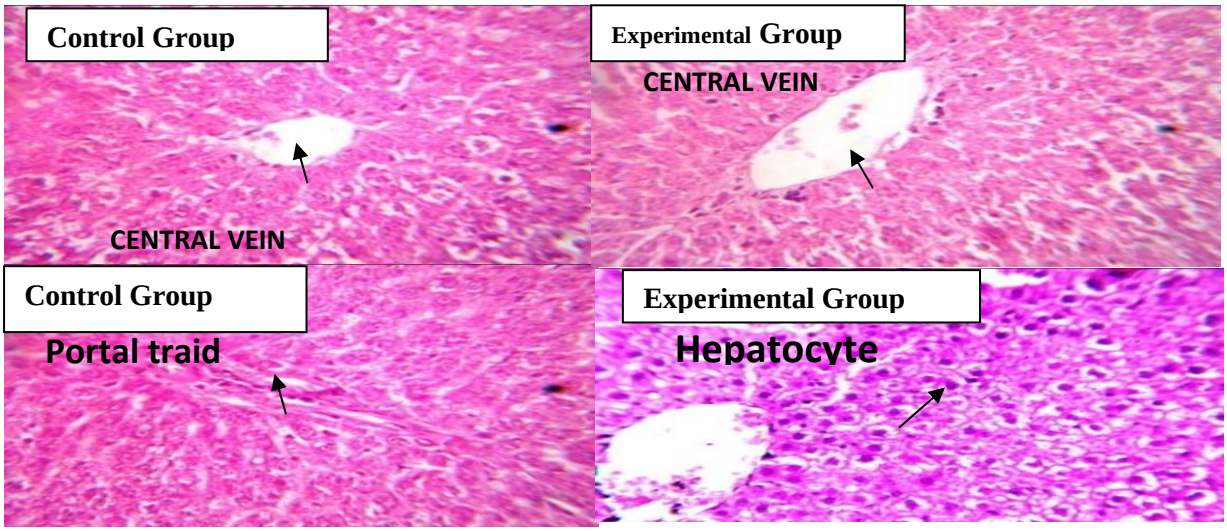

PLATE 3: Photomicrographs of liver tissue stained with haematoxylin and eosin x 400 magnification

No toxicity was observed in the liver tissue of the experimental group of the *Rattus norvegicus* exposed to 15 mg concentration of J-Kim mosquito coil smoke for a period of 90 (ninety) days as compared to that of the control group which showed a normal status. This histological observation is in alignment with the measured biochemical marker which also revealed no statistically significant alteration

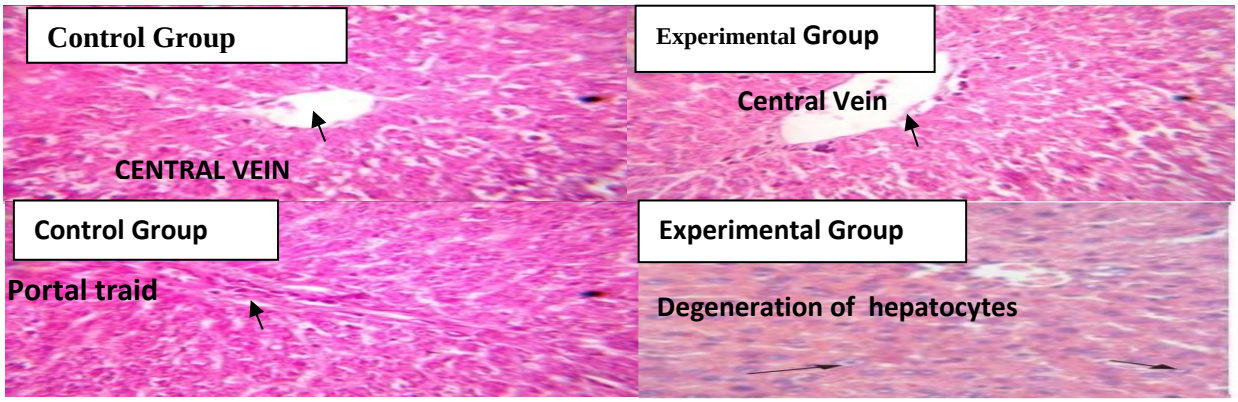

PLATE 4: Photomicrographs of liver tissue stained with haematoxylin and eosin x 400 magnification

Degeneration of hepatocytes showing necrosis, ischema and few balloons as indicated by the arrows was observed in the liver tissue of the experimental group of the *Rattus norvegicus* exposed to 15 mg concentration of J-Kim mosquito coil smoke for a period of 120 (one hundred and twenty) days as compared to that of the control group which showed a normal status. This severe toxic histological observation is in alignment with the measured biochemical marker which also revealed statistically significant elevation

Histomorphological Assessment of Renal Tissue Exposed to J-Kim Mosquito Coil

The histomorphological revelations on kidneys excised from the *Rattus norvegicus* exposed to 15 mg concentration of smoke generated from J-Kim mosquito coil on daily basis for a period of 30 (thirty) days (experimental group one), 60 (sixty) days (experimental group two), 90 (ninety) days (experimental group three) and 120 (one hundred and twenty) days (experimental group four) as compared with that of the *Rattus norvegicus* not exposed to smoke generated from mosquito coil or any other source of smoke (control group) are shown in plates 5-8 respectively

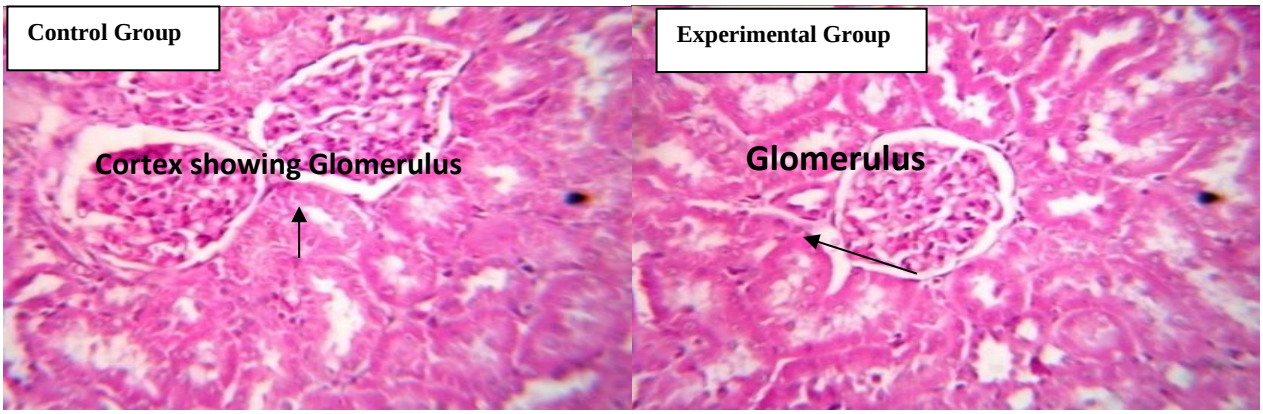

PLATE 5: Photomicrographs of kidney tissue stained with haematoxylin and eosin ×400 magnification

No toxicity was observed in the kidney tissue of the experimental group of the *Rattus norvegicus* exposed to 15 mg concentration of J-Kim mosquito coil smoke for a period of 30 (thirty) days as compared to that of the control group which showed a normal status. This histological observation is in alignment with the measured biochemical marker which also revealed no statistically significant alteration

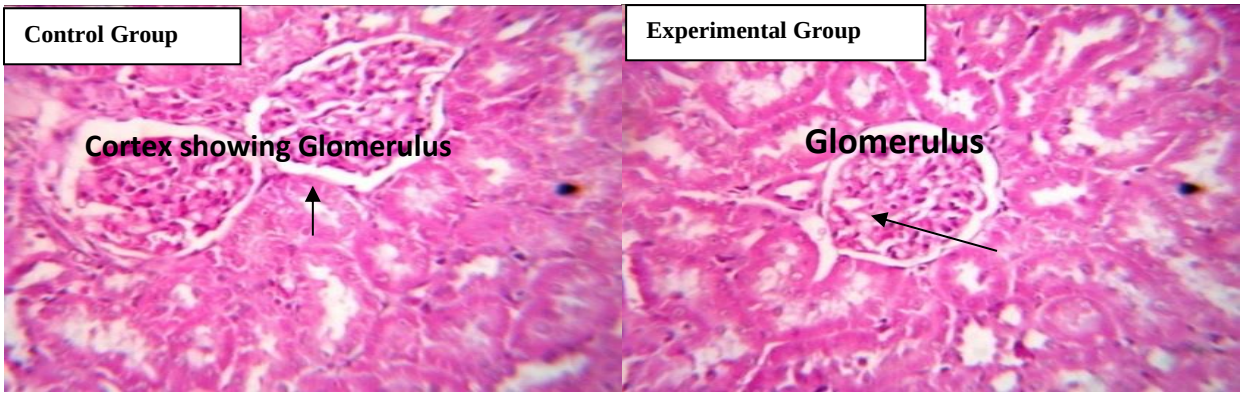

PLATE 6: Photomicrographs of kidney tissue stained with haematoxylin and eosinx 400 magnification

No toxicity was observed in the kidney tissue of the experimental group of the *Rattus norvegicus* exposed to 15 mg concentration of J-Kim mosquito coil smoke for a period of 60 (sixty) days as compared to that of the control group which showed a normal status. This histological observation is in alignment with the measured biochemical marker which also revealed no statistically significant alteration

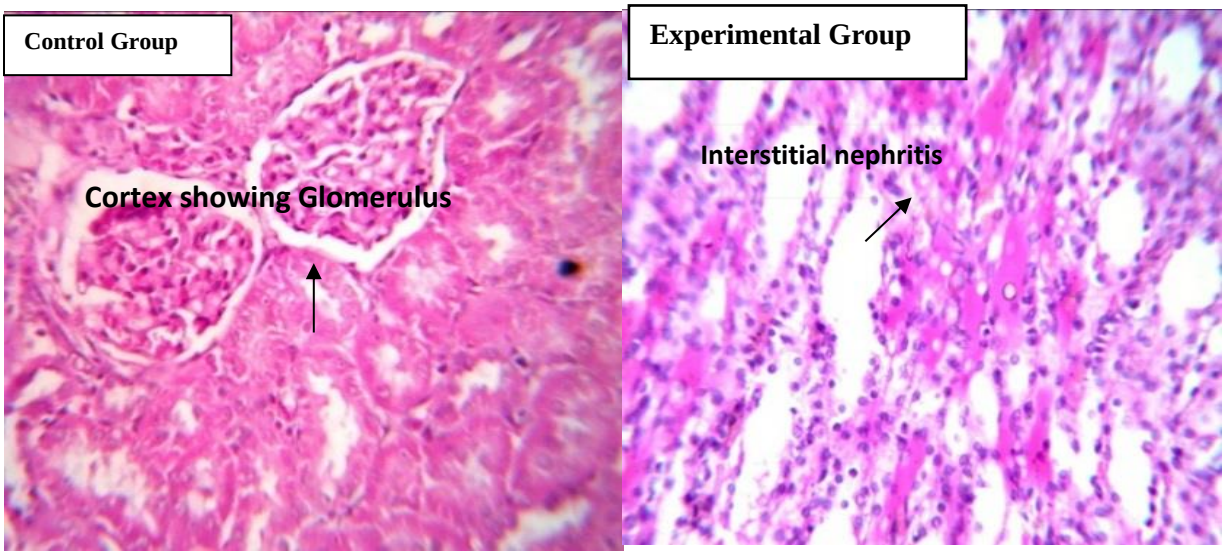

PLATE 7: Photomicrographs of kidney tissue stained with haematoxylin and eosin x 400 magnification

Interstitial nephritis was observed in the experimental group of the *Rattus norvegicus* exposed to 15 mg concentration of J-Kim mosquito coil smoke for a period of 90 (ninety) days as compared to that of the control group which showed a normal status. This histological observation is in alignment with the measured biochemical marker which also revealed statistically significant elevation.

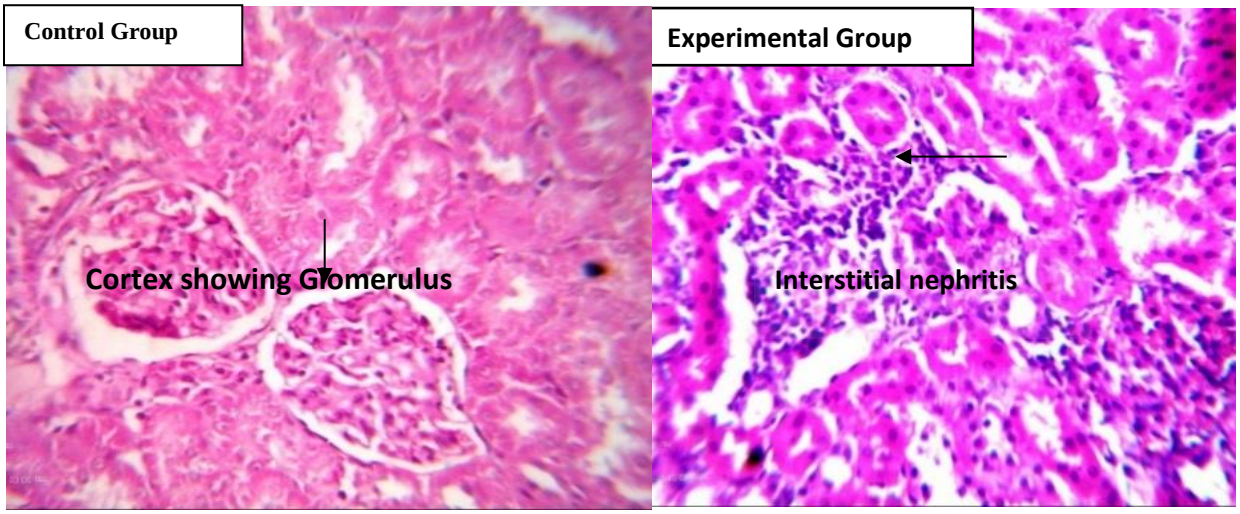

PLATE 8: Photomicrographs of kidney tissue stained with haematoxylin and eosin x 400 magnification

Interstitial nephritis of the kidney tissue as indicated by the arrow was observed in the kidney tissue of the experimental group of the *Rattus norvegicus* exposed to 15 mg concentration of J-Kim mosquito coil smoke for a period of 120 (one hundred and twenty) days as compared to that of the control group which showed a normal status. This histological observation is in alignment with the measured biochemical marker which also revealed statistically significant elevation

4.DISCUSSION

In developing countries, malaria is recognised as a public health concern that contributes to an increase in infections and mortality rates. Humans have decided to employ various control technologies (aerosols, mosquito coils, mosquito nets, etc.) to prevent bites from malaria-transmitting mosquitoes in an effort to address this problem (Khadijah *et al.,* 2020).

Mosquito coils release smoke that comprises numerous harmful compounds upon ignition. Nonetheless, there is a dearth of literature on this subject, thus requiring further research in this area (John *et al.,* 2016). Only d-cis-transallethrin at a standard concentration of 0.25 percent is identified as the active ingredient, while the additional chemicals are often categorised as "others." Nonetheless, Dubey *et al.* (2014) and Yang *et al.* (2015) found that the particulate matter from mosquito coils contains both toxic heavy metals and concerning organic compounds. Heavy metals are harmful to human health even at low concentrations due to their high density (Monisha *et al.*, 2014).

The liver is the major organ involved in nutritional metabolism in humans and certain animals, rendering it vulnerable to metabolic diseases (Ozougwu and Eyo, 2014; Mutiara and Dwi, 2018). Enzymes are proteins that enhance the speed of a chemical process while staying unaltered at the end of the reaction. In healthy persons, enzyme levels typically remain steady. Consequently,

increased plasma activity may signify tissue injury (Offor, 2014) far before histological identification. Evaluating its activity is essential for identifying tissue damage induced by chemical agents (Nafiu *et al.*, 2016).

This study quantified the levels of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase to evaluate the hepatic function of *Rattus norvegicus* subjected to a 15 mg concentration of J-Kim mosquito coil smoke throughout periods of 30, 60, 90, and 120 days, as outlined in Table 1.

This biochemical finding, indicative of a normal liver state, corresponds with the histomorphological assessment of the liver status in the exposed *Rattus norvegicus*, which exhibited no toxicity relative to the control group, as depicted in Plates 1-3. These findings, however, contradict Offor's (2014) earlier research, which showed a significant increase ($p<0.05$) in these enzymes in male Wistar albino rats exposed daily to mosquito coil smoke for a two-week period. A plausible explanation for this may arise from discrepancies in the experimental conditions, such as the number of coils ignited daily, room size, ventilation rates, exposure patterns, and type of mosquito coil, among other factors.

This biochemical finding, suggestive of a hepatotoxic condition, may arise from the inhalation of toxic heavy metals like cadmium and/or lead released by burned mosquito coils, potentially leading to liver damage and subsequent enzyme release into the plasma. The process by which it causes liver damage is its attachment to albumin in the bloodstream, subsequently transported to the liver, where it interacts with glutathione and metallothionein-1 (MT-1) to create a complex referred to as cadmium metallothionein-1 (Cd-MT-1). This complex is released into bile and subsequently reabsorbed into the bloodstream via enterohepatic circulation, resulting in the release of free cadmium, which causes an overproduction of free radicals that trigger the apoptotic process in the liver (Hafiza, 2018).

This conclusion corresponds with the histomorphological assessment of liver condition in *Rattus norvegicus*, which revealed hepatocyte deterioration compared to the control group, as depicted in Plate 4.

The kidneys are paired, bean-shaped organs. They serve a crucial role in the removal of deleterious substances due to their filtration, secretion, and reabsorption properties. This role exposes them to toxic substances (Khadijah *et al.*, 2020).

This study assessed renal biomarkers, namely creatinine and urea, to determine the renal condition of *Rattus norvegicus* following exposure to a 15 mg concentration of smoke from J-Kim mosquito coils for periods of 30, 60, 90, and 120 days, respectively.

The biochemical finding ($p>0.05$) in rats subjected to mosquito coil smoke for 30 and 60 days is consistent with the previous study by Emmanuel *et al.* (2013) and correlates with the histomorphological assessment of the rats' kidneys, which demonstrated no toxicity compared to the control rats, as depicted in Plates 5 and 6.

However, there was a significant rise in these renal markers in the group of rats exposed to the smoke generated by this coil for ninety and one hundred twenty days, respectively. These

significant biochemical elevations, in accordance with the previous study by Rukmini *et al*. (2004), may indicate renal impairment. Cadmium can cause nephrotoxicity when the low molecular weight cadmium-metallothionein-1 (Cd-MT-1) complex, under 7 kDa, is filtered by the glomerulus and then reabsorbed in the proximal convoluted tubule. This mechanism leads to lysosomal dissociation, releasing free cadmium that produces free radicals, therefore triggering apoptosis (Ansari *et al.*, 2017; Hafiza, 2018).

This biochemical finding corresponds with the histomorphological assessment of the kidneys of *Rattus norvegicus* subjected to 90 and 120 days of exposure, revealing interstitial nephritis, unlike the control *Rattus norvegicus*, which displayed normal kidney morphology, as depicted in Plates 7 and 8, respectively.

CONCLUSION

In conclusion, this study has revealed the following:

Prolonged exposure to 15 mg concentration of J-Kim mosquito coil smoke on daily basis for 90 (ninety) days and 120 (one hundred and twenty) days respectively may trigger renal disorder in *Rattus norvegicus.*

Prolonged exposure to 15 mg concentration of J-Kim mosquito coil smoke on daily basis for 120 (one hundred and twenty) days may trigger hepatic disorder in *Rattus norvegicus*.

Disclosure of conflict of interest

No conflict of interest

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Recommendations

It is therefore recommended as follow:

(i) The biochemical parameters assessment of this study in a well control manner should be reproduced in human in order 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.

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