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# TOXICOLOGICAL EFFECT OF INHALED MOSQUITO INCENSE STICKS SMOKE ON THE HISTOLOGY AND BIOCHEMICAL RESPONSES IN EXPERIMENTAL RATS

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# ABSTRACT

This study was carried out to investigate the toxicological effects of four brands of mosquito incense stick smoke (DR insecticide stick (AS-1987), Incense upgraded formula (BM-102), Fragrance (B3-254) and Citronella (A7-435) on selected biochemical and histological profile of male albino wistar rats. Fifteen (15) wistar rats with mean weight (174.6 ±0.91g) were randomly divided into five (5) groups. Group I were considered as negative control while group II, III, IV and V were challenged with the brands of mosquito incense stick smoke for 8 hours daily by inhalation for 21 days. Laboratory investigations were carried out using standard protocols. It was observed that the activities of the liver enzymes (Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) increased significantly (p<0.05) in all the experimental rats when compared with the control. The histopathological alterations examined in the liver tissues of the experimental rats during the study period include mild necrosis, mild degeneration of hepatocytes and cellular infiltration from group III, IV and V respectively. Lung tissues of the challenged rats of group II, III and V revealed mild cellular infilterations around alveolar septa, hyperplasia and bronchiolar epithelial wall degeneration respectively. Kidney tissues revealed glomeruli distortion, mild haemorrhage and renal damage from group III and IV respectively. The alterations examined in the liver, kidney and lung tissues of the experiment rats were period dependent but independent of mosquito sticks brands which inferred that continuous exposure to mosquito stick by inhalation has the potentials to facilitate different pathological effect with multiple organs toxicity in the long run.

Keywords: Histopathology, liver enzymes, incense stick based-insecticides, albino wistar rats

# INTRODUCTION

Mosquito sticks are slow-burning devices which emit smoke containing one or more insecticides for hours. They are applied in close proximity to persons requiring protection against mosquitoes in order to prevent malaria, a disease of wide distribution (Abdullah *et al.*, 2017). The scourge of malaria had led citizens in low income communities to adopt the use of mosquito stick or coil to control the mosquito populations around residential areas because they are cheap and readily available (El-Demerdash, 2011).

The activities of mosquito stick have been demonstrated against *Aedes, Anopheles* and *Mansonia* (Lawrence and Croft, 2004). The health implications of burning one mosquito stick is equivalent to the release of the same amount of particulate matter as burning 75 to 137 cigarettes and emitting formaldehyde equivalent to 51 cigarettes (Chen *et al.*, 2008). The gas phase of mosquito stick smoke have been reported to contain carbonyl compounds (formaldehyde and acetaldehyde)

with properties that can produce strong irritating effects on the upper respiratory tract (Garba *et al.*, 2007).

Histopathological investigations have demonstrated that mosquito smoke leads to focal inflammation of the tracheal epithelium, metaplasia of the epithelial cells and morphological alteration of the alveolar macrophages (Garba *et al.*, 2007 and Fetoui *et al.*, 2010).

Moquito coil smoke is considered as a potent air pollutant in indoor environment and prolong exposure to the smoke could alter blood biochemical parameters in mice (Idowu *et al.*, 2016). For instance, exposure of the albino rats to pyrethroid-based insecticides smoke has been reported to cause significant increase in the liver enzyme (AST, ALT and ALP) activities with a decrease in the levels of total protein and serum albumin(Ugwu *et al.*, 2016). According to Abubakar and Hassan (2007), liver enzymes such as AST, ALT and ALP activities were significantly increased due to exposure to different brands of mosquito coil. Ugwu *et al.* (2016) reported increased in the levels of urea and creatinine in rats exposed to mosquito smoke. In view of the forgoing this research aimed at evaluating the toxicity profile of four new brands of mosquito incense sticks smoke on the histology and biochemical responses in experimental rats.

#### **Test Materials**

Four (4) new brands of mosquito incense sticks were procured from registered store at Sabongari market in Kano state, Nigeria. The country of origin NAFDAC number, Batch number and the list of active ingredient were obtained from the package. Measurement was obtained from the mosquito incense sticks such as diameter 3.5cm, 30cm for length and 1.6g for weight.

# MATERIALS AND METHODS

Table 1: Brands of Mosquito incense Sticks Used for the Research

Brand	Country	Active ingredient
DR insecticide stick (AS-1987)	Nigeria	Meperfluthrin 0.2%
Incense upgraded formula (BM-102)	China	Rich-d-transllethrin 0.25%
Citronella (A7-435)	India	Citronella
Fragrance (B3-254)	China	Pyrethrum

### **Experimental Animals**

A total of fifteen (15) adult male albino rats with mean weight of 174.6±0.91g were procured from animal room of Biological Science Department, Bayero University Kano and were kept in animal cage with a dimension of (40.20cm by 23.40cm by 18cm) at room temperate of  $\pm 28.7^{\circ}$ C and relative humidity of <30% with a 12 hour light/dark cycle. The animals were acclimatized on standard pelletized grower feed and allowed access to free drinking water for one week as adopted by Muhammed and Yakasai (2017).

### **Determination of Physical Changes in Animals**

The weight of experimental and control rats were taken before and after the study for any Physical and behavioral changes such as; body weight changes, loss of hairs, coughing and sneezing as adopted by Muhammed and Yakasai (2017).

# Experimental Design and Exposure to Mosquito Incense Sticks Smoke

The experimental rats were randomly grouped into five (5) groups that is I-V of three rats each. They were kept in undisturbed cages of size 40.20cm by 23.40cm by 18cm with 5 meters distance to each other and a cross ventilation for 8 hours daily for 21 days. The control group was not challenged to any mosquito stick smoke inhalation. Groups II-V was used for the sub chronic investigation as adopted by Garba et al. (2007). At the end of the exposure period, the experimental rats were sacrificed by humane killing as described by Organization for Economic Co-operation and Development (OECD) (2011).

#### **Histopathological Investigation**

The biopsies of rat's tissues (lung, liver and kidney) were carried out at Histopathology Laboratory, Aminu Kano Teaching Hospital, Kano-Nigeria using the method described by Ochei and Kolhatkar (2007). The experimental rats' tissues were euthanized with 40% ethyl alcohol before individually excising their lungs, liver and kidney for histopathological investigation. The tissues were fixed with 10% formal saline dehydrated with

ascending grade of alcohol, cleared with toluene, infiltrated with molten paraffin wax. Microtome sections (5µm) of the tissue were stained with Haematoxylin and Eosin stains and examined microscope (LEICA DM 750 model) with and photomicrographed with HD camera (LEICA ICE 50 model). **Biochemical Analyses** 

At the end of the exposure period of 21 days, the experimental rats were put to sleep with chloroform and prominent jugular vein was incised as adopted by Nafiu et al. (2020).Blood samples were collected from challenged and control group with heparinized plastic syringe, fitted with 21gauge hypodermic needle. The blood sample was collected in lithium heparin bottles and EDTA bottles for biochemical analysis (The aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activities). ALT, ALP, AST were determined at Biochemistry Laboratory, Bayero University Kano, using Randox kits as adopted by Ezenwaji et al. (2013).

#### **Statistical Analysis**

Statistical analysis was conducted using SPSS (Version 23.0). Data from the 3 replicates of the experiment was subjected to one-way Analysis of Variances of (ANOVA). Treatment means was separated using DMRT at 5% probability level. Probability level of less than 5% (p< 0.05) was considered significant.

#### RESULTS

# Toxicological implication of inhaled mosquito incense sticks smoke on Mean Body Weight

During the study period, there was significant difference (p<0.05) between the mean body weight of the rats challenged with group III mosquito incense sticks smoke when compared to the control group which recorded increase in weight (Table 1). With regards to the physical and behavioural changes at the beginning of the treatment, the experimental rats were examined to be active, with normal feeding on the first day. However, after sneezing, coughing and body weakness which continued for the changes was observed in the control group.

155.36±3.00

141.50±1.85

127.13±1.11

2 weeks exposure feeding habit decreased with regular remaining period of exposure. None of the above behavioural

 $-22.8\pm0.52$ 

 $-20.5 \pm 1.43$ 

-14.13±0.81

Table 1. Weah weight of Experimental Rats before and after Exposure with Mosquito incense Sticks Smoke					
Group ID	Mean weight before exposure	Mean weight after exposure	Mean weight gain/loss		
			after exposure		
Group 1	282.5±2.46	293.32±2.64	10.8±1.81		
(Contol)					
Group II	$166.40 \pm 1.50$	147.50±3.64	-18.9±0.31		
(DR)					

Table 1: Mean Weight of Experimental Rats before and after Exposure with Mosquito Incense Sticks Smoke

Group I= Control, II= DR insecticide stick (AS-1987), III =Incense upgraded formula (BM-102), IV= Fragrance (B3-254), V= Citronella (A7-435)

133.18±1.92

120.66±1.33

113.38±1.46

### **Biochemical Analysis**

Group III

(IGP) Group IV

(CIT) Group V

(FRG)

The mean liver AST of the experimental rats increased at 21 days when challenged to different brands of mosquito incense sticks smoke (Table 2). Lowest activity was obtained in the control group of  $32.70\pm1.02$  U/L and the highest of  $45.40\pm$ 0.71U/L was obtained in group V. The AST activity between control and treatment did not differ significantly (P>0.05).

The activity ALT was highest (56.41±0.97U/L) in experimental rats challenged in group III incense stick smoke (Table 2). The lowest values were observed at Group II, IV, and Group V

which 43.34±1.45U/L, 49.00±1.48U/L were and 48.70±8.37U/L respectively as against the control value of  $42.05 \pm 0.43$  U/L. Statistically there was no significant difference between the groups (P>0.05).

The activity of ALP also show no mosquito stick dependent in which the highest value obtained at Group III of 29.29±1.81u/l followed by the Group II with 23.20±1.83u/l and Group IV 22.24±1.24u/l respectively as against the lowest value from the control group of 21.70±0.62u/l. The value obtained differed significantly (P<0.05).

	Table 2: Mean Conce	ntrations of Biochemical	Indices of Experi	mental Rats Expose	d to mosquito sticks Smoke
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Group/parameters	AST (U/L)	ALT (U/L)	ALP (U/L)	
GROUP I	42.05 ±0.43 <sup>a</sup>	32.70±1.02 <sup>a</sup>	21.70±0.62ª	
GROUP II	$43.34{\pm}1.45^{a}$	33.70 ±0.11 <sup>a</sup>	23.20±1.83ª	
GROUP III	56.41±0.97 <sup>a</sup>	38.56±1.07 <sup>a</sup>	23.29±1.81ª	
GROUP IV	$49.00 \pm 1.48^{a}$	42.19±0.35 <sup>a</sup>	$22.24 \pm 1.24^{a}$	
GROUP V	48.70±8.37ª	45.40±0.71 <sup>a</sup>	$26.10\pm0.41^{d}$	

Means with same superscripts in a column did not differ significantly (p>0.05).

Sub-chronic toxicity revealed treatment-related tissue alterations in the experimental rats challenged with brands of mosquito incense sticks smoke. Liver tissue had normal appearance with normal hepatocytes, morphology, vacuolation and hepatic cord arrangement in the control samples without any discernible changes (Plate 1a). Histological analysis of the rat's liver challenged with mosquito incense sticks smoke for 21 days showed mild necrosis (plate 1b), degeneration of hepatocytes (plate 1c) and cellular infiltration (plate 1d) from group III, IV and V respectively.



Plate 1a Photomicrograph of normal liver tissue structure of wistar rat (H & E mag x 100)

Plate 1b Photomicrograph of wistar rat liver tissue challenged with group III mosquito incense stick smoke showing areas of mild necrosis (H & E mag x 100)



Plate 1c: Photomicrograph of wistar rat liver tissue challenged with group IV mosquito incense stick smoke showing areas of degeneration of hepatocytes (H & E mag x 100)

Plate 1d: Photomicrograph of wistar rat liver tissue challenged with group V mosquito incense stick smoke showing areas of cellular infilteration (H & E mag x 100).

Photomicrograph section of control rat's pulmonary tissues showed normal alveolar sacs architecture without any discernible lesions (plate 2a). Nevertheless, mosquito sticks smoke inhaled by experimental rats from group II, III and V revealed inflammatory cells disruption around alveolar septa (plate 2b), hyperplasia (plate 2c) and degeneration of bronchiolar epithelial wall (plate 2d) respectively.



Plate 2a Photomicrograph section of wistar rat lung showing normal lung tissue alveolar sacs architecture without any discernible lesions

Plate 2b Photomicrograph section of wistar rat lung challenged with group II mosquito incense stick smoke showing areas of inflammatory cells disruption around alveolar septa (H & E mag x 100)



Plate 2c Photomicrograph section of wistar rat lung challenged with group III mosquito incense stick smoke showing areas of hyperplasia (H & E mag x 100)

Plate 2d Photomicrograph section of wistar rat lung challenged with group V mosquito incense stick smoke showing areas of degeneration of bronchiolar epithelial wall (H & E mag x 100)

Photomicrograph section of wistar rat kidney challenged with different groups of insecticides mosquito incense stick smoke for 21 days revealed normal tissue architecture without any visible lesion in the control samples (Plate 3a) when compared with experimental groups. Glomeruli distortion (Plate 3b) and mild haemorrhage and renal damage (Plate 3c) were the significant changes examined in the kidney tissues of group III and IV rats. Statistically there is no significant difference (P>0.05) between experimental samples.

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Plate 3a: Photomicrograph of wistar rat kidney tissues architecture without any visible lesion(H & E mag x 100)

Plate 3b: Photomicrograph of wistar rat kidney tissues challenged with group III mosquito incense stick smoke showing areas of glomeruli distortion and group III (H & E mag x 100)



Plate 3c: Photomicrograph of wistar rat kidney tissues challenged with group IV mosquito incense stick smoke showing areas of mild haemorrhage and renal damage (H & E mag x 100)

# DISCUSSION

The present findings revealed that crucial liver biomarkers (AST, ALT and ALP) in the blood of all the experimental rats were altered significantly (P < 0.05) when challenged with mosquito incense stick smoke. Increased activity of these hepatic enzymes when compared with control could be associated with hepatic damage as reported by Idowu *et al.* (2013) and Abdullah *et al.* (2017). This might be due to the effect of active ingredient in the mosquito incense sticks smoke allethrin or its by-product pyrethrum derived from pyrethroid metabolism on the membrane phospholipids of hepatocytes as obtained in group III incense sticks.

Allethrin has been reported to increase membrane fluidity in a living cell which allows passing through cellular enzymes within the extracellular matrix which facilitate transaminases activity in blood (Akunna *et al.*, 2013 and Abdullah *et al.*, 2017). Similar observations were reported by Karthikeyan *et al.* (2006) and Anvita *et al.* (2006) in mice, exposed to mosquito repellent mat vapour. In the present study, increase in the activity of ALT,

AST and ALP in the challenged groups insecticides sticks smoke especially group III, IV and V could also be attributed to hepatic injury, myocardial infarction and cardiovascular diseases as reported by Narendra *et al.* (2007). This is in tandem with the finding of Abubakar and Hassan (2007) who reported increased in liver enzymes (ALT, AST and ALP) due to exposure to different brands of mosquito coil (Swam, Rambo and Cork) smoke. Mossa *et al.* (2013) depicted that increased level of serum enzymes (ALT, AST and ALP) were due to exposure to pyrethriod-based insecticides and combination of different pyrethriod-based insecticides (Tetramethrin and Sumithrin).

The increased activity of liver enzymes in the experimental rats exposed to group II, III, IV and V might also be due to the presence of toxic chemicals in incress stick smoke that possibly interfere with catalytic interconversion of amino acids and  $\alpha$ -ketoacids by amino group in ALT as reported by Molina *et al.* (2005). During the study period, liver histology of experimental rats revealed hepatic disruption characterized by mild necrosis,

degeneration of hepatocytes and cellular infiltration. The mild necrosis could be due to intracellular accumulations within the liver tissues as reported by Muhammad *et al.* (2014) and Abdullah *et al.* (2017). Histological alterations identified in the liver tissues of group II, III, IV and V mosquito incense sticks smoke inferred that it contains toxic compounds as reported by Sani and Ibrahim (2016). The hepatic cellular infiltrations examined in group III mosquito incense stick could be attributed to the inflammatory reaction in the liver cells as reported by Uthman *et al.* (2016).

Histological studies revealed mild lung damage evidenced by inflammatory cells disruption around alveolar septa, hyperplasia and degeneration of bronchiolar epithelial wall in challenged rats when exposed with group II, III, IV and V mosquito sticks smoke. Disruption of inflammatory cells around alveolar septa, hyperplasia might be due to inflammatory processes taking place as a result of irritation of pulmonary tissues by toxic chemicals from the incense sticks smoke as reported by Idowu *et al.* (2013), and Ghani and Shahbaz (2014). Similar observation was reported by Abubakar and Hassan (2007) in their work on toxicological effects of some mosquito coils brands in experimental rats. The body weakness experienced by the experimental rats may perhaps be due to toxic chemicals in mosquito sticks smoke.

Abdullah *et al.* (2017) reported the presence of carbon particles, heavy metals and aldehydes in the peripheral region of lung which leads to cellular injury and mucus membrane destruction in mice model exposed to mosquito coil. In the present findings, degeneration of bronchiolar epithelial wall might cause reduced oxygen carrying capacity of erythrocytes, leading to reduced cellular metabolism which may explain the body weakness experienced by the experimental rats as reported by Okine *et al.* (2004) and Yuonis *et al.* (2015). Epidemiological studies have shown that long-term exposureto mosquito coils smoke can induce asthma and persistent wheeze in children (Pankaj and Prahlad, 2004).

Damage to the kidney tissues with group III and IV mosquito sticks smoke could be due to decline in erythropoietin production which leads to high urea within kidney tissue and could lead to glumeruli distortion as reported by Nwanio et al. (2005). This may perhaps revealed degeneration of fat as a result of alteration in metabolic activities within the kidney tissues. Similar observation was reported by Liu et al. (2003). Mild haemorrhage haemorrhage and renal damageexamined in the kidney tissues with group III mosquito sticks smoke may be due to Inflammatory response the kidney cells are exposed to or due to the presence of irritant in the insecticides incense stick such as pyrethrins and sulphates which have been reported to induce inflammatory response in kidney tissues (Abubakar and Hassan, 2006). Taiwo et al. (2008) reported severe multifocal congestion, interstitial mononuclear cellular infiltration and wide spread fibrosis in kidney tissues of exposed rats to mosquito coil.

# CONCLUSION AND RECOMMENDATIONS

From the present finding it can be concluded that inhaling smoke from burning new brands of mosquito incense stick containing meperfluthrin, pyrethrum and allethrin for long term may not be safe as it revealed different pathological effect (biochemical and histopathological) in experimental rats. It is therefore recommended that further research should be carried out to assess the mechanism of toxicity, reversibility or irreversibility of the damage. Further study should also look into the effects of the new brands of mosquito incense stick smoke using other bioindicators of pollution.

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