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Research Article

Pulmonary Oxidative Stress and Inflammation in Wistar Rats Exposed to Fume of Alletrin Based Mosquito Coil

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Abstract
The use of alletrin-based mosquito coil in developing countries to eliminate mosquitoes is still on the increase. Information on deleterious effects of prolonged exposure to mosquito coil fume on pulmonary tissue is limited. Thus, this study assessed the mosquito coil fume (MCF)-induced damage to pulmonary tissue. Thirty-six male Wistar rats were divided into 6 groups (n=6). Rat in groups 1, 3 and 5 were exposed to 8 hours of MCF for 30, 60 or 90 days via whole body inhalation in wooden iron gauze cage. Rats in groups 2, 4 and 6 served as unexposed groups and had access to natural air. Rats were euthanized on day 30, 60 or 90 and lungs removed aseptically. The biochemical and histopathological effects of MCF on the lungs were assessed. Exposure of experimental rats to MCF resulted in increased lipid peroxidation and nitrite levels in the lungs from day 30 to day 90 compared to the unexposed rats ($P < 0.05$). Furthermore, there was 2-fold decrease in lung's glutathione levels in MCF exposed rats compared to the unexposed control rats, ($P < 0.05$). In addition, the lung TNF-α level of MCF exposed rats was significantly increased starting from day 30 to day 90 relative to the unexposed rats ($P < 0.05$). Histological analysis of the lungs of MCF exposed rats showed erosion of the bronchial epithelium, numerous macrophages defoliating from the wall of the alveoli septae and foci of mild desquamation. Continuous exposure to allethrin-based mosquito coil fume promotes oxidative stress and inflammation in the lungs of Wistar rats.

Key Words: Mosquito coil, Wistar rat, Pulmonary, inflammation, oxidative stress

INTRODUCTION

The spread of mosquito borne diseases such as malaria, yellow fever, Zika virus, dengue fever and filariasis in the developing world still remains a challenge. Ghandi *et al.*, (2019), Lee *et al.*, (2018). To repel or kill this disease carrying vector, increased use of mosquito repellants like mosquito coils, spray, mats and vaporizers have been observed. Naz *et al.*, (2019). Mosquito coils are most preferred repellent in most areas due to their affordability Ijaz *et al.*, (2018) and they are widely used in Africa, Asia, and South America. Idowu *et al.*, (2013), McCallum *et al.*, (2003). Pyrethrins/pyrethroids are the main active ingredients in coils and mats and these accounts for 3-4% of coil mass. Other constituents of coils are dyes, binders and organic fillers. Members *et al.*, (2015). Pyrethrins are the natural components of Chrysanthemum plants such as *Chrysanthemum coccineum* and *Chrysanthemum cinerariefolium* flowers while the synthetic analogs which is a derivative of pyethrins is pyrethroids. Pyrethrin is preferred to pyrethroids as mosquito repellant because it is biodegradable and less toxic. Macan *et al.*, (2006). Allethrin is a synthetic pyrethroid (a Type I pyrethroid) used as insecticides in commercially available mosquito repellents (mats, coils, sticks and liquidators). The air level following conventional household aerosol in allethrin spraying is not expected to exceed 0.5 mg/m^3. Sivard *et al.*, (1989). Coils sold in Nigeria contain allethrin concentration 0.05 - 0.1% w/w and is reported to be a weak to moderately toxic pyrethroid with inhalation LC_{50} >1500 mg/m^3 in mouse and rat. Tomlin *et al.*, (1994). However, staying/sleeping indoors with the lit mosquito coil can be injurious, because of increased inhalation of the fume can occur. In fact, pyrethroid-based mosquito coils, which is commonly used in developing countries have been reported to expose human to severe toxicity when used in closed and poorly ventilated areas. Lukwa *et al.*, (1998). Lou *et al.*, (2006) reported that mosquito coils emit particulate matter, carbon monoxide, nitrogen oxides and methane when burned. The gas phase of the coil has also been found to contain carbonyl compounds like formaldehyde and acetaldehyde. Chang *et al.*, (1998). Inhalation of fumes of the mosquito repellent like liquid vaporizers may get entry into the brain by breaching the developing blood-brain barrier, hence deleterious to

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developing nervous system and can lead to long-term functional deficits. Biswal *et al.*, (2006).

Hematological, histological and biochemical changes in some tissues, sperm abnormality and sinusoidal congestions when rats were exposed to mosquito coils fume have been reported. Idowu *et al.*, (2013). Chronic exposure of allethrin-based mosquito coil in mice has been shown to result in pulmonary emphysema and hyperplasia, while hepatic tissue showed apoptosis mediated damage of hepatocytes along with severe form of necrosis. Bhuyan *et al.,* (2017). In addition, toxic effects of exposure to allethrin-based mosquito coil smoke in male offspring of pregnant female rats exposed for 111 days post parturition (21-day weaning plus up to 90 days post-weaning) showed increased oxidative stress, distorted antioxidant enzyme status, down- regulation of genes involved in spermatogenesis, sperm maturation as well as steroidogenesis. Madhubabu *et al.*, (2017). Despite the limited studies on the toxic effect of the mosquito coil fume on pulmonary tissue, the mechanism by which MCF causes deleterious effect has not been fully elucidated. Thus, this study reports the mechanisms by which MCF induced pulmonary tissue damage.

MATERIALS AND METHODS

Experimental Animals: Thirty-six (36) adult male Wistar rats weighing between 150 and 200 g were obtained from Central Animal Facility, University of Ibadan. The rats were acclimatized for 2 weeks before commencement of the study. Rats were kept in cages at room temperature, fed with standard rat pellets (ACE® Feeds Nigeria Limited) and provided with access to clean drinking water *ad libitum*.

Ethical approval: The study followed both the international (Publications of NIH volume 25 no 28. 1996 revised edition) and National guidelines (Animal Care and Use Research Ethics Committee of the University of Ibadan UI-ACUREC/19/046).

Chemicals and reagents: Mosquito coil was purchased from a supermarket in Ibadan, Oyo state, Nigeria. Tumor necrosis factor kit (TNF-α) was bought from Biolengend®,UK. The remaining chemicals were purchased from Sigma-Aldrich®, UK.

Experimental design: Thirty-six (36) male Wistar rats were divided into 6 groups (n=6). Rats in groups 1, 3 and 5 were exposed to MCF via whole body inhalation in wooden iron gauze cage in a separate room for 8 hours every day for 30, 60 or 90 days. Then, returned to the regular animal facility room after exposure on a daily basis. Rats in groups 2, 4 and 6 were maintained under normal animal house conditions and served as control.

Biochemical evaluation of the toxic effects of mosquito coil fume on the lung: The toxic effect of allethrin-based mosquito coil fume in Wistar rats was assessed on day 30, 60 and 90 post exposure. Briefly rats in group 1 (MCF exposed rats) and 2 (un exposed control rats) were euthanized on D30, lungs were harvested, weighed, rinsed with cold saline and blotted on paper. Samples from whole lungs were taken, preserved in -80ºC till when needed for tumour necrosis factor alpha (TNF-α) estimation and biochemical analysis. The same above process was repeated on 60 and 90 with rats in groups 3 & 4 and 5 & 6 respectively.

Briefly, minced lung tissue was homogenized in phosphate buffer saline pH 7.4 (50 mg/mL) and centrifuged at 12,000 rpm for 10 minutes at 4^0C. The supernatant was stored at -80ºC till when needed for analysis. The lipid peroxidation (LPO) was quantified as malondialdehyde (MDA) and expressed as micromoles MDA/g tissue Varshney *et al.*, (1990). Reduced glutathione (GSH**)** level was estimated according to the recycling method (Anderson, 1985) and expressed as nanomoles per gram tissue. Estimation of nitrite level was done using the Griess reagent Piao *et al.*, (2011). The nitrite concentration was extrapolated from sodium nitrite standard curve and expressed as micromoles per gram tissue.

Determination of tumor necrosis factor alpha (TNF-α) level in the lung: Minced tissue sample was homogenized in homogenizing solution containing protease inhibitor cocktail and lysis buffer (50 mg/mL). This was centrifuged at 12,000 rpm for 10 minutes at 4^0C. The TNF-α was quantified from the supernatant using rat TNF-α (BioLegend®, San Diego. USA) according to the manufacturer's instruction and results expressed as picogram per gram of wet tissue.

Histological evaluation of the lungs: Samples of the lungs were individually fixed in 10% buffered formalin, dehydrated in ethanol and embedded in paraffin. The lung sample was sectioned to thickness of 5 µm. Sections were stained with hematoxylin and eosin and examined under a light microscope by a histopathologist blind to the treatment Liu *et al.*, (1987).

Statistical analysis: The data obtained were all expressed as mean ± S.E.M (standard error of mean) and analyzed with Graph Pad Prism software version 5.0. Statistical analysis of data was done using one-way analysis of variance (ANOVA) and post hoc multiple comparison test was further done by Tukey post hoc test. P-value less than 0.05 was considered statistically significant.

RESULTS

The effect of the fume of mosquito coil on markers of oxidative stress and inflammation in the rat's lung: Malondialdehyde, a bye-product of lipid peroxidation was significantly increased in the lungs of MCF exposed rats on days 30, 60 and 90 (9.34 ± 0.66; 12.07 ± 0.65 & 26.76 ± 3.76 µmol/g tissue) compared to unexposed rats (3.89 ± 0.26, 5.96 ± 0.12 & 8.0 ± 0.58 µmol/g tissue, Fig. 1a) respectively ($p \leq 0.05$). Likewise, A two-fold increase in nitrite level was observed in the lungs of MCF exposed rats compared to the control on day 60 (0.83 ± 0.08 vs 0.47 ± 0.05 µmol/g tissue) and 90 (0.93 ± 0.17 vs 0.44 ± 0.09 µmol/g tissue) repectively ($P \leq 0.05$; fig. 1b). In addition, unexposed control rats lungs had higher GSH levels (6.35 ± 0.68, 6.00 ± 0.47 & 5.34 ± 0.20 nmol/g tissue) than the lungs of MCF exposed rats (3.61± 0.70, 3.07 ± 0.18 & 2.74 ± 0.18 nmol/g tissue; $P \leq 0.05$) on day 30, 60 & 90 respectively (fig 1c). There was significant increase in TNF-α level in MCF exposed rats compared to the unexposed control rats on day 30 (5.45 ± 0.38 vs 1.02 ± 0.03 pg/mg tissue) 60 (12.24 ± 1.07 vs 0.93 ± 0.1 pg/mg tissue) and 90 (18.6 ± 1.03 vs 2.01 ± 0.40 pg/mg tissue; $p \leq 0.0001$; fig. 1d).

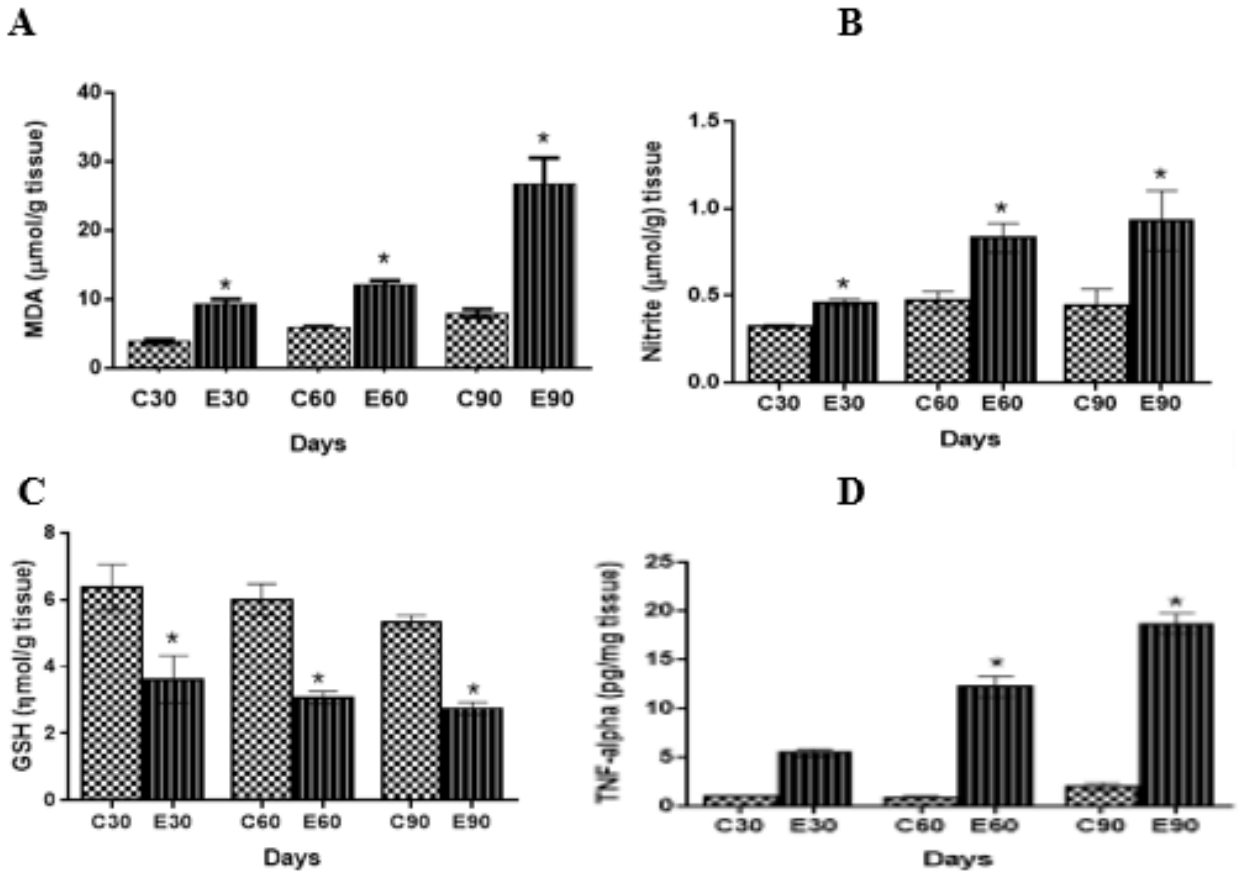

Figure. 1:
Effect of mosquito coil fume on lungs (A). Malondialdehyde level. (B). Nitrite level. (C). Glutathione (GSH) level (D). Tumor necrosis factor alpha (TNFα) level in Wistar rats.
Each bar represent mean ± SEM. C30, C60 & C90 are unexposed control rats, while E30, E60 and E90 are MCF exposed rats.
**P< 0.05.*

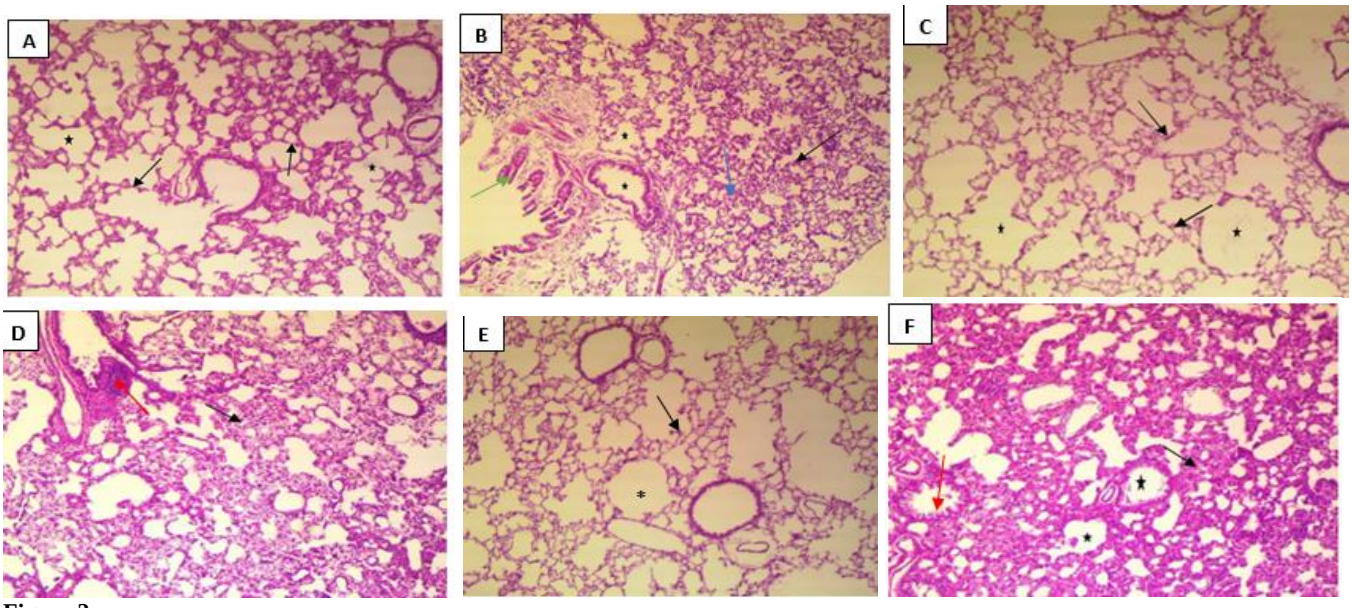

Figure 2:
Histological section of lung tissue of unexposed (control) and rat exposed to mosquito coil fume on day 30, 60 & 90 magnification (x100). A). Day 30 - control rat, the airways (star) are clear, the alveolar interstitium (black arrows) appears normal. B). Day 30 - MCF exposed rat, moderate thickening of the alveolar walls (black arrows), loss of the epithelial lining of the bronchioles (green arrows) and macrophages defoliating from the wall of the alveoli septae (blue arrows). C). Day 60 - control rat, the airways (star) are clear, the alveolar interstitium (black arrows) appears normal. D). Day 60 - MCF exposed rat, widespread marked proliferative thickening of the alveolar interstitium (black arrows), ballooning degeneration of the pneumonocytes lining the alveolar wall (black arrows) and expansion in bronchial-associated lymphoid tissue (red arrow). E). Day 90 - control rat, the airways (star) are clear, the alveolar interstitium (black arrows) appears normal. F). Day 90 - MCF exposed rat, widespread severe thickening of the alveolar wall (black arrows), foci of mild desquamation and loss of the epithelial cells lining the bronchioles (red arrow).

Histopathological effect of mosquito coil fume in the lungs of exposed rats

Histopathology of the lungs of rat exposed to mosquito coil fume showed erosion of of the bronchial epithelium, numerous macrophages defoliating from the wall of the alveoli septae on day 30, 60 and 90 (Fig 2B, 2D, 2F). Interstitial pneumonia and foci of mild desquamation and loss of the epithelium cells lining of the bronchioles was observed (Fig 2B, 2D, 2F). Airways are clear and empty for the control animals on all the days under investigation (Fig 2A, 2C, 2E).

DISCUSSION

Allethrin a pyrethroid is the active ingredient in mosquito coil used as repellent to prevent mosquito bites in most developing countries including Nigeria especially among the low-income earners. Despite effectiveness of the fume to kill or wade away mosquitoes, the fume is toxic and might have enormous adverse health effects, this is because it releases over 60 organic compounds, heavy metals and particulate matters. Hsiang *et al.*, (1988)

This study demonstrated that there was a significant increase in the levels of mmalondialdehyde, nitrite and tumour necrosis factor alpha, accompanied by decreased in antioxidant molecule gluthatione in the lungs of rat exposed to mosquito coil fume. These markers of oxidative stress and inflammation increased as the period of exposure to the mosquito coil fume increased. Metabolism of pyrethroids is said to generate reactive intermediates which contribute to oxidative stress in a variety of organ system (Wang *et al.*, 2016). The elevated reactive oxygen species and overexpression of stress responsive gene, p53 by mosquito coil fume have been documented Madhubabu *et al.*, (2012). An excessive amount of reactive oxygen species can result in the damage to DNA, proteins, and lipids, which can induce cell death, implicated in the pathogenesis of cancer, cardiovascular diseases and many general neurodegenerative pathologies (Agil *et al.*, 2006), Castegna *et al.*, 2002; Tapia-Paniagua *et al.*, 2014).

The epithelial lining of the lower respiratory tract may be the first line of defense against oxidative challenge occurring as a result of the inhalation of the fume of the mosquito coil. As a result, depletion of the glutathione (GSH) and GSH-associated enzymes located around this area might occur. Continuous exposure to oxidative stressors might lead to reduction in GSH and other antioxidant in the lungs Bunnell *et al.*, (1993). Glutathione is an antioxidant that acts either by interacting directly with reactive oxygen species/reactive nitrogen species or by acting as a co-factor for different enzymes (Gradishar *et al.*, 2017). Reduced glutathione level seen in this study is an indication of its free radical scavenging capacity which was depleted as a result of overwhelming release of reactive oxygen species.

Free radical generation as a result of oxidative stress modulate signaling pathways such as apoptosis, necrosis, carcinogenesis and inflammatory (Bhuyan *et al.*, 2017). In this study, increased level of inflammatory cytokine (TNF-α) and nitrite were observed in the lungs of rats exposed to mosquito coil fume, this suggests that there is significant inflammation in those animals. Overexpression of stress responsive gene, p53 caused by mosquito coil fume has been strongly linked to pulmonary and hepatic inflammation (Tiwari *et al.*, 2016).

By day 30 histological examination of the lungs of rats exposed to mosquito coil fume showed moderate thickening of alveolar walls, loss of epithelial lining of the bronchioles and macrophages defoliating from the wall of alveoli septae. The recruitment of inflammatory cells along with proteolytic enzymes can cause destruction of the alveolar septa resulting in emphysema Al Mamun *et al.*, (2017). Continuous exposure till day 60 to mosquito coil fume increased the injury caused to the lungs, with widespread marked proliferative thickening of alveolar interstitium, severe ballooning, degeneration of the pneumocytes lining the alveolar wall and moderate expansion in bronchial-associated lymphoid tissue were observed. This observation is a clinical feature of chronic obstructive pulmonary disease (COPD) which can result in airway resistance and remodeling (Al Mamun *et al.*, 2017). People with COPD are at increased risk of developing heart disease, lung cancer and a variety of other conditions (Chou *et al.*, 2018). Finally, by day 90, pronounced damage were observed in lungs of rats exposed to mosquito coil. There were foci of mild desquamation and loss of the epithelial cells lining the bronchioles, widespread severe thickening of the alveolar wall, with findings indicative of interstitial pneumonia. Oedema, inflammation, exudative pneumonia, anthracosis, thrombosis and vasculitis have been previously reported in rats exposed to the fume of pyrethroid mosquito coil (Idowu *et al.*, 2013; Taiwo *et al.*, (2013).

In conclusion, continuous exposure to allethrin-based mosquito coil fume promotes oxidative stress and inflammation which are implicated in the pathogenesis of chronic diseases

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