

Toxicological effects of prolonged and intense use of mosquito coil emission in rats and its implications on malaria control

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Abstract: Efectos toxicológicos del uso prolongado e intenso de emisiones de espirales contra mosquitos en ratas y sus implicaciones sobre el control de la malaria. Mosquito coil is a vector control option used to prevent malaria in low income counties, while some studies have addressed this issue, additional research is required to increase knowledge on the adverse health effects caused by the prolonged use of coils. In this study we investigated the toxicological effects of fumes from two locally manufactured mosquito coil insecticides (with pyrethroids: transfluthrin and d-allethrin as active ingredients) on male albino rats. For this, we recorded the haematological and biochemical indices, and made histopathology and mutagenicity evaluations in rats exposed to mosquito fumes during 2, 4, 8, 12 and 16 week periods. Haematological determination was performed using automated hematology analyzer to determine White Blood Cell (WBC), Packed Cell Volume (PCV), Red Blood Cell (RBC) and Platelet (PLT) counts, while biochemical evaluations were determined using available commercial kits. Gross histopathological changes were studied for the kidney, liver and lungs in sacrificed rats. The rat sperm head abnormalities assessment was used to evaluate mutagenicity. Mosquito coil fumes produced significant increase ($P<0.05$) in the levels of total protein, total albumin and bilirubin, when animals were exposed from two weeks to 16 weeks with transfluthrin. Similarly, elevation in the activities of aspartate amino transferase, alanine amino transferase and alanine phosphatase, increased significantly in both insecticides. Increase in WBC, RBC and PCV were recorded for all the exposure periods, however PLT count showed no significant increase ($P>0.05$). Mutagenicity assessment revealed sperm abnormality was statistically significant ($P<0.05$) compared with the control at 8, 12 and 16 weeks post exposure to transfluthrin. Histological studies revealed severe lung damage evidenced by interstitial accumulations, pulmonary oedema and emphysema in exposed rats. Intracellular accumulations and severe sinusoidal congestion of liver cells were observed from 12 weeks exposure, indicating liver damage. Our studies indicate that mosquito coil fumes do initiate gradual damage to the host. These pathological effects must be taken into consideration by the malaria control program, particularly when regulating their long term and indoor usage. *Rev. Biol. Trop.* 61 (3): 1463-1473. Epub 2013 September 01.

Key words: mosquito coil, insecticide, hematology and biochemical parameters, prolong usage, mutagenicity, histopathology.

Malaria is a public health problem in many countries of the world especially in tropical and subtropical regions. An 85% of estimated annual 225 million cases of malaria worldwide are caused by *Plasmodium falciparum* and *P. vivax*, and are recorded within the African region. It also accounts for approximately one million deaths annually and 89% of the malaria death occurring in Africa South of the Sahara (WHO 2010). In Nigeria, malaria is highly

endemic accounting for 60% of outpatient visit (Anonymous 2010a).

The methods employed in the control of malaria are solely based on the administration of antimalarial drugs and vector control which include utilization of Insecticide Treated Net (ITN), Long lasting Insecticide mosquito Nets (LLIN) and Indoor Residual Spray (IRS) as recommended by the World Health Organization (WHO). However, these options have been



set back largely due to development of resistant parasites and insecticide resistant strains of mosquitoes (Wernsdorfer 1994, Trigg & Wernsdorfer 1999, Chandre *et al.* 1999, Awolola *et al.* 2003, Nwane *et al.* 2009).

Mosquito coils (mosquito repelling insecticides) are essentially made up of pyrethrum powder and are widely used in Asia, Africa and South America, particularly among the low income earners, because of its affordability (Mulla *et al.* 2001, Weili *et al.* 2003). The activities of mosquito coil have been demonstrated against *Aedes*, *Anopheles* and *Mansonia* (Yap *et al.* 1996, Lawrence & Croft 2004). The health implications of burning one mosquito coil 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, Liu *et al.* 2003).

Histopathological investigations have demonstrated that mosquito fumes leads to focal deciliation of the tracheal epithelium, metaplasia of the epithelial cells and morphological alteration of the alveolar macrophages (Liu & Sun 1988, Liu & Wong 1987, Cheng *et al.* 1992, Chang & Lin 1998). Kidney tissues of exposed rats have revealed severe multifocal congestion, cystic dilation in the medulla, interstitial mononuclear cellular infiltration and wide spread fibrosis (Garba *et al.* 2007a, Taiwo *et al.* 2008), while damage to spleen revealed severe sinusoids hyperplasia and regression of red and white pulps (Garba *et al.* 2007b).

Elevated levels of urea and creatinine have been reported in rats exposed to mosquito smoke (Garba *et al.* 2007a) and significant increase in white blood cell count Garba *et al.* 2007b). Mutagenicity effect of mosquito coil smoke have been reported to cause chromosomal aberrations in metaphases and a significantly higher incidence of chromosomal aberration frequency in exposed rats and mice (Das *et al.* 1994, Moorthy & Murthy 1994). Besides, long term exposure to mosquito coil smoke has been demonstrated by some workers to induce asthma and persistent sneezing

in children (Azizi & Henry 1991, Fagbule & Ekanem 1994, Koo & Ho 1994).

Presently, the use of mosquito coil as a control option is widespread in Nigeria, despite the fact that inhaling mosquito coil fumes may have potential adverse health effects. Here we investigated the histopathological alteration on various organs, the changes in haematological indices and the mutagenicity induced in mice with a model of prolonged use of coil.

MATERIALS AND METHODS

Tests materials: Two different mosquito coils were sourced from Nigerian markets and were used for the experiment namely Baygon and Raid. Baygon brand (containing 0.03% transfluthrin and 99.7%w/w inert ingredients) manufactured by Turare N'Hausawa Ltd. in Kano State. Raid brand (containing pyrethroids (d-allethrin) 0.2%w/w and other ingredients 99.8%) manufactured by Johnson Wax Nigeria Ltd, Isolo, Lagos State.

Test animals: A total of 60 albino rats (*Rattus rattus norwegicus*), all adult males weighing 210±30g were used for this study. The rats were purchased from the Animal Facility Centre of Biochemistry Department, Nigerian Institute of Medical Research, Yaba, Lagos. The rats were kept in the Zoological Garden of the University of Lagos for two weeks to acclimatize to their new environment, and were observed for physical deformity or any ailment that may render them unfit for the study. The exposure to the insecticide coil, namely Baygon and Raid brands, was performed in two different rooms, which were far apart such that the emission driven in one room did not affect the other. The animals were kept in locally fabricated cages with a size of 30x60cm=180sqcm². The cages were made of plastic at the base and sides, and the dimension of wire mesh used to cover the cages were 0.5x0.5cm (1/4"x1/4") square openings. The animals were kept at a room temperature of 30±3°C and a relative humidity of about 35% with a 12hr light/dark cycle. They had

access to drinking water and standard laboratory diet *ad libitum*.

Experimental design: For the exposure of two different mosquito coils (transfluthrin and d-allethrin), the rats were divided into six groups. The rats were kept in different plastic boxes of four rats each. The rats in groups one to five were exposed, while those in group six were unexposed and served as the control group. The rats in group one were exposed for two weeks, group two for four weeks, group three for eight weeks, group four for twelve weeks, and group five for 16 weeks; after exposure, all rats were slaughtered. The rats in group 6 were not exposed to the fumes at all; each time rats from any group were slaughtered, two rats from the control group were also slaughtered for comparison. The rats in groups one through five were kept in a separate location from the control, to prevent accidental or low level exposure.

The cages were demarcated with wire mesh to allow a small space for the mosquito coil which was in a separate compartment from the rats to prevent the rats from knocking over the coil and starting a fire, but allowing the smoke to penetrate and saturate the box allowing maximum exposure of the rats to the fumes. The box was covered round about and a wire mesh on a window cut on top of the box to allow for ventilation. They were exposed for eight hours a day for the period of study, as an attempt to mimic average period of time that man sleeps in a day (Anonymous 2012b).

The rats in each group were observed for any clinical signs associated with the exposure to the active ingredient from the fumes. The rats were killed using the method of spinal paralysis and dissected to obtain the kidneys, lungs and liver for histopathological studies while 3mL of blood samples were obtained for determination of haematological and quantitative determination of total Protein (PR), Albumin (ABL), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), Serum Bilirubin (BIL) and blood Urea nitrogen (UR).

Haematological determination: Blood samples were collected in appropriate heparinised blood containers for determination of the Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (RBC) using automated hematology analyzer (Minray Model BC 3200).

Biochemical analysis: The biochemical assessment was done with rat blood samples. Alanine aminotransferase level was measured by commercially available standard blood ALT kit by Randox Reitman and Frankel ALT level 2 control (cat. No. SC 2643). The method of Young (1975), was used to determine alkaline phosphatase (ALP). The BCG method of Doumas *et al.* (1985) was used to estimate Albumin. The plasma urea level was measured by commercially available standard blood urea kit (Maizy, France) by Biolabo South Africa. The plasma creatinine level was measured by commercially available standard blood creatinine kit (Maizy, France) by standard protocol for photometric determination of creatinine based on Jaffe kinetic method. The biuret method was used for the measurement of protein level. The Sulfanilic acid method of Tietz & Shuey (1993) was employed for the estimation of bilirubin.

Mutagenicity determination: Mutagenicity was determined by examination of the sperm head abnormalities after testicles dissections that followed the methods of Wyrobek & Bruce (1975). The control and experimental groups were in replicates (A and B). A thin smear of the spermatozoa was made on a slide and fixed with 95% ethanol and allowed to air dry for about 5-10min. The smear was washed with sodium bicarbonate formalin solution to remove any mucus that may be present. The slide was rinsed several times in water and the smear covered with dilute carbon fuels in solution (1:20), and allowed to stain for about 3min, washed off with water subsequently stained again with dilute Loeffler's methylene blue. The slides were then coded, randomized and examined cytologically under X100 oil immersion binocular light microscopy. Three

separate slides were prepared for each group out of which two slides were selected for scoring using the tally counter. For each group, 800 sperm cells were assessed for morphological aberration according to the criteria of Wytobek & Bruce (1975). Mutagenicity assessment was only carried in animals exposed to d-allethrin.

All data from this study were expressed as mean±standard error. A probability level of less than 5% ($p < 0.05$) was considered significant in all instances. Statistical software version 5.03 (1992-2010).

RESULTS

Haematological indices: The effects of mosquito coil smoke inhalation on haematological indices are presented in table 1. RBC and PCV increased in all groups exposed to mosquito coil smoke and was observed to be significant ($p < 0.05$) in the groups of rats exposed for two and eight weeks and not significant ($p > 0.05$) for 12 and 16 weeks when compared with the control for rats exposed to transfluthrin. However rats exposed to d-allethrin were observed not to be significant ($p > 0.05$) for two and four weeks but significant ($p < 0.05$) as from eight to 16 weeks when compared with control.

Platelets counts showed no significant change ($p > 0.05$) for various experimental groups when exposed rats were compared to

non-exposed rats for both transfluthrin and d-allethrin.

WBC counts increased in all the exposed groups when compared with control groups, and was not significant for all groups of rats exposed to transfluthrin ($p > 0.05$), but significant ($p < 0.05$) for all groups exposed to d-allethrin from four to 12 weeks.

Biochemical assessments: Table 2 presents some biochemical assessment of mosquito coil emission in albino rats. AST, ALP and ALT levels increased significantly in all animals groups exposed (two to 16 weeks) to mosquito coil smoke ($p < 0.05$) when compared with the control animals for both transfluthrin and d-allethrin.

Significant levels ($p < 0.05$) of total protein, albumin and creatinine were recorded for rats exposed to transfluthrin when all experimental groups were compared with control animals. Serum levels of creatinine, albumin and total protein were not significantly affected for animals exposed to d-allethrin.

Blood urea levels were not significant for all groups of rats exposed to d-allethrin and two and four weeks exposed to transfluthrin but significant for eight and 16 weeks when they were compared with the control. Bilirubin level were increased significantly ($p < 0.05$) in the groups of rats exposed to transfluthrin mosquito coil

TABLE 1

Effect of mosquito coil emission on hematological indices in albino rats exposed to transfluthrin and d-allethrin

| Groups (Duration of exposure) | Insecticides | | | | | | | |
|-------------------------------------|---------------|------------|-------------|------------|-------------|------------|-------------|------------|
| | Transfluthrin | | | | d-allethrin | | | |
| | Parameters | | | | Parameters | | | |
| | WBC | RBC | PCV | PLT | WBC | RBC | PCV | PLT |
| Control | 8.3±0.1 | 6.85±0.15 | 47.50±1.5 | 300±50.0 | 8.55±0.50 | 7.60±0.10 | 45.15±1.25 | 731.5±1.25 |
| 2 Weeks | 10.10±1.0 | *8.55±0.25 | *55.0±0.5 | 355±5.0 | 9.75±0.05 | 8.60±0.2 | 49.25±0.05 | 732.0±1.00 |
| 4 Weeks | 12.5±1.5 | *9.06±0.26 | *57.50±0.50 | 451.5±6.50 | *14.30±1.0 | 8.25±0.05 | 45.75±1.55 | 732.5±0.50 |
| 8 Weeks | 10.75±0.25 | *9.15±0.05 | 66.50±1.5 | 425±25 | *14.50±0.1 | *8.55±0.05 | *52.05±0.05 | 729.5±0.50 |
| 12 Weeks | 9.80±0.30 | 9.25±0.25 | 72±1.0 | 175±25 | *11.10±0.10 | *10.3±0.15 | *53.50±0.10 | 730.5±0.50 |
| 16 Weeks | 7.90±0.40 | 10.45±0.15 | 76.5±1.5 | 225±25 | 9.90±0.10 | 11.10±0.10 | *54.05±0.05 | 731.5±0.50 |

WBC=White blood cell, RBC=Red blood cell, PCV=Packed cell volume, PLT=Platelets.

* Significant difference ($p < 0.05$).

TABLE 2
Biochemical assessment of mosquito coil emission in albino rats exposed to d-allethrin and transfluthrin

| Insecticides | Groups (Duration of exposure) | AST (μ/L) | CRE (μmol/L) | ALT (μ/L) | UR (mmol/L) | ALB (g/L) | ALP (μ/L) | BIL (μmol/L) | PR (g/L) |
|---------------|-------------------------------|-----------|--------------|-------------|-------------|-----------|-----------|--------------|------------|
| d-allethrin | Control | 34.0±0 | 70.5±8.5 | 79.0±1.0 | 7.5±0.5 | 36.0±0.0 | 15.5±0.5 | 2.50±0.5 | 84.5±0.5 |
| | 2 | *39.0±0.5 | 61.0±1.0 | *83.0±1.0 | 6.0±0.0 | 37.0±0.0 | *18.5±0.5 | 3.0±0.0 | 82.0±2.0 |
| | 4 | *40.5±0.5 | 62.0±0.0 | *84.5±0.5 | 6.5±0.5 | 37.0±1.0 | *19.5±0.5 | 3.0±0.0 | 85.0±1.0 |
| | 8 | *42.0±1.0 | 64.0±2.0 | *86.0±0.0 | 7.0±0.0 | 38.0±1.0 | *20.5±0.5 | 5.0±1.0 | 84.0±1.0 |
| | 12 | *42.5±0.5 | 64.0±0.0 | *88.5±0.5 | 7.5±0.5 | 35.0±1.0 | *26.5±0.5 | *9.0±1.0 | 84.0±4.0 |
| | 16 | *43.0±1.0 | 65.5±0.0 | *90.50± 0.5 | 8.0±0.0 | 35.0±0.0 | *29.0±1.0 | *10.5±1.5 | 80.5±0.5 |
| Transfluthrin | 2 | *65.0±3.0 | *66.5±1.5 | *55±5.0 | 6.6±1.0 | *48.0±1.0 | 13.5±2.5 | 2.7±0.0 | *87.5±2.5 |
| | 4 | *73.5±1.5 | *73.6±2.2 | *124.9±2.6 | 7.8±0.3 | *57.2±1.9 | *25.0±2.0 | *7.0±0.0 | *86.2±0.9 |
| | 8 | *75±3.0 | *144.0±4.0 | *132.9±8.5 | *9.6±1.0 | *58.5±0.5 | *28.7±0.7 | *7.5±0.5 | *103.5±1.5 |
| | 12 | *77±2.0 | *189.0±1.0 | *138.5±2.5 | *10.2±1.4 | *54.5±2.5 | *26.0±2.0 | *10.0±1.0 | *105.0±4.0 |
| | 16 | *82.5±1.5 | *276.0± 3.0 | *140.0±6.0 | *2.5±0.0 | *55.5±2.5 | *22.5±0.5 | *12.0±0.0 | *104.0±1.0 |

AST=Aspartate aminotransferase, CRE=Creatinine, ALT=Alanine aminotransferase, UR=Urea, ALB=Albumin, ALP=Alanine phosphatase, BIL=Bilirubin, PR=Protein. *Significant difference (p<0.05).

smoke for four and 16 weeks, and 12 and 16 weeks for rats exposed to d-allethrin.

Histopathological findings: The histopathology for the two mosquito coil (transfluthrin and d-allethrin) used followed the same pattern for the organs investigated namely kidney, liver and lungs.

Kidney: Kidney tissue of albino rats exposed to mosquito coil smoke showed no morphological changes until eight weeks, when mild interstitial congestion were first observed, however, full congestion around the glomerular tuft were observed in the 16 weeks post exposure (Fig. 1A). Figure 1B evidenced the control albino rats showing normocellular glomerular tufts in a background of tubules with cuboidal cell epithelial lining with no necrosis.

Liver: Histological appearance of liver tissues exposed to mosquito coil smoke for two weeks showed extensive intracytoplasmic accumulations and moderate hydropic change. The liver tissues of rats exposed for four weeks showed generalized intracellular accumulations, and the cytoplasm appeared

frosted and granular; there was also a mild hydropic change. At eight weeks of exposure, the liver tissues showed preserved architecture hepatocytes displayed as radiating plates and uniformly eosinophilic cytoplasm, while liver tissues of experimental animals exposed for 12 and 16 weeks, showed generalized intracellular accumulations and severe sinusoidal congestion (Fig. 1C). Histological appearance of liver tissue of control animals showed normal hepatocytes, arranged as plates, nucleus surrounded by a nuclear membrane and nucleolus (Fig. 1D).

Lungs: Histological appearance of lung tissues of animals exposed to mosquito coil smoke for two weeks showed mixed inflammatory cells, giant cell reaction, stromal fibrosis, and four weeks group showed inflammation and congestion of the interstitium, and hyperplasia of peribronchial lymphoid aggregates. At 16 weeks of exposure, the lung tissues showed peribronchial lymphoid hyperplasia, interstitial inflammation and congestion with pulmonary oedema (Fig. 1E). Fig. 1F shows histological appearance of control lung tissue.

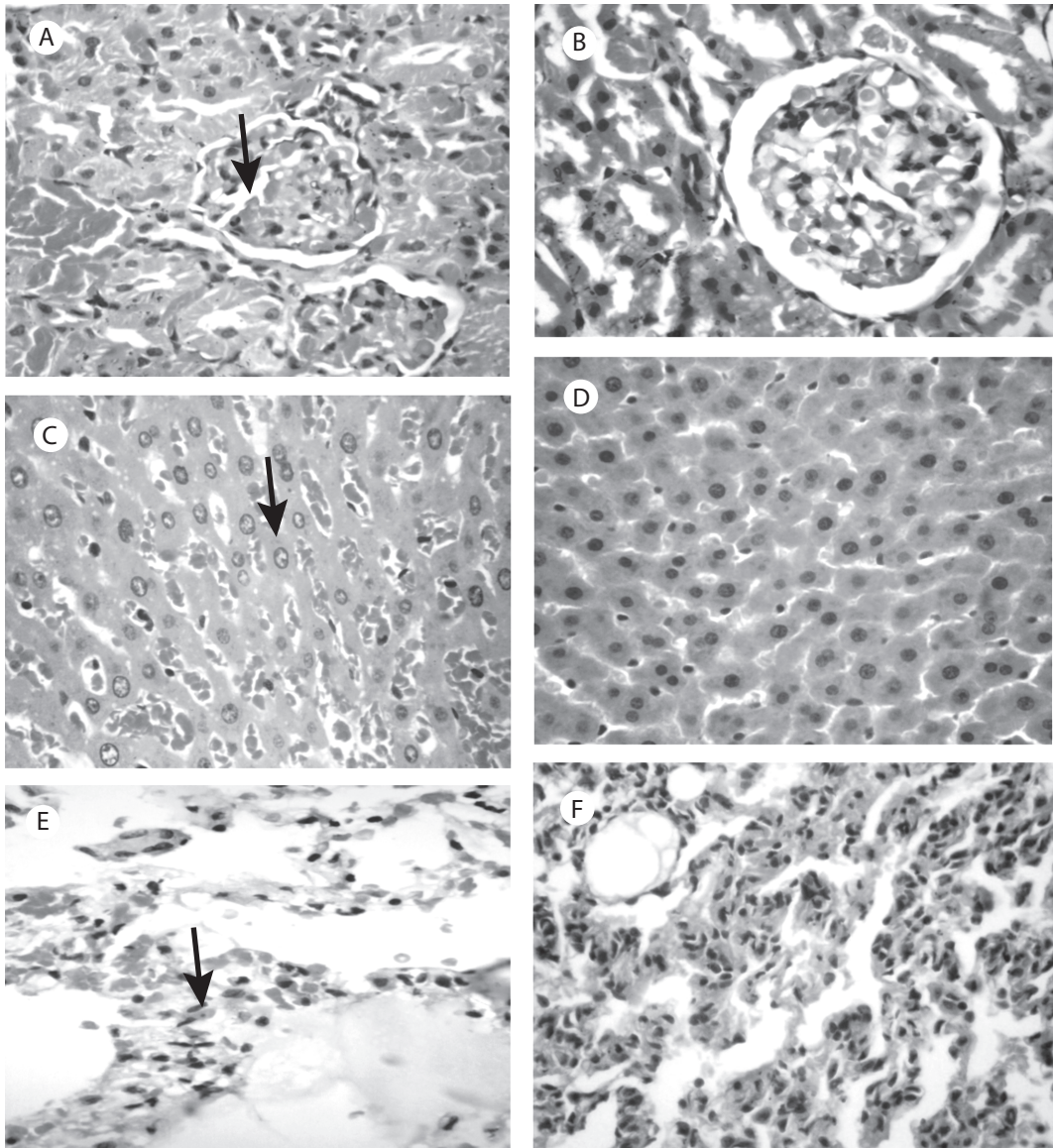


Fig. 1. A. Kidney tissue of albino rat exposed to mosquito coil fumes for 16 weeks showing congestion around the glomerular tuft. B. Kidney tissues of control albino rats showing normocellular glomerular tufts in a background of tubules with cuboidal cell epithelial lining with no necrosis. C. Liver tissue of albino rat exposed to mosquito coil smoke for 16 weeks showing severe sinusoidal congestion. D. Liver tissue of the control albino rat showing normal hepatocytes arranged as plates. The cytoplasm is eosinophilic. E. The lung tissue of albino rats exposed to mosquito coil smoke for 16 weeks showing: peribronchial lymphoid hyperplasia, interstitial inflammation and congestion pulmonary oedema. F. Lung tissue of control albino rat showing normal peribronchiallymphoid follicles not enlarged (H&E)x400.

Mutagenicity assessment: Nine different forms of sperm head abnormality were observed from sperm squashed in the sperm head abnormality assay. The abnormal sperm showed rudimentary tail, bent mid-piece, curved mid-piece, bent tail, curved tail, normal head without tail/tailless head, normal tail without head/headless tail, coiled tail and looped tail. Table 3 shows the mean and percentage of normal sperm cells and abnormal sperm cells of control and experimental animals. There was no statistical difference in the percentage of sperm head abnormality of animals exposed to mosquito coil smoke during two and four weeks ($p>0.05$), when compared with that of the control, however for eight, 12 and 16 weeks post exposure, it was resulted highly significant ($p<0.05$).

DISCUSSION

The significant increase in RBC and PCV in rats exposed to transfluthrin at two and four weeks and d-allethrin at two to 12 weeks, may be due to cyanide which is a by product of mosquito coil smoke which is known to cause reduction in oxygen carrying capacity of RBC leading to reduced metabolism. The reduction of oxygen stimulates erythropoietin which in turn stimulates the bone marrow to produce RBC. These findings are in agreement with earlier workers (Parker *et al.* 1984, Schoeinig

1995, Shakouri *et al.* 1992, Garba *et al.* 2007a) which similarly observed increase in RBC and PCV in rats exposed to pyrethrins however it is contrary to work of Saka *et al.* (2011) which demonstrated no significant increase in haematological parameters (WBC, RBC and PLT) when they exposed rats for one to three daily hours; however, the present study exposed the rats for eight hours daily mimicking daily exposure of man to mosquito coil in normal setting. Since the study further reveals the RBC and PCV levels dropped and became insignificant compared with control at 16 weeks exposure to both transfluthrin and d-allethrin insecticide, it can be concluded that haematological parameters observed do not suggest anemia as a risk factor in mosquito coil exposure. However marked increase in WBC recorded in rats exposed to d-allethrin in this study confirm observation of Garba *et al.* (2007a).

The elevated activities of AST, ALT ALP enzymes observed in rats exposed to both transfluthrin and d-allethrin insecticides suggest hepatic damage or dysfunction; high concentrations of these enzymes in hepatic tissues of dogs, cats and primates have been linked to hepatocellular damage (Kaneko & Cornelius 1980, Hall *et al.* 2001). This was confirmed by histopathological examination of rats exposed to transfluthrin and d-allethrin mosquito coil smoke for eight, 12 and 16 weeks in this study, which indicates

TABLE 3
Percentage Sperm head abnormality of control and test animals exposed to d-allethrin

| Sperm Head Abnormality Number | Groups (Duration of Exposure in Weeks) | | | | | | | | | | |
|-------------------------------|--|-------|-------|-------|-------|--------------|-------|-------|-------|-------|-------|
| | Control Animals | | | | | Test Animals | | | | | |
| | Groups | 2 | 4 | 8 | 12 | 16 | 2 | 4 | 8 | 12 | 16 |
| NSC | A | 551 | 542 | 516 | 508 | 474 | 555 | 553 | 485 | 403 | 321 |
| | B | 550 | 553 | 519 | 492 | 471 | 551 | 552 | 489 | 422 | 323 |
| | Mean | 550.5 | 547.5 | 517.5 | 500.0 | 472.5 | 553.0 | 552.5 | 487.0 | 412.5 | 322 |
| | % of NSC | 68.81 | 68.44 | 64.69 | 62.50 | 59.06 | 69.13 | 69.06 | 60.88 | 51.56 | 40.25 |
| ASC | A | 245 | 247 | 284 | 292 | 245 | 249 | 258 | 315 | 397 | 479 |
| | B | 249 | 248 | 281 | 308 | 249 | 258 | 247 | 311 | 378 | 477 |
| | Mean | 247.0 | 247.5 | 282.5 | 300.0 | 247.0 | 253.5 | 252.5 | 313.0 | 387.5 | 478.0 |
| | % of ASC | 30.88 | 30.94 | 35.31 | 37.50 | 30.88 | 31.69 | 31.56 | 39.13 | 48.44 | 59.75 |

NSC=Normal Sperm Cell, ASC=Abnormal Sperm Cell.

generalized intracellular accumulations and severe sinusoidal congestion, which also have been demonstrated (Garba *et al.* 2007b, Taiwo *et al.* 2008, Aslam *et al.* 2010). This study therefore demonstrates potential damage to the liver arising from the use of transfluthrin and d-allethrin mosquito coil. Fetoui *et al.* (2010) demonstrated increase in the enzymatic activities of aminotransferases AST and ALT when exposed to Lambda-Cyhalothrin (pyrethroid) which is ameliorated with co-administration of vitamin C, similarly Cypermethrin, a synthetic pyrethroid insecticide, have been shown to increase liver enzymes levels of broiler chicks which were ameliorated by combination of Vitamin E and selenium (Aslam *et al.* 2010). It is therefore suggested that the administration of Vitamins C and E, may reduce liver damage and may be recommended as routine intakes for those using pyrethroid based insecticide for mosquito control.

The inflammation resulting from damage to the liver cells, which are sites of the protein synthesis, may lead to the increase level of plasma protein. The coil smoke could lead to elevated plasma urea levels; the elevation could probably be due to the increase in activities of urea enzymes, ornithine carbomoyl transferase and arginase, which provide evidence of liver damage in many animal species. The concentration of total protein, total albumin and creatinine which are a measure of liver and kidney function, are significantly higher when compared to the control groups in rats exposed to transfluthrin, while the reverse is the case in animal exposed to d-allethrin. Exposure to transfluthrin also demonstrated significant levels of bilirubin and blood urea in some exposed groups which agrees with the findings of (Liu *et al.* 1989, Garba *et al.* 2007b) the observation of this study therefore suggest that transfluthrin can cause more damage to the liver and kidney than raid but these differences is not noticeable in histological findings.

Histopathological evaluation of mosquito coil effect had shown the impact on the kidney, 16 weeks post exposure, which demonstrates full congestion around the glomerular tuft,

the study agrees with Taiwo *et al.* 2008 which demonstrated glomerula and tubular degeneration, necrosis, thrombosis and vasculitis to mosquito coil and varying insecticidal spray fumes in experimental rats. The study is also in line with earlier published work of Garba *et al.* 2007a which demonstrated serve multifocal congestion, cystic dilation in the medulla of kidney tissue exposed to pyrethroid based mosquito coil.

The lung tissue of rats exposed to mosquito coil smoke for the longest period (16 weeks) showed peribronchial lymphoid hyperplasia, interstitial and congestion pulmonary oedema; this observation has been documented by Okine *et al.* 2004. Oedema could have resulted from the inflammatory processes taking place as a result of irritation of various organs by toxic chemicals from coil smoke. Other pathological manifestation that has been associated with pyrethroid mosquito coil but not observed in this study, includes exudative pneumonia, anthracosis, thrombosis and vasculitis, as observed by Taiwo *et al.* 2008. The sneezing that resulted at the initial stage of exposure could be the result of irritants released in the coils smoke such as aldehydes, sulphates and polycyclic aromatic hydrocarbons such as acenaphthene, penanthrene, benzo(a)pyrene (EPA 1998, Liu *et al.* 2003).

The present study demonstrates that mutagenicity was induced and was statistically significant as from eight weeks exposure to mosquito coil smoke. The abnormal spermatozoa can therefore cause significantly reduction in the reproductive potential of the male rats. High frequency of chromosomal aberration in metaphase have been reported in rats and mice exposed to mosquito coil (Das *et al.* 1994, Moorthy & Murthy 1994). The potential of induction of mutagenicity of mosquito coil in humans is therefore a major concern, because it could account for infertility in males, as was also demonstrated by Madhubabu & Yenugu (2012) in a study of the effect of continuous inhalation of allethrin based mosquito smoke in the male reproductive tract of rats. The findings of this preliminary studies therefore

demands further investigation on experimental mice and human.

Malaria control heavily rely on the use of pyrethroids, however the use of pyrethroids in form of mosquito coil is an available option especially to the rural populace. Control effort aiming at reducing man contact with the smoke should be encouraged in view of histopathological damage and the mutagenicity it can produce due to long term exposure.

RESUMEN

Las espirales contra los mosquitos se utilizan en los países de bajos ingresos como una opción para prevenir la malaria controlando el vector de esta enfermedad. A pesar de que algunos estudios han abordado este tema, se requiere más investigación para incrementar el conocimiento sobre los efectos adversos en la salud, causados por el uso prolongado de las espirales. En este estudio se investigaron los efectos toxicológicos de los gases de las espirales a partir de dos insecticidas fabricados en el país (con piretroides: transflutrina y d-aletrina como ingredientes activos) en machos de ratas albinas. Para esto, se registraron los índices hematológicos y bioquímicos, y se hicieron evaluaciones histopatológicas y de mutagenicidad en ratas expuestas a los gases de las espirales durante periodos de 2, 4, 8, 12 y 16 semanas. La determinación hematológica se realizó mediante un analizador de hematología automatizado para determinar el conteo de los Glóbulos Blancos (WBC), el Hematocrito (PCV), Glóbulos Rojos (RBC) y las Plaquetas (PLT), mientras que las evaluaciones bioquímicas se determinaron utilizando kits comerciales disponibles. Los cambios histopatológicos fuertes se estudiaron en el riñón, el hígado y los pulmones de ratas sacrificadas. Las anomalías en la cabeza de los espermatozoides de las ratas se utilizaron para evaluar la mutagenicidad. El humo de las espirales contra los mosquitos producen un aumento significativo ($p < 0.05$) en los niveles de proteína total, albúmina total y bilirrubina, cuando los animales fueron expuestos de dos semanas a 16 semanas con transflutrina. Del mismo modo, la elevación en las actividades de aspartato amino transferasa, alanina amino transferasa y alanina fosfatasa, aumentó significativamente con ambos insecticidas. Se registro un aumento en los leucocitos, eritrocitos y el hematocrito para todos los periodos de exposición, sin embargo el recuento de las plaquetas no mostró un aumento significativo ($p > 0.05$). Las pruebas de mutagenicidad revelaron que las anomalías en el espermatozoides de las ratas fue estadísticamente significativa ($p > 0.05$) al comparar el control a las 8, 12 y 16 semanas post exposición a la transflutrina. Los estudios histológicos revelaron una serie de daños pulmonares graves en las ratas expuestas al humo de la espiral, evidenciados por la acumulación intersticial, edema pulmonar y enfisema. Las

acumulaciones intracelulares y la congestión sinusoidal severa de las células del hígado se observaron a partir de las 12 semanas de exposición, lo que indica daño hepático. Nuestros estudios indican que los vapores de las espirales contra mosquitos inician el daño gradual al huésped. Estos efectos patológicos deben ser tomados en cuenta por el programa de control de la malaria, particularmente a la hora de regular su uso a largo plazo y bajo techo.

Palabras clave: espirales contra mosquitos, insecticida, parámetros hematológicos y bioquímicos, uso prolongado, mutagenicidad, histopatología.

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