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Research Article

Hazardous Effects of Smoke and Smokeless Mosquito Coils on the Fertility and Respiratory Functions in Rats

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ABSTRACT

Although the same pyrethroid insecticide is impregnated in smoke and non-smoke coils, the non-smoke one emits fewer PICs (products of incomplete combustion) due to its different base materials, hence gaining popularity among the enlightened. However, the impacts of non-smoke mosquito coils on the respiratory and male reproductive systems in an insufficiently ventilated room simulating human's closed bedrooms is yet to reveal. In this experiment, a total of eighteen male rats (age: 10-12 weeks) were randomly divided into three groups, each group containing six animals. The control group was exposed to normal environmental air, the non-smoke coil group inhaled D-transallethrin based coil smoke in a closed atmosphere and the smoke group was exposed to meperfluthrin based coil smoke in a well-ventilated room, for 8 hours per night. After 4 weeks of exposure, sperm parameters as well as testicular and pulmonary histopathology were accessed. The findings indicated that both mosquito coils considerably deteriorated sperm quality along with testicular and pulmonary architecture, with little distinction between them. Furthermore, the degenerated seminiferous tubules and distorted respiratory bronchioles lead us to conclude that if the room is not well-ventilated, the pollutants produced by burning non-smoke mosquito coils greatly exceed and it becomes as deleterious as conventional smoke emitting ones for the respiratory and male reproductive system.

Introduction

Phobia about mosquito-borne diseases like dengue and chikungunya has prompted a surge in the use of mosquito repellents in Bangladesh, especially coils, vaporizers, and aerosols. Among them, the mosquito coils with pyrethroid pesticide are preferred due to their affordability and accessibility (Karim et al., 2020). However, in addition to repelling mosquitoes, these insecticides represent serious health dangers that have not received sufficient attention from the general public and policymakers (Al-Mamun et al., 2017). Moreover, their non-compliant manufacturing process, unprofessional monitoring by the competent authority, and inappropriate way of use exacerbate health concerns.

Mosquito coils repel insects from the room by slow and steady emission (smoldering) of insecticides impregnated in their base material. A higher incidence of infertility and respiratory disorders has been linked to this pesticide exposure. Moreover, the smoldering of base material releases products of incomplete combustion (PIC) such as microscopic particles, aldehydes and polycyclic aromatic hydrocarbons (PAHs). Among the PICs, formaldehyde, benzene, and polycyclic aromatic hydrocarbons (PAHs) are carcinogens, while aldehydes are known to cause respiratory and eye irritation (Zhang et al., 2010).

Although the same pyrethroid insecticide is included in both smoke and non-smoke coils in various forms, the difference in base materials results in lower PICs emission from the smokeless coils (Zhang et al., 2010). Since coconut husks or sawdust serve as base materials for smoke-emitting conventional mosquito coils, burning one of them produces the same number of fine particles as 75-135 cigarettes (Liu et al., 2003). Conversely, $PM_{2.5}$ mass (particulate matter < 2.5 µm in width), aldehyde, PAHs, and total particle number emissions are reduced by at least a factor of ten from

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the burning of charcoal or carbon powder-based non-smoke coils (Zhang et al., 2010). This advantage has increased the popularity of smokeless coils among educated people. However, if the room is not well-ventilated to remove pollutants, the quantities of pollutants produced by burning non-smoke mosquito coils may significantly exceed. Moreover, in a closed environment, the PICs emitted by non-smoke coils can linger in the surrounding air for a prolonged period, where anyone nearby has no choice but to breathe (Liu et al., 2003).

According to the recommendations of the World Health Organization, mosquito coils should only be used outside, or the user should vacate the room while the coil burns and then return with the windows open (Kyaw, 2016). Unfortunately, for maximum effect, many unconscious people use mosquito coil in their confined living rooms and bedrooms, where the residual concentrations are nearly ten times higher than in the outdoor space (Atmakuru and Vijayalakshmi, 2002; Karim et al., 2020). Moreover, during overnight burning, nearby subjects continuously become exposed to sub-micron particles, vapors, and metal fumes from mosquito coil smoke. These substances eventually enter the pulmonary alveoli and irritate the upper respiratory tract (Chang and Lin, 1998). In addition to asthma and persistent wheezing in children, the tracheal epithelium has undergone ultrastructural changes following mosquito coil exposure (Liu and Sun, 1988; Fagbule and Ekanem, 1994).

While there have been studies documenting the negative effects of mosquito coils on different physiological systems, the anti-fertility effect has received less attention from researchers. However, it has been observed that pyrethroid exposure reduces testicular sperm counts and increases the quantity of defective sperm (Kamijima et al., 2004). Moreover, mosquito coil smoke compromised germ cell production in rats' testes following extended exposure (Madhubabu and Yenugu, 2012).

On the above background, the experiment was designed to investigate the effects of smokeless mosquito coils in a closed environment on the fertility and respiratory functions of exposed animals in comparison to conventional smoke-emitting one.

Materials and Methods

Coil composition

Two BSTI (Bangladesh Testing and Standard Institution) approved mosquito coils- Night Guard® (meperfluthrin-based smoke coil) and Baoma® (D-transallethrin-based non-smoke coil), were purchased from the regional supermarket. Details of the compositions of the two coils are given in the Table 1.

Table 1. Coil composition

Compositions	Non-smoke coil (% w/w)	Smoke coil (% w/w)
D-transallethrin	0.3	-
Meperfluthrin	-	0.05
Carbon Powder	60	-
Wood Powder	30	14.33
Binder	9.7	-
Coconut Shell	-	65.2
Gum Powder	-	20
Color	-	0.47

Animals

The experiment was lasted from July to September 2021. Long Evans rats procured from icddr'b were reared in the polypropylene rat cages under typical settings with sufficient food and water in the anatomy laboratory of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur. Animals were allowed two weeks for acclimatization in the experimental environment. Animal Research Ethics Committee (AREC), BSMRAU (AREC/BSMRAU/2021-06) approved the study in accordance with the standards on the humane treatment of animals in research and teaching.

Experimental procedures

Eighteen (N=18) male rats (10-12 weeks old) were divided into three groups; each having six animals (n=6):

Group-A: Normal Control- exposed to normal environmental air

Group-B: Non-Smoke Coil- exposed to D-transallethrin-based coil smoke

Group-C: Smoke Coil- exposed to meperfluthrin-based coil smoke

Polypropylene rat cages were separated into two halves by a wire partition. The rats were housed in the first half while the mosquito coils were lit on the other. The wire made separation spared rats from burning and allowed smoke to enter the other side. In the non-smoke coil group, the cage was draped with thin cotton garments to create a closed atmosphere, whereas no draping was given in smoke coil group. The animals were exposed to the coil for eight hours/night (9 pm - 5 am) for 28 days via whole-body inhalation to reflect human settings.

Histopathological study

After 4 weeks of mosquito coil exposure, all the rats were sacrificed by cervical subluxation method following light anesthesia (pentobarbitone @ 35 mg / kg, i.p.). Later, the lungs, caudal epididymis and testes of the rats were collected. Following collection, 10%

formalin preserved, alcohol dried and paraffin-embedded tissue blocks were sliced into 6 µm thickness using a Leica, RM2245 rotary microtome (Germany). Finally, the hematoxylin and eosin stained sections were viewed, and images were captured with a microscope (Leica ICC50 E, Germany) coupled with a digital camera (Alam and Kurohmaru, 2014).

Sperm count

The cauda epididymis of the rat was finely minced in normal saline (1 ml) and then squeezed to release the spermatozoa into the medium. Then, the aforementioned sample (100 µL) was diluted with distilled water (100 µl). A drop (10 µL) of the above liquid was added to the Marienfeld hemocytometer (Paul Marienfeld GmbH & Co. KG., Germany). Using a microscope (Leica ICC50 E, Germany), the number of sperms in each chamber was counted (Heidari et al., 2021).

Sperm motility

The motility of the sperm was evaluated by pouring one drop of fresh semen and two drops of warm 0.9% normal saline on a slide that was held close to body temperature (37-38 °C). Two hundred spermatozoa were chosen at random, and graded as motile or non-motile based on their forward movement under a microscope at 400X magnification (Haredy et al., 2017).

Motile sperm (%) = (number of motile sperm/ 200) X 100

Sperm viability

Twenty microliters of eosin solution were mixed to an equal volume of sperm suspension and left for five minutes at ambient temperature. Sperm viability (%) was assessed by examining 200 randomly selected sperms under 200-fold optical magnification. White (unstained) sperm was considered alive; however, sperm with any pink or red sign was considered dead (Abbasi et al., 2010).

Sperm morphology

For morphological examinations, a drop of epididymal semen sample was smeared on a slide and stained with Diff-Quick. In a count of 200 randomly picked spermatozoa, those having abnormalities in the head, neck, or tail were labelled as “abnormal” (Heidari et al., 2021).

Statistical analysis

Graph Pad Prism software (version 9.0) was used to evaluate all the recorded data. Statistical analysis included one-way ANOVA and Tukey's multiple comparison test. Mean ± SEM (standard error of the mean) was used to present the results. At $p < 0.05$ level, group differences were considered statistically significant.

Results and Discussion

Sperm Parameters

Table 2. Sperm Parameters

Groups	Sperm Count ($x10^6$/mL)	Sperm Motility (%)	Sperm Morphology (Abnormal %)	Viability (Live %)
Normal Control	152.3±5.1	85.1±3.2	10.7±1.5	85.4±2.3
No Smoke Coil	99.3±4.5*	66.0±9.2*	28.5±3.1*	70.9±1.8*
Smoke Coil	89.1±2.4*	57.3±1.7*	34.6±4.2*	65.1±3.6*

Results are Mean ± SEM of 6 animal; * p < 0.05 compared to control

Rats exposed to mosquito coils exhibited substantial oligospermia than the control group (Table 2). Additionally, their sperm motility and viability were notably affected ($p < 0.05$), and the percentage of aberrant sperm was dramatically higher. Although the non-smoke coil group had higher sperm count, motility and viability than the smoke coil exposed group; their difference was not statistically significant ($p < 0.05$).

The active metabolites of D-transallethrin or meperfluthrin may have contributed to the formation of lipid peroxides. These lipid peroxides could limit mitochondrial function, lead to oxidative degeneration and finally result in sperm cell death due to its polyunsaturated fatty acids enriched membranous structure (Akunna et al., 2013a). Our findings on sperm parameters are consistent with those of Akunna et al. (2013a), who noted a comparable drop in sperm motility, spermatozoa concentration, and normal sperm number in animals exposed to mosquito coils. Oyeniran et al. (2021) also reported similar decreases in the cauda epididymal sperm count and normal sperm number. Fertility has also been shown to be negatively affected by the products of incomplete combustion, such as formaldehyde. In the sperm quality and quantity of formaldehyde inhaled experimental animals, Zhou et al. (2006) found a remarkable decrease. Statistically insignificant sperm parameters between the coil groups indicated that in a closed environment smokeless mosquito coil produces higher amounts of pollutants and results in a similar toxic effect as the smoke coil.

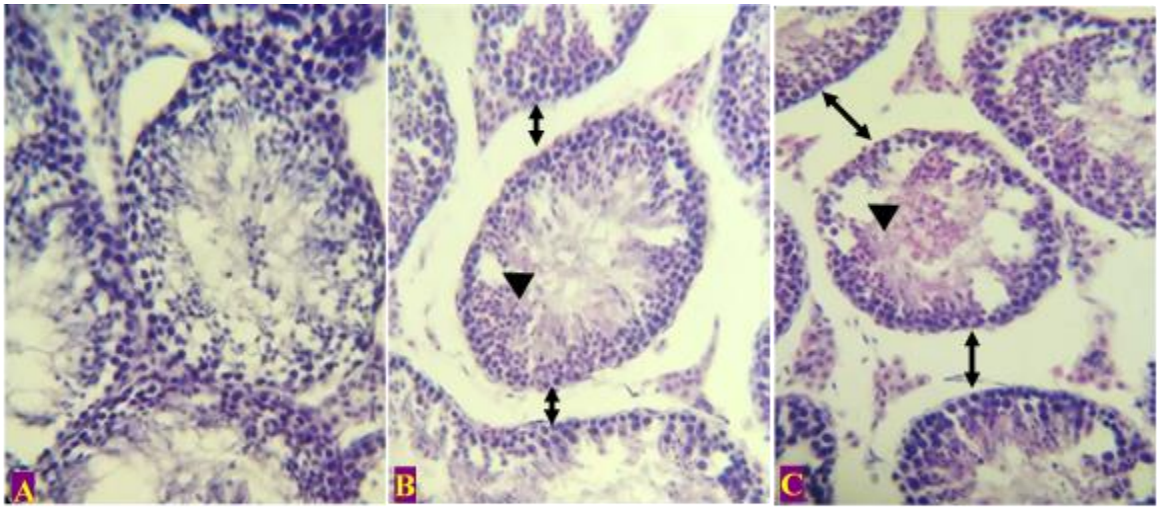

Figure 1. Histological features of the testes from control (A), non-smoke (B), and smoked coil (C) group stained with H & E (40X). (A) Normal appearance of testes in the control group. (B, C) Degenerated seminiferous tubules (arrowhead) and increased interstitial spaces (double arrow) were present in the non-smoke and smoked coil exposed testes.

Histology of testes

Typical seminiferous tubules with spermatogenic cells at various phases were seen in the testicular section of the control group (Figure 1). Moreover, seminiferous tubules' lumen, sertoli cells, interstitial cells, and interstitial spaces were normal. However, exposure to D-transallethrin or meperfluthrin-based mosquito coil smoke induced moderate to severe degenerative changes in seminiferous tubules. Also, the interstitial spaces between the seminiferous tubules were lengthy. Besides, the seminiferous tubules' number in a randomly selected field dropped drastically in the mosquito coil treated rats in comparison to control.

Even though non-smoke coils are hypothesized to cause fewer pathological changes of seminiferous tubules, an inadequately ventilated room could significantly increase pollutant concentrations, resulting in the same pathological lesions as smoke coils (Liu et al., 2003). The histological evidence presented here is consistent with Sakr and Azeb (2001), who evaluated the toxicological effects of pyrethroid on seminiferous tubules and spermatogenic cells. Benedict et al. (2017) described maturation arrest as a result of allethrin-based repellant treatment in the seminiferous tubules. Moreover, Akunna et al. (2013a) documented seminiferous tubules with distinct and gradual degenerative changes in mosquito coil exposed rats. Besides arresting spermatogenesis, formaldehyde, one of the products of incomplete combustion, causes testicular tissue degeneration (Majumder and Kumar, 1995). Moreover, Razi et al. (2013) reported adverse effects on seminiferous tubules, sperm viability and spermatogenesis after chronic inhalation of formaldehyde. Increased oxidative stress and decreased testicular antioxidant system may be the underlying cause of the degenerative alterations of seminiferous tubules. In fact, sperm membranes' polyunsaturated fatty acids are extremely vulnerable to oxidative stress induced by pyrethroid or formaldehyde via the production of free radicals (Akunna et al., 2013b).

Histology of lungs

The lungs in the control group were histologically normal, with alveoli and thin inter-alveolar septa (Figure 2). In contrast, the different extents of histo-architectural alterations were identified in the pulmonary tissue of the mosquito coil inhaled rats. Nevertheless, meperfluthrin and D-transallethrin inhaled rats revealed a massive enlargement of air space, mass infiltration of inflammatory cells, distortion of respiratory bronchioles, congestion, and thickening of the bronchiolar epithelial wall and inter-alveolar septa.

Even if the smokeless coils reduce visible smoke, the PICs released by them might linger for an extended duration in a closed environment and penetrate the pulmonary alveoli of neighboring subjects, causing the same respiratory issues as smoke coils. The toxic products of the mosquito coils irritate pulmonary tissues, initiating inflammatory processes and accumulating inflammatory cells around the alveolar septa (Nafiu et al., 2020). Inflammatory cell accumulation further contributes to bronchial epithelial wall thickening. Free radicals and volatile organic compounds of D-transallethrin or meperfluthrin-based mosquito coil smoke are primarily responsible for the substantial thickening of the alveolar septa and bronchial epithelial wall (Al-Mamun et al., 2017).

Moreover, distortion of respiratory bronchioles might reduce the surface area of the lungs and, in turn, impede normal respiratory function. Because of this, Låg et al. (2020) found strong associations between PAHs (polycyclic aromatic hydrocarbons) and respiratory diseases, including asthma and COPD.

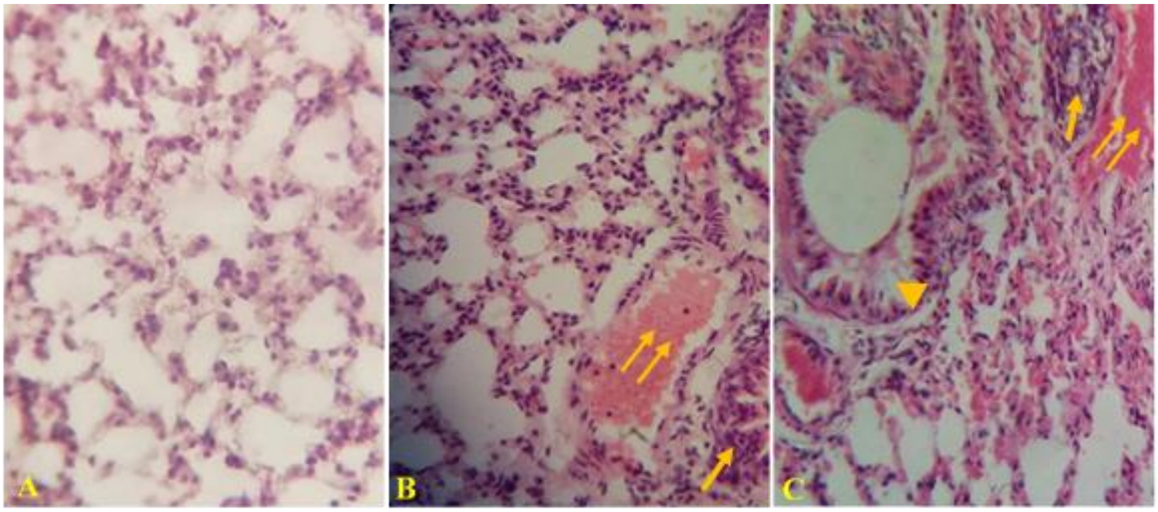

Figure 2. Photomicrographs of lungs from the control (A), non-smoke (B), and smoked coil (C) treated rats stained with H & E (40X). (A) Normal congestion-free lungs of the control group. (B, C) The non-smoke and smoked coil exposed group presented enlarged air spaces, inflammatory cells infiltration (arrow), congestion (double arrow), and distortion of the respiratory bronchioles (arrowhead).

Findings from this research are in agreement with Karim et al. (2020), who reported alterations of lung tissue alignment in the mosquito coil exposed mice. Moreover, Al-Mamun et al. (2017) suggested that chronic exposure to mosquito coil leads to air space enlargement, inflammatory cell infiltration, and thickening of alveolar septa. As well as Nafiu et al. (2020) revealed moderate cellular infiltration surrounding alveolar septa, bronchiolar epithelial wall degeneration, and hyperplasia on the mosquito sticks inhaled rats.

Conclusion

In poorly ventilated spaces, the non-smoke mosquito coils pose almost identical risks to the respiratory and male reproductive systems as conventional ones. The deterioration of sperm quality and impairments of histological architecture of the testes and lungs suggest a significant loss in fertilization and respiratory function. Thus, mosquito coils should be avoided to use in a closed enclosure.

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