

## **Histopathological and Biochemical Effects of Selected Mosquito Chemical Repellents on the Liver of Adult Wistar Rats**

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### **Abstract**

**Background:** Repeated outbreak of malaria compelled many into the use of mosquito chemical repellents in Africa most populous nation, Nigeria. Despite successes achieved, human health issues are major concern nowadays regarding the volatile-active agents and by-products of most mosquito chemical repellents. Hence, the need to evaluate histopathological and biochemical effects of commonly used mosquito repellents on the liver as body's centre for detoxification.

**Materials and Methods:** Forty five Wistar rats were divided into five groups of nine rats kept in plastic cages measuring 53.5 cm x 39.5 cm to inhale vapours of selected brands of mosquito repellents for 5 minutes/day over a period of 21 days. A group of animals without exposure to repellent vapours served as normal control. Three animals from each group were sacrificed at week 1, 2 and 3 respectively. Biochemical and histopathological studies were performed following physical observations of toxicity signs.

**Results:** Exposure of Wistar rats to mosquito repellents for 5minutes/day over a period of 21 days did not cause mortality. Liver enzymes showed significant increase in rats exposed to liquid form of repellents (Rambo and Sharp) ( $P < 0.05$ , Bonferroni test = 0.04) corroborated by sinusoid dilatations, cytoplasmic vacuolation and on-going apoptotic cells. Coil form of repellents (Wave tide and Baoma) did not show significant change in liver enzymes ( $P > 0.05$ ) but there was histopathological changes compared to normal liver.

**Conclusion:** Wistar rats exposed to liquid and coil vapours of mosquito repellents for 5minutes/day over a period of 21 days caused liver injury.

**Keywords:** Malaria; Mosquito Chemical Repellents; Wistar Rats; Liver Enzymes; Histopathological Changes

### **Introduction**

Insect-borne diseases are global health problem, especially in tropical and subtropical climates [1]. Malaria infection is among the most severe public health problem confronting Africa, where Nigeria has the greatest number of cases [2], contributing to a quarter of malaria burden in Africa [3]. Subtropical climate, Poor drainage system and dense population size are cogent factors triggering mosquito scourge day by day [4]. The repeated episodes of malaria outbreak in Nigeria has compelled many to resolve into the use of different brands of mosquito repellents, including locally fabricated brands as control measures [5,6]. However, human health issues are major public concerns nowadays regarding the use of volatile organic compounds present in mosquito repellents [7,8]. Most active agents and bye products of mosquito repellents are spiteful malicious to man's health [9]. They have potential to cause inflammation, necrosis,

carcinogenesis and apoptosis [10]. There are irrefutable cases of tissue-organs damaged following repeated exposure to mosquito repellents, including the liver which serves as seat for detoxifications of harmful substances in the body [10,11,13]. Despite multiple scientific reports on effects of mosquito repellents, there is still no report on the histopathological and biochemical effects of commonly used mosquito repellents in Sokoto, Nigeria.

**Materials and Methods**

**Ethical issues**

The Institutional Animal Ethics Committee for the use of laboratory animals provided necessary permission to carry out the present study and protocol followed was in accordance with the use of laboratory animals at Usmanu Danfodiyo University Sokoto, Nigeria.

**Procurement of mosquito repellents**

Both Coil and Liquid forms of mosquito repellents were procured from commercially available store at Diplomat area of Sokoto South Local Government Area, Nigeria.

**Test mosquito repellents**

Commonly used mosquito repellents in Sokoto are the coil and liquid (spray) forms. Four brands of mosquito repellents were selectively employed using Simple Random Sampling Technique in this study. The two Coil forms include: Wave tide manufactured by Hangzhou Jiadchao Commodity Co Limited and Baoma manufactured by Fujian Benma Daily Chemical Limited. Each of these coils weighs 12 grams; measuring 70 cm long and contains 0.2% w/w composition of d-Allethrin and Rich-d-Trans allethrin. The two Spray forms include: Rambo, manufactured by Gongoni Company Limited Nigeria containing 0.25% Transfluthrin and 0.20% Permethrin (which are all synthetic Pyrethrin in a 300 ml pressurized liquid Canister) and Sharp spray form manufactured by Onitsha Company Nigeria composed of 70% Dichlorvos.

**Experimental animals**

A total of forty five (45) adult male and female Wistar rats, weighing 150-180 grams were used in this experiment. Animals were procured from the Animal House, Ahmadu Bello University Zaria Kaduna State. They were kept in well ventilated room with optimum environmental conditions of temperature (22 ± 2°C), relative humidity and 12 hours dark/light cycle. The animals were fed with standard pellets and tap water *ad libitum* and were allowed to acclimatize for two weeks before the experiment.

**Experimental design**

Forty five rats were divided into five groups of nine rats each kept in plastic cages measuring 53.5 cm x 39.5 cm to inhale vapours of four selected brands of mosquito repellents in a closed room for 5 minutes/day over a period of 21 days [14]. A group of animals kept under similar conditions but without exposure to any repellent vapours served as normal control. Three animals from each groups exposed to each of the four mosquito repellents were sacrificed at week 1, week 2, week 3 respectively. The biochemical parameters and histopathological studies were performed following physical observations of toxicity signs [15-17].

Brands of mosquito repellents	Dose of repellents... (5 minutes daily)	Period of exposure in Weeks		
		Week 1	Week 2	Week 3
Normal control	None	3 rats	3 rats	3 rats
Wave tide	10 kg/w	3 rats	3 rats	3 rats
Baoma	10 kg/w	3 rats	3 rats	3 rats
Rambo	0.45 ml/w	3 rats	3 rats	3 rats
Sharp	0.45 ml/w	3 rats	3 rats	3 rats

**Table 1:** Showing different brands of mosquito repellents, dose and durations of exposure of experimental animals (n = 9).

**Biochemical studies**

Blood sample was drawn from the heart of each rat by cardiac punching of syringe. Collected blood sample was kept for half an hour on plane, clean tubes to clot and then centrifuged at 5000 rpm for 10 minutes at 4°C to separate serum as clear supernatant and stored at -20°C for biochemical analysis. The activity of ALT, ALP and AST was determined by colorimetric method [18].

**Histopathological analysis**

Animals from each group were anesthetized with chloroform vapour and abdominal incisions were made. Intact liver tissues were surgically removed and fixed in 10% formal saline overnight. Tissues were washed in running tap water and passed through increasing concentrations of alcohol, followed by tissue embedding in molten paraffin wax. Tissue blocks were sectioned at 4 µm thickness via rotary microtome and stained by routine haematoxylin and eosin solution. Finally, the slides were visualized under light microscope and photographs were taken by the camera attached to it.

**Statistical analysis**

SPSS 23.0 software was employed for this analysis. Data entry was done using Microsoft excel version 13 and Shapiro-Wilk test to verify data distribution. Mean and standard deviation (SD) was performed to assess whether the data was parametric or non-parametric distribution and a two-way analysis of variance (ANOVA) using Bonfeeroni adjustment test to compare experimental parameters of the animals. P ≤ 0.05 was considered statistically significant.

**Results**

**Morbidity and mortality**

Exposure of wistar rats to different vapours of mosquito repellents (Wave tide, Baoma, Rambo and Sharp) for 5 minutes/day over a period of 21 days did not cause mortality in all experimental animals. However, some animals expressed behavioural signs of toxicity such as sneezing, gurgling, ruffled fur appearance, nostrils with eyes irritations and lethargy.

**Biochemical finding**

Liver enzymes (AST, ALP and ALT) did not show significant change in rats exposed to the coil form of mosquito repellents (Wavetide and Baoma brands, P > 0.05, table 2). However, liver enzymes of both male and female rats following inhalation of liquid form of mosquito repellents (Rambo and Sharp) showed significant increase in liver enzymes compared to the normal liver profiles (P < 0.05, table 2, Bonfeeroni test = 0.04, table 2).

Repellents	Duration	AST	ALT	ALP
Normal Control	1 Week	253.00 ± 50.91	166.00 ± 26.87	274.50 ± 61.52
	2 Weeks	212.50 ± 10.61	168.50 ± 43.13	339.00 ± 49.50
	3 Weeks	247.50 ± 70.00	172.50 ± 78.49	352.00 ± 62.23
Coil	1 Week	183.50 ± 0.71	224.00 ± 90.51	248.50 ± 6.36
	2 Weeks	237.00 ± 72.12	132.00 ± 41.01	315.50 ± 7.78
	3 Weeks	194.50 ± 54.45	184.50 ± 44.55	291.50 ± 14.85
Spray	1 Week	171.00 ± 0.00*	222.00 ± 0.00*	382.00 ± 0.00*
	2 Weeks	225.00 ± 0.00*	222.00 ± 0.00*	147.00 ± 0.00*
	3 Weeks	244.00 ± 0.00*	189.00 ± 0.00*	257.00 ± 0.00*

**Table 2:** Biochemical Effects of commonly used coil and liquid mosquito repellents on liver enzymes and normal profile of control group of rats for three weeks (n = 9).

Liver enzymes were significantly increased in animals exposed to spray forms of mosquito repellents (P < 0.05, at 0.00\*). Post Hoc Test Multiple Comparisons (Bonfeeroni test = 0.04, P < 0.05).

There was marked increase in liver enzymes of rats exposed to liquid mosquito repellents compared to the coil form of mosquito repellents and normal liver profile (Figure 1-3).

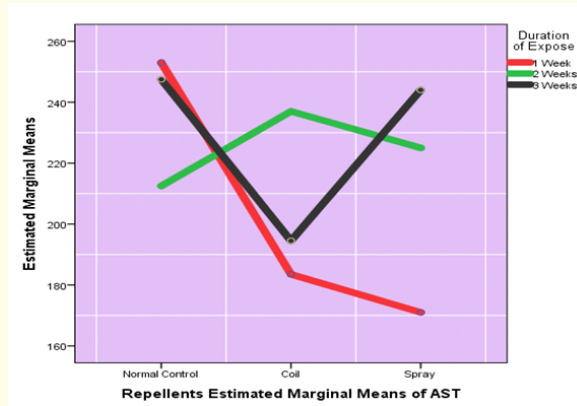


Figure 1: Changes in liver enzyme (AST) following inhalation of commonly used coil and spray mosquito repellents.

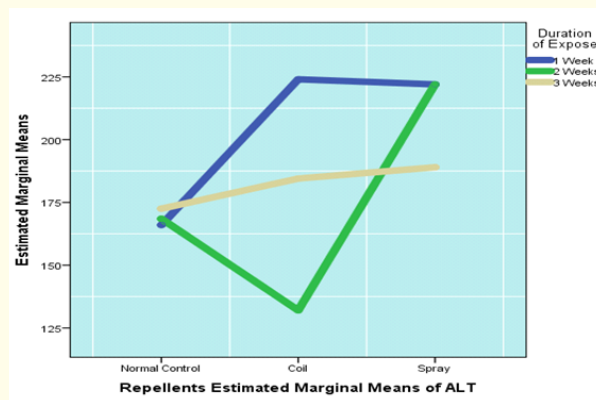


Figure 2: Figure Changes in liver enzyme (ALT) following inhalation of commonly used coil and spray mosquito repellents.

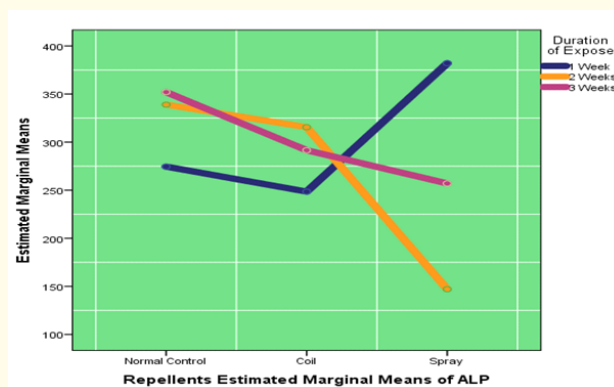
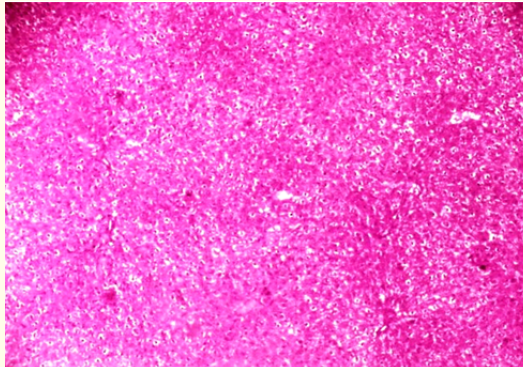
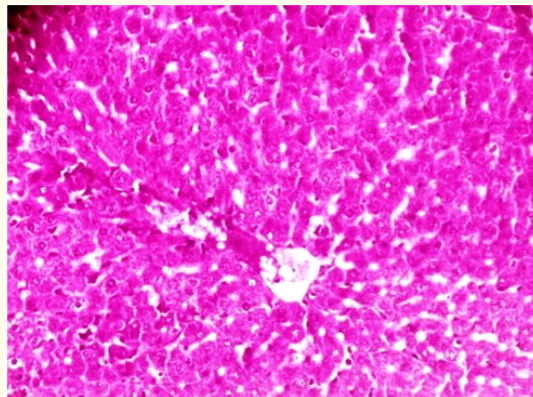


Figure 3: Changes in liver enzyme (ALT) following inhalation of commonly used mosquito repellents.

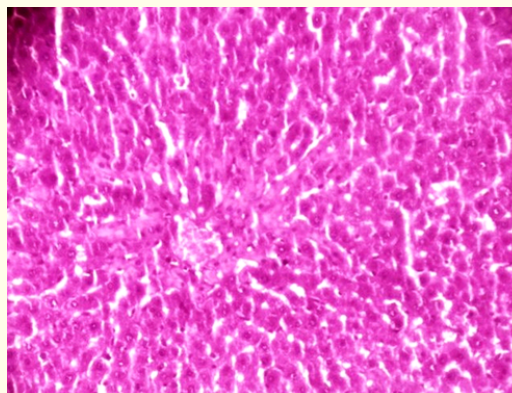
**Histopathological studies**



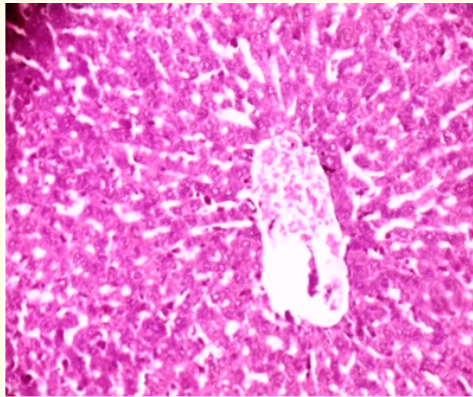
**Plate 1:** Liver tissue of normal wistar rat shows radiating chords of hepatocytes arranged in hexagonal plates with eosinophilic cytoplasm and pyknotic nuclei (H&E. X100).



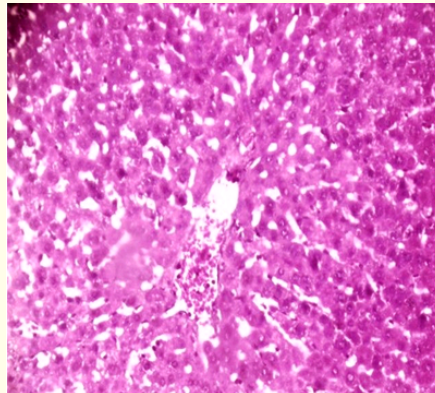
**Plate 2:** Liver tissue of wistar rat exposed to Baoma brand of mosquito coil smoke for a week showing mild sinusoidal dilatations and on-going apoptotic cells (H&E. X400).



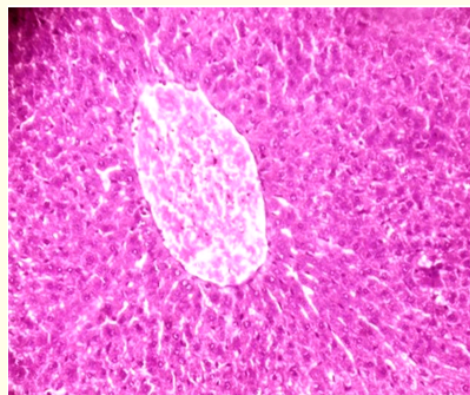
**Plate 3:** Liver tissue of wistar rat exposed to Wave tide brand of mosquito coil smoke for a week showing sinusoidal dilatations and congestion of central vein with on-going apoptotic cells (H&E. X400).



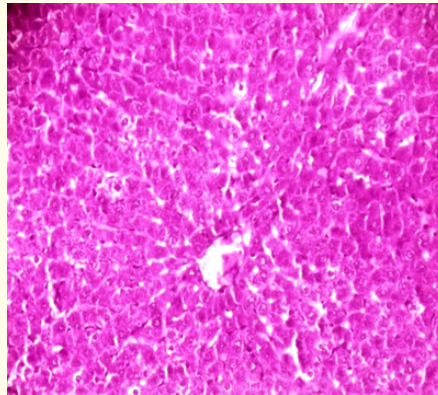
**Plate 4:** Liver tissue of wistar rat exposed to Rambo brand of mosquito spray for a week showing marked dilatations of central vein and sinusoids with on-going apoptotic cells (H&E. X400).



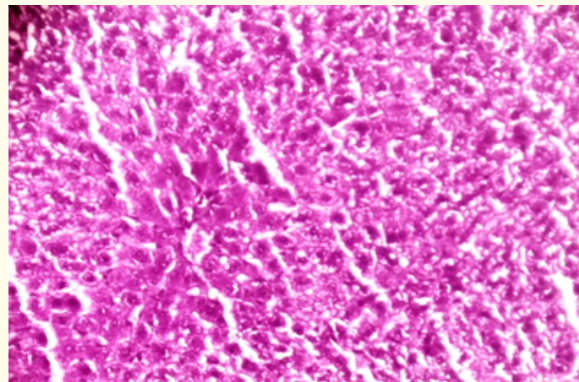
**Plate 5:** Liver tissue of wistar rat exposed to Sharp brand of mosquito spray for a week showing dilatation of the central vein and sinusoids, inflammatory cells with marked cytoplasmic vacuolation (H&E. X400).



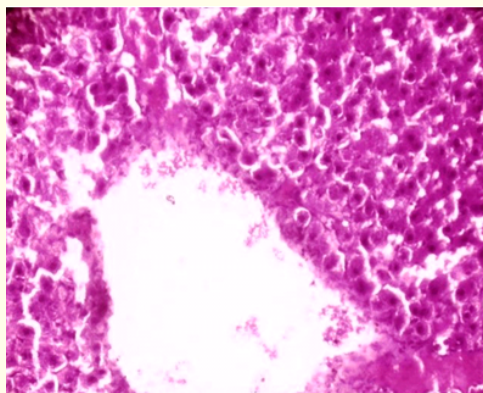
**Plate 6:** Liver tissue of wistar rat exposed to Baoma brand of mosquito coil smoke for 2 weeks showing marked dilatation of central vein (H&E. X400).



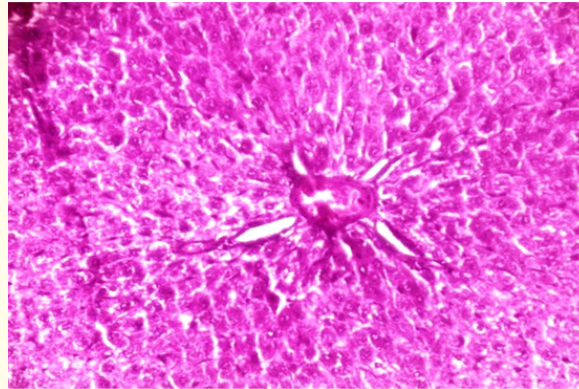
**Plate 7:** Liver tissue of wistar rat exposed to Wave tide brand of mosquito coil smoke for 2 weeks showing mild dilatation of sinusoids (H&E. X400).



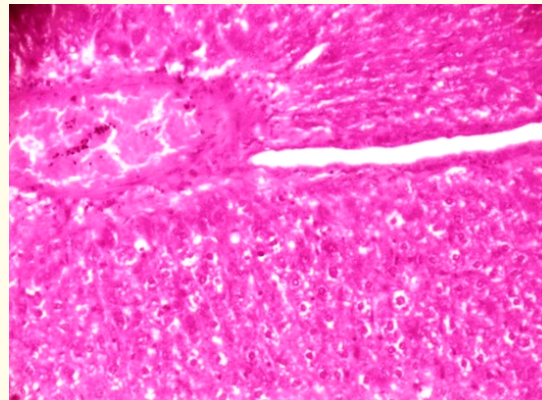
**Plate 8:** Liver tissue of wistar rat exposed to Rambo brand of mosquito spray for 2 weeks revealing marked dilatation of the sinusoids, constriction of central vein and condensation of on-going apoptotic cells (H&E. X400).



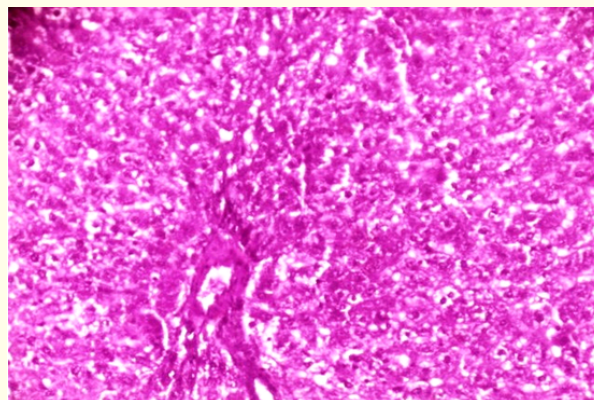
**Plate 9:** Liver tissue of wistar rat exposed to Sharp brand of mosquito spray for 2 weeks showing marked dilatation of the central vein and sinusoids with cytoplasmic vacuolation (H&E. X400).



**Plate 10:** Liver tissue of wistar rat exposed to Baoma brand of mosquito coil smoke for 3 weeks showing mild congestion of central vein (H&E. X400).

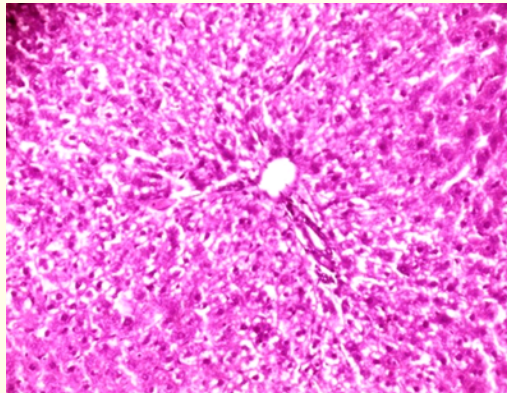


**Plate 11:** Liver tissue of wistar rat exposed to Wave tide brand of mosquito coil smoke for 3 weeks showing congestion of central vein (H&E. X400).



**Plate 12:** Liver tissue of wistar rat exposed to Rambo brand of mosquito spray for 3 weeks showing central vein constriction, cytoplasmic vacuolation and condensation of on-going apoptotic cells (H&E. X400).





**Plate 13:** Liver tissue of wistar rat exposed to Sharp brand of mosquito spray for 3 weeks showing dilatation of sinusoids with cytoplasmic vacuolation (H&E. X400).

## Discussion

The strategy for malaria prevention especially to rural populace, focused mainly on the use of mosquito repellents [6,19]. However, several types of repellents are known to elicit spiteful malicious effects similar to cyanide poisoning [19]. All the types of mosquito repellents used in this study induced behavioural signs of toxicity such as sneezing, gurgling, ruffled fur appearance, irritations and lethargy on repeated exposure of experimental animals for 5 minutes/day from week 1, week 2 and week 3, but did not cause mortality. Our finding is in agreement with several reports that repeated exposure of experimental animals to mosquito repellents causes persistent sneezing and lethargy [20-23]. Electrolytes channels ( $\text{Na}^+$ -K<sup>+</sup> Pump) is a major target sites for binding free radicals emitted from repellents vapours and when this occur,  $\text{Na}^+$  channel remain opened, inciting influx of  $\text{Na}^+$  ions which causes hyper-excitation of the nervous system leading to fatigue [24].

The biochemical effects following repeated exposures of experimental animals to the coil form of mosquito repellents (Wavetide and Baoma) showed no significant change in the levels of liver enzymes (AST, ALT, and ALP) compared with normal control (where  $P > 0.05$ , Table 2). Although, our finding does not agree with Emmanuel's report on effects of mosquito coils on liver enzymes [25] but reported similar histopathological changes on sinusoidal congestion of the liver. This disparity could arise from the different brands of mosquito coil used Baygon and Raid.

Liver enzymes following repeated exposures of experimental animals to the spray form of mosquito repellents (Rambo and Sharp) showed significant increase in the levels of liver enzymes (where  $P = 0.00$ , table 2), compared to the enzymes levels of animals exposed to coil repellents and normal control. Ordinarily, when liver cells are damaged, liver enzymes leak out of the cells into the blood stream indicated by raised serum levels of enzymes compared with normal control [26]. The raised levels of liver enzymes in serum is proportionate to the extent of tissue damaged [23]. This implies that liquid (spray) brands of Rambo and Sharp caused more severe liver damaged than coil forms of Wave tide and Baoma (Figure 1-3). Our finding agreed with the histopathological changes caused by liquid base mosquito repellents [25]. Therefore, our study indicated that spray form of mosquito repellents causes more severe liver injury compared to the coil form of mosquito repellents, indicated by gradual damage to the host liver tissues (Plates 2, 3, 6, 7, 10, 11). However, this result does not agree with recent report of Mamuna who reported that mosquito coil caused more severe liver damage compared to the spray form of mosquito repellents [27]. The active constituents of these mosquito repellents, duration of animal's exposure and species of experimental animals used could be possible sources of disparity.

## Conclusion

The results of our research have shown that exposure to mosquito repellents caused liver cell damage. Active agents and by-products in both the liquid and coil vapours of mosquito repellents are injurious to the liver with different degrees of damage. The liquid form of Rambo and sharp pose the worst damage revealing marked dilatation of the sinusoids, cytoplasmic vacuolation and apoptotic cells. Hence, prolong exposure may cause more damage to organs.

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## Conflict of Interest

None.

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