Effects of Mosquito Coil Smoke (d-transallethrin) on the parenchyma of testis of adult Wistar albino rat

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Significance:
The use of mosquito coils increases exponentially in summer and rainy seasons, in the under developed countries. These coils are emerging as a major health risk, due to unawareness in general population about their adverse effects. These coils and inhalation of their smoke have been proved to cause extensive tissue damage in respiratory tract leading to upper and lower airway tract infections. It was uncertain if inhalation of coil smoke can be responsible for decreased fertility by damaging the gonadal organs. This study proves Testicular cytotoxicity is an adverse effect of prolonged inhalation of d-transallethrin.

Abstract
Background: The use of mosquito coils has increased exponentially, especially in the under developed countries. Many researches have been conducted over the past few years to advocate both the possible risks and potential benefits. These coils and the inhalation of their smoke have been proved to cause upper and lower airway tract infections. But still the possible side effects of inhalation of these coil smoke on other organ systems of the body were unclear.

Methods: Adult male Wistar rats were divided into two groups each containing eight rats. Group A was control group while group B was allowed to inhale mosquito coil smoke for four weeks. The experimental group was exposed to MCS for 8 hours / day. Histopathological analysis of testis was carried out.

Results: Histopathological studies of rats exposed to MCS revealed changes in parenchyma of testis. Decrease in the height of germinal epithelium and diameter of seminiferous tubules and increase in and vascular congestion was observed.

Conclusion: The results of present study suggest that all ethrin based mosquito coil smoke has harmful effects on testis

Introduction
Mosquito coils are slow burning devises which emits smoke. Mosquito coils are used in under developed countries to control mosquito-borne diseases like malaria, dengue fever, West Nile fever, and yellow fever. They have low cost and are easy to use. Mosquito coils are considered to be safe but can be harmful, when used in closed spaces.

Pyrethroids and pyrethrins are main active ingredients present in MCS. D-transallethrin is type I pyretheroid and most commonly used in mosquito coils. It has potential to cause toxicity in humans through various routes like skin contact, inhalation and even with food and water ingestion. It is already proved that the smoke evaporate by burning 75-137 cigarettes were same as smoke emitted by burning one mosquito coil (2).

Research suggest that Type I pyretheroid can cause tremor like syndrome, aggressive behavior, hypersensitivity and ataxia (3). Literature review suggested that mosquito coil smoke has toxic effect on various organ like kidney, heart, lungs, and liver (4,5,6). It also induces adverse changes in female and male reproductive system (7,8)

This study was designed to study the effect of mosquito coil smoke inhalation on the histology of parenchyma of testis.

Materials and Methods
Study Design & Settings: A randomized control experimental study was conducted in animal house of PGMI Lahore. Albino rats of Wistar strain were use. After acclimatization, the animals were randomly divided into two groups A and B having 8 rats in each. Group A served as a control and was not exposed to mosquito coil smoke. Group B was experimental group and was exposed to mosquito coil smoke inhalation for 8 hours/ day for four weeks (table 1).

Table 1. Detail of animal groups and duration of therapy

<table>
<thead>
<tr>
<th>Groups</th>
<th>Duration of therapy</th>
<th>Number of animals</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0 Weeks</td>
<td>8</td>
<td>Control</td>
</tr>
<tr>
<td>B</td>
<td>4 Weeks</td>
<td>8</td>
<td>Exposed to mosquito coil smoke</td>
</tr>
</tbody>
</table>

The experiment was conducted in a well ventilated room of size 26.2 m² (3.0 x 3.5 x 2.5). The rats were kept in their respective cages and allowed to inhale the MCS. The mosquito coil was burnt and placed in the center of room and cages placed equidistant around the mosquito coil so that rats inhaled equal amount of smoke. Rats were dissected after the duration of therapy and testis were removed. Stained histological sections were prepared after proper tissue processing and observed under Labomed binocular microscope.

Ethical Considerations: Institutional ethical approval was obtained from Institutional Research Ethics committee of Post Graduate Medical Institute, Lahore prior to the conduct of this study.

Statistical Analysis: Data was analyzed using SPSS 21.0. Normality of data was checked with Shapiro Wilk Test. Mean ± SD was calculated for quantitative variables. One-way ANOVA was applied to determine the mean difference among the groups for the diameter
of seminiferous tubules and epithelial height. Chi-square was used to observe association among qualitative variable of vascular congestion. A p-value ≤ 0.05 was considered as statistically significant.

**Results:**

**Effect of D-Transallethrin on Diameter of Seminiferous tubules:** Significant decrease was observed in the value of mean diameter of the seminiferous tubules (p-value= 0.000*) when compared between the control and experimental groups (Table 2).

**Effect of D-Transallethrin on Epithelial Height:** The difference was also statistically significant when mean epithelial height (p-value= 0.000*) in the seminiferous tubules of testis section of mice of control and experimental groups was compared (Table 2).

**Table 2:** Showing comparison of mean diameter of seminiferous tubules and mean epithelial height between groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group Mean ± SD</th>
<th>Experimental group Mean ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter of Seminiferous Tubules (µm)</td>
<td>282.5± 16.5</td>
<td>56.8± 2.3</td>
<td>0.000*</td>
</tr>
<tr>
<td>Epithelial Height (µm)</td>
<td>82.5± 4.7</td>
<td>14.1± 1.8</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

* p-value ≤ 0.05 is considered to be statistically significant

**Effect of D-Transallethrin and vascular congestion:**

In experimental group section blood vessels were markedly congested. Chi square test was applied as it is a qualitative parameter which showed that p-value for vascular congestion was statistically significant.

**Table 3:** Showing comparison of percentage of vascular congestion between groups

<table>
<thead>
<tr>
<th>Vascular Congestion</th>
<th>Percentage of vascular congestion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group n=8</td>
</tr>
<tr>
<td>Present</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Absent</td>
<td>6 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
</tr>
</tbody>
</table>

**Chi-Square test p-value= 0.000**

* p-value ≤ 0.05 is considered to be statistically significant

**Effect of D-Transallethrin on Histological section of Testis:** Testis sections of control group (Figure 1) showed tunica albuginea and tunica vasculosa surrounding lobules separated by septas. Each lobules has multiple sections of seminiferous tubules lined by modified stratified epithelium of spermatogenenic and Sertoli cells. Seminiferous tubules are surrounded by intertubular connective tissue in which blood vessels, nerves and clusters of interstitial/Leydig cells are present. Marked germinal epithelial cell loss with deranged contour of tubules was observed in histological sections of experimental group.

**Discussion**

Health hazards have been well attributed to the commonly used mosquito repellents. These repellents constitute synthetic pyrethroids amongst which d-transallethrin is the main culprit with pronounced toxic potential. Hepatotoxicity, ophthalmic discomfort and upper respiratory tract irritation have been associated with this stereoisomer where its ability to induce inflammation can be visualized by the presence of...
vascular congestion (9). The key mechanism behind this hyperemia is the expression of pro-inflammation mediators TNF-α and IL-1β which are influenced by the allethrin (10).

Reproductive tract malformations from allethrins have been documented in literature conferring us with their anti-gonadal potency (11). In this study the decrease in the epithelial height and subsequent decrease in the diameter of seminiferous tubules could be the consequence of formation of the free radicals leading to degradation of the parenchymal cells via oxidative stress (10).

Damage to testicular epithelia can also be based on allethrin compound’s ability to depolarize the membrane of mitochondria and release Cytochrome c. This Cytochrome c in turn inclines the cell to the apoptotic pathway by unbalancing Bax/Bcl-2 ratio. Thus, the apoptosis caused by the decrease in Bcl-2 levels and activation of caspase 3 can be held accountable for the disfigurement of germinal epithelium (10).

Figure 3: H & E stained sections showing seminiferous tubules of Group B (experimental) showing marked vascular congestion (green arrow), germinal epithelial cell loss (black arrow) with deranged contour of tubules (red arrow) Note the presence of empty spaces and desquamation. X10

Conclusion
From the foregoing results, it is clear that d-transallethrin via mosquito coil smoke exposure caused histopathological changes in the microarchitecture of the testicular tissue. Hence it is recommended to restrict the use of mosquito coils containing d-transallethrin.

Conflict of interest: Authors declare no conflict of interest.

Disclosure: None
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References