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Some Toxicological Effects of a Commonly Used Mosquito Repellent in Lagos State, Nigeria

[1,3]A.F. Ayorinde, [1]B.O. Oboh, [2]O.A. Otubanjo, [1]A.C. Alimba and [1]P.C. Odeigah
[1]Department of Cell Biology and Genetics, University of Lagos, Akoka, Lagos, Nigeria
[2]Department of Zoology, University of Lagos, Akoka, Lagos, Nigeria
[3]Department of Biological Sciences, Redeemer's University, Mowe, Ogun State, Nigeria

Corresponding Author: A. Ayorinde, Department of Cell Biology and Genetics, University of Lagos, Akoka, Lagos. Nigeria

ABSTRACT

Although, toxicity of some mosquito repellents has been reported, effects of different doses on some parameters and different sexes is not clear. This study was aimed at determining the toxicity of a commonly used mosquito repellent in Lagos State, Nigeria. The effects studied in rats were based on some hematological parameters, body weight/organ indices (Relative organ weights) and histopathological analysis. The rats were divided into two groups of 6 males and 6 females each and a control of 2 males and 2 females. The two groups were exposed to one and two mosquito repellents per day for one, two and four weeks, respectively. Hematological analysis showed increase in White Blood Cell (WBC) counts in the females and males exposed for 2 weeks and above ($p<0.05$). Increase was also recorded in the percentage of Mixed Population of White Blood Cells (MXD%) of females and males rats exposed for 2 weeks and above ($p<0.05$). Platelets (PLT) counts in the female rats (after 2 weeks) also increased when compared with the control ($p<0.05$). For relative organ weights, significant difference was observed in relative spleen ($p<0.05$), heart ($p>0.05$) of both female and male animals and thymus ($p<0.05$) of the female animals only when compared with control. The histopathological studies showed a severe damage to the lungs whereas no damage was observed in the liver and kidney in both male and female rats. These results show that exposure to Black coil could be detrimental to health.

Key words: Hematological, histopathology, indices, mosquito repellent, rats, exposure

INTRODUCTION

Bencsits (1996) defined mosquito repellents as chemical substances having a repellent effect on mosquitoes and some other insects. Mosquito repellents can be grouped into 3 types; these are coil, mat and liquid (Sahu and Ranjit, 1998). The active substance in these mosquito repellents is allethrin (Okine *et al*., 2004). Insecticides or smoke generated from burning mosquito coils are mostly used to protect people from nuisance of mosquitoes (Chen *et al*., 2008). Most mosquito coils sold in Nigeria contain allethrin, which is a pyrethroid. The insecticide used for this study contains D-transallethrin as the active ingredient.

Release of volatile, semi-volatile organic compounds, polyaromatic hydrocarbons or aerosols in its gaseous phases from burning of mosquito coil in-door has been reported to be similar to that of smoking (Karthikeyan *et al*., 2006). Smoke has been reported to affect blood hematological parameters (Al-Malki *et al*., 2008). Hematological studies by Sunyer *et al*. (1996) observed increase

in WBC counts in smokers when compared with non-smokers. Hematological studies on rats exposed to pyrethroid insecticide carried out by Krishnappa *et al.* (2000), observed decrease in Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) while RBC was high in some of the rats. No effect was recorded in WBC count. Red Blood Cell (RBC) was observed to be transitory and returned to normal after some days. Al-Awadhi *et al.* (2008) reported the effects of smoke on white blood cell count.

Mosquito coil smoke has been reported to have toxic effects on the lungs of exposed female rats (Okine *et al.*, 2004). It has also been reported that the toxicity of mosquito coil smoke is caused by its combustion products such as sub-micron particles coated with heavy metals, allethrin and a wide vapour like phenol, o-cresol, benzene and toluene (Okine *et al.*, 2004). Apart from allethrin, mosquito coils contain 70 other compounds which include phenol, benzene, toluene and xylene.2 (Dworkin, 2002). Benzene is a naturally occurring chemical found in crude petroleum. Studies however have shown that benzene is toxic in numerous animals (Panhwar, 2005). Liver and kidney damage as well as respiratory effects have also been reported as the main short term exposure effects of phenol (Panhwar, 2005). Animal studies with long term exposure to mosquito coil showed abnormal growth of skin cells, poor weight gain and lung damage (Dworkin, 2002).

In this study, the toxic effects of a commonly used mosquito repellent in lagos state, Nigeria, called black coil was studied. The effects of inhalation in male and female rats on the hematology, relative organ weight and histopathology of 3 organs were studied.

MATERIALS AND METHODS

Chemical and reagents: Mosquito repellent used is Black coil repellent incense and purchased from the open market. It contains Rich-D-Transallethrin as the active ingredient.

Animals and treatment: Adult albino rats of 14 males and 14 females weighing between 54 and 156 kg were purchased from Department of Biochemistry animal house University of Ibadan, Oyo state, Nigeria. They were housed in rat houses specially built for this experiment (80×50×70 cm). The rats were fed on pelleted food and tap water. The rats were divided into two groups of 12 made up of 6 male rats and 6 female rats. Also a control group of 4 rats made up of two female rats and 2 male rats. Group 1 animals were exposed to one mosquito repellent per day for 6 hours. The group 2 animals were exposed to 2 mosquito repellents per day. The control animals were kept in the animal house in cages of similar ventilation without mosquito coil for the period of the experiment.

Evaluation of the toxicity of the mosquitoes repellent on the experimental animal: Four rats (2 males and 2 females) were euthanized by cervical dislocation from each group that is 8 rats from the two groups the 1st week, 2nd week and 4th week. For the control, 2 males and 2 female rats were euthanized after the 2nd week.

Hematological (full blood count): Blood samples were collected from the animals (rats by carotid artery cannulation) into potassium EDTA bottles. Cell counter (model: systimex) was used to analyse the following: Red Blood Cell (RBC), Mean Corpuscular Hemoglobin (MCH), White Blood Cell (WBC), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), Mean Corpuscular Hemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW), Neutrophil (Neut), Hemoglobin (HGB), Hemocrit (HCT), Lymphocytes (LYM), Mixed Population of White Blood Cells-Monocyte, basophil, eosinophil (MXD).

Body weight/organ indices: Each animal was weighted before sacrifice and lungs, liver, kidneys (left and right), heart, Thymus, spleen were also weighted. The relative organ weight of each animal was calculated.

Histopathology: The animals were sacrificed by cervical dislocation at baseline. The lungs, liver, kidneys were excised in formal aldehyde dehydrated with alcohol. The tissues were cleared with xylene and impregnated with paraffin wax and sections cut and stained with haematoxylin and eosin. These were mounted on slide for light microscopic examination.

Statistics: The Data are expressed as Mean±SEM. Statistical analysis was performed using t-test. Significance was set at 0.05.

RESULTS

Hematological: Table 1 shows the result of the hematological analysis. Increase in WBC counts was observed in all the female groups when compared with control but significant difference ($p<0.05$) was observed in female rats exposed to 2 repellents for 2 weeks (17.5±0.42) compared with the control (2.2±0.14). Significant difference ($p<0.05$) was also observed in WBC of males exposed to 2 repellents for 4 weeks (23.4±0.42) when compared with the control (14.55±0.07). Significant difference ($p<0.05$) was observed in MXD% of the female groups exposed to 2 repellents for 2 weeks (23.55±2.56) when compared with the control (9.55±1.77). MXD% increase and significant difference ($p<0.05$) also in the males exposed to two repellents for 1 week (24.2±4.85), 2 weeks (27.2±1.46) and 4 weeks (18.55±2.90) when compared with the control (7.0±1.70). Increase in LYM counts ($p>0.05$) was observed in female groups exposed to two repellents for 2 weeks (12.9±3.68)

Table 1: Effects of different doses of mosquito repellent at different weeks on hematological parameters of the female and male rats

Groups	WBC	RBC	HBG	HCT (%)	MCV	MCH	MCHC	PLT	LYM	MXD (%)	NEUT
Female											
1F1 and 2	5.45±2.47	6.115±0.30	12.05±1.20	38.5±1.84	62.8±0.14	19.65±1.06	31.25±1.63	414±231.93	4.65±2.47	6.6±3.11	0.5±0
1F3 and 4	5.8±0	5.71±0.085	11.7±0	36.6±2.12	64.15±2.62	20.8±0	33.3±0	427±264.46	4.5±0	6.9±0	0.9±0
2F1 and 2	9.65±2.05	6.525±0.25	12.7±0.28	44.25±1.20	67.8±0.71	19.5±1.13	33.7±5.66	658±103.24	6.35±0.92	23.55±2.56	0.8±0.28
2F3 and 4	17.5±0.42	6.64±0.28	13.35±0.21	43.35±0.64	62.8±0.14	20.1±0.57	36.8±9.48	627±43.840	12.9±3.68	13.3±9.90	0.95±0.35
Control-f	2.2±0.14	6.44±0.34	12.7±0.57	34.15±2.05	62.45±0.35	19.6±0	22.9±12.16	406±0	2.6±1.41	9.55±1.77	0.5±0.28
Male											
1M1 and 2	12.05±4.17	6.33±0.95	12.1±.99	40.15±3.75	63.7±3.68	19.2±1.27	30.15±0.35	834.5±99.70	6.4±0	35±3.47	0.95±0.07
1M3 and 4	12.2±4.24	6.99±1.43	12.8±2.83	44±9.05	62.95±0.07	18.3±0.28	29.05±0.49	834.5±50.20	8.8±5.37	22.65±8.46	1.0±0.14
1M5 and 6	9.6±0	5.265±0.39	6.85±3.89	32.75±2.47	62±0.14	13.3±8.34	21.5±13.44	451.5±75.66	5.8±2.97	5.85±4.88	0.5±0.42
2M1 and 2	5.45±1.63	6.285±0.50	12.35±1.06	41.6±4.67	66.1±2.12	19.65±0.07	29.75±0.78	747±428.51	3.4±0.14	24.2±4.85	0. 6±0.28
2M3 and 4	9.95±2.05	6.28±0.89	12.15±0.21	40.3±0.85	64.75±7.85	19.5±2.40	30.15±0.07	722±349.31	6.45±2.47	27.2±1.46	0.9±0.14
2M5 and 6	23.4±0.42	5.16±1.82	11.05±2.05	38±2.97	61.7±0.71	17.9±2.12	28.95±3.18	781±0	11.45±4.60	18.55±2.90	3.25±2.62
Control-m	14.55±0.07	6.9±1.16	11.85±0.21	44.25±8.56	64±1.70	17.35±2.62	27.25±4.74	795±176.78	10.95±1.77	7±1.70	2.6±1.41

Values are Mean±SD, 1F1 and 2: (Female groups exposed to 1 repellent for 1 week) WBC-White Blood Cell NEUT- Neutrophil, 1F3 and 4: (Female groups exposed to 1 repellent for 2 weeks) RBC-Red Blood Cell, 2F1 and 2: (Female groups exposed to 2 repellent for 1 week) HBG- Hemoglobin, 2F3 and 4: (Female groups exposed to 2 repellents for 2 weeks) HCT-Haemocrit, 1M1 and 2: (Male groups exposed to 1 repellent for 1 week) MCV-Mean corpuscular volume, 1M3 and 4: (Male groups exposed to 1 repellent for 2 weeks) MCH-Mean corpuscular 1 hemoglobin, 1M5 and 6: (Male groups exposed to 1 repellent for 4 weeks) MCHC-Mean Corpuscular haemoglobin concentration, 2M1 and 2: (Male groups exposed to 2 repellents for 1 week) PLT-Platelet, 2M3 and 4: (Male groups exposed to 2 repellents for 2 weeks) LYM-Lymphocytes and 2M5 and 6: (Male groups exposed to 2 repellents for 4 weeks) MXD- Mixed population of White Blood Cells

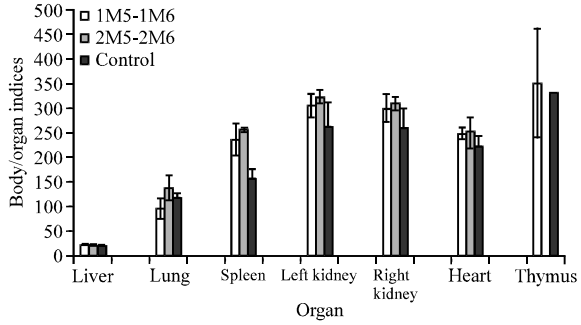

Fig. 1: Body weight/organ indices of male rats exposed to repellents for longer periods (4 weeks), 1M5-1M6: Group exposed to 1 repellent for 4 weeks, 2M5-2M6: Group exposed to 2 repellents for 4 weeks) Mean±SE

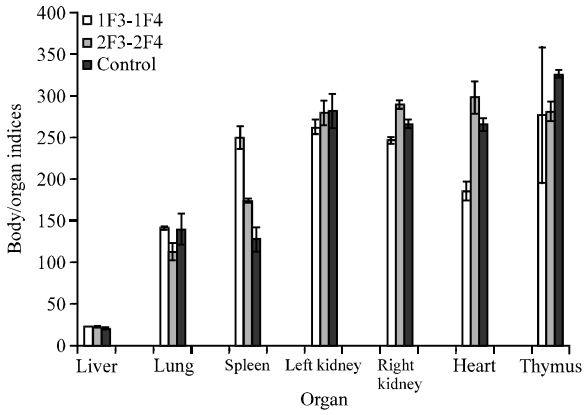

Fig. 2: Body weight/organ indices of female rats exposed to repellents for longer periods (2 weeks), 1F3-1F4: Group exposed to 1 repellent for 2 weeks, 2F3-2F4: Group exposed to 2 repellents for 2 weeks) Mean±SE

when compared with control (2.6±1.41). Platelet counts show significance difference ($p<0.05$) in female groups exposed to 2 repellents for 2 weeks (627±43.8) compared with the control (406±0). Other hematological parameters appear to be normal when compared with the control.

Bodyweight/organ indices (relative organ weight): The effects of inhalation of the mosquito repellent on some body weight/organ indices after exposure are shown in Fig. 1 and 2. A significant difference ($p>0.05$) was observed in the bodyweight/lung indices in males exposed to 2 repellents for 4 weeks (Fig. 1). Also a significant difference ($p>0.05$) in body weight/heart indices in both male and female groups exposed to 2 repellents for 2 weeks and 4 weeks, respectively when compared with the control. Significant differences ($p<0.05$) also observed in Body weight/spleen indices in both males (Fig. 1) and females (Fig. 2) exposed to 1 and 2 repellents for 2 and 4 weeks, respectively. Relative thymus weight was only significant ($p<0.05$) in females exposed to 1 repellent for 2 weeks (Fig. 2).

Histopathological effect: Histological appearance of liver to 1 and 2 repellents for 1, 2 and 4 weeks in both male and female rats show normal hepatocytes and presence of portal trial in all the groups.

Also no observable difference in kidney of all the groups compared with control. The glomeruli and proximal tubule appeared normal in both exposed and the control. Histological appearance of the lung tissues exposed to 1 repellent for 1 week, 2 weeks and 4 weeks show inflammation response consolidation in alveolar areas and septa thickening. Exposure to 2 repellents for 1, 2 and 4 weeks also show septa thickening, consolidation of alveolar area and thickening of bronchiolar epithelial wall in both male and female exposed animals. The control had a thin bronchiolar epithelial wall and wide alveolar spaces.

Mortality effect: Four females died, two from the group exposed to two repellents a day on the 4th and 5th day and two in the group exposed to one repellent a day on the 7th day.

DISCUSSION

A normal level of Haemocrit (HCT), Red Blood Cell (RBC), Haemoglobin (HGB) in the groups exposed to the repellent was observed when compared with the control. This is an indication that the rats were not anaemic Sunyer *et al.* (1996). Hematological studies on rats exposed to pyrethroid insecticide carried out by Krishnappa *et al.* (2000), observed that RBC was high in some of the rats studied. Also in this study, WBC, lymphocytes, MXD% show higher values when compared with the control, however no effect was recorded in WBC count in the study by Krishnappa *et al.* (2000). Sunyer *et al.* (1996) recorded a higher White Blood Cell count in smokers compared with non smokers and Al-Awadhi *et al.* (2008) reported the effects of smoke on white blood cell count. Aboderin and Oyetayo (2006) reported that White blood cells are important in defending the body against infection. Also that presence of Basophils counts is an indication of increase in sensitization to an antigen or (allergen). According to Oladunmoye (2006), T-lymphocytes and other key cells of the immune system are known to activate production of antibody to destroy evading pathogen. The increase in the lymphocyte count, WBC and other white blood cells in the exposed animal in this study show sign of immunostimulatory effect of the mosquito repellent on the animals.

Increase in platelets count was observed in the female group exposed to one repellent for one week and two repellents for one week before a decrease in the second week. Damoah (2010) also documented increase in the activation of platelets in smokers. It has been shown that platelet activation and aggregation increase within 20 minutes of exposure to passive smoke. Elevated platelet counts can lead to blood clots developing in the blood vessels, which can lead to stroke, myocardial infraction and embolisms (Damoah, 2010). Elevation in platelets in the exposed group could have led to the clotting of the blood vessels probably could have caused mortality recorded in the female rat groups.

Previous studies by Okine *et al.* (2004) using albino rats exposed to mosquito coil smoke showed that the smoke caused morphological changes in lungs, liver but did not affect the kidney. In this study, effects of mosquito coil on histology of lung, liver and kidney show that the smoke only affected the lungs and did not show any significant effect on the kidney and liver when compared with the control The lung tissue showed thickening of the bronchiolar epithelial wall, alveolar thickening and consolidation in alveolar areas after 1, 2 and 4 weeks of exposure which are indicative of toxicity of the mosquito repellent to the lung.

It was observed that Body/lung weight index in animals increased, especially in males exposed to the repellents for longer weeks when compared with the control. Okine *et al.* (2004) reported an increase in mean lung wet weight in rats exposed to mosquito coil smoke. They went on to explain that an increase in mean lung weight in rats exposed to mosquito coil smoke could be due to activities of pneumooxicants like naphthalene butylated hydroxytoluene and 1,1-dichloroethylene (DCE) which cause time-dependent damage to Clara and alveolar cells of the lung. These can further lead to a concormitant reduction in some monooxygenase activities and thus increase in lung weight. Also this may be an indication that apart from D-allethrin which is the active agent of this particular mosquito repellent used (Black coil), other ingredients that made up the mosquito repellent might be some of the pnuemotoxicants mentioned above.

Resch *et al.* (2005) reported that long term exposure to moderate doses of carbonmonoxide is consistently associated with congestive heart failure. They also reported that exposure to smoke for a long-term can lead to increased blood flow velocity in the central retinal artery and retinal vessel diameter. Thus the increase in heart weight observed in this study could be as a result of increase blood flow to the heart.

The spleen and thymus are organs in the body where lymphocytes and monocytes are produced. In this study, increase in body/spleen and thymus was recorded in animals exposed for longer weeks. De Oliveira *et al.* (2007) reported that under certain circumstances, the lung may become immune-priviledged. Thus, the immune system detects and combat invaders to restrict local inflammatory responses. This can explain the increase in these organs (spleen, thymus) weight when compared with the control in this study. This is because of large production of these antibodies for the above purposes.

CONCLUSION

Exposure to mosquito repellent for a long period of time can have toxic effects on the hematological parameters and histopathology of the lungs. The hematological effects were higher in females compared with males while histopathological effects were the same in both sexes. Thus exposure to these repellents by the human populace should be controlled especially among market women who are exposed to the repellents in the market places (used to repel insects) and also in the homes at night (used to repel mosquitoes).

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