Histomorphological Changes in Testis of Rats with Prolonged Exposure to Allethrin-Based Mosquito Coil Smoke

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ABSTRACT

Objective: To explore the potential adverse effects of prolonged inhalation of mosquito coil smoke on the testicular histomorphology and serum testosterone levels in rats.

Study Design: An experimental study.

Place and Duration of the Study: Department of Anatomy, Army Medical College, National University of Medical Sciences (NUMS), Rawalpindi, Pakistan, from January to December 2020.

Methodology: This study was carried out on 20 male Sprague-Dawley rats, divided into control Group A (n = 10) and experimental Group B (n = 10). Rats in Group B were exposed to mosquito coil smoke (allethrin-based) for 4 hours / day for 12 weeks. After 12 weeks of exposure, serum testosterone levels and testicular morphology (seminiferous tubule diameter, germinal epithelium height, and testicular capsule thickness) were compared between the groups.

Results: Rats exposed to mosquito coil smoke showed significantly reduced serum testosterone levels ($p < 0.001$) along with testicular histological disruption in terms of significantly dilated seminiferous tubules ($p < 0.001$), reduced germinal epithelial height ($p < 0.001$), and thickened testicular capsule ($p < 0.001$), as compared to the control group.

Conclusion: Prolonged inhalation of allethrin-based mosquito coil smoke reduced serum testosterone levels and caused testicular histological disruption in the exposed group of rats.

Key Words: *Allethrin, Germinal epithelium, Mosquito coil, Seminiferous tubules, Testicular capsule, Testosterone, Testis.*

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INTRODUCTION

Among all vector-borne illnesses, mosquito-borne diseases account for the highest morbidity and mortality.[1] Various methods are used at the domestic level to avoid mosquito exposure, including aerosols, mosquito coils, and liquid vaporisers.[2] Mosquito repellant action of coils includes spatial action of evaporated pyrethroid particles achieved *via* burning that repels mosquitoes.[2] Experimentation has shown that adequate deterrence, feeding / biting inhibition, and insecticidal role can be efficiently achieved *via* burning pyrethrin-based mosquito coils.[3]

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Mosquito coils are mostly used in low-income societies because they are cost-effective and easily available. Although relatively safer outdoors, mosquito coils are frequently used indoors too in Asia, Africa, and Australia regions. This potentially causes indoor air pollution.[4] Coil users are exposed to this smoke approximately for eight hours at night. As mosquito infestation is an endemic problem in these societies, this exposure lasts for several months every year and it continues for many years.[4]

Exposure to mosquito coil smoke affects vital organs such as the liver, testis, kidneys, and lungs.[5] Few descriptive and cohort studies have also suggested the association of deranged male reproductive functions with exposure to mosquito coil smoke, in terms of decreased sperm count, increased sperm abnormalities, sperm DNA damage, and endocrinal disruption.[5,6] However, the quest for conclusive results is still going on because of the paucity of information and non-standardisation of data available regarding the toxicological impact of mosquito coils on the male reproductive tract of animals and humans.[7,8]

Also, there is no substantial research data available on the adverse effects of long-term allethrin-based mosquito coil smoke exposure on histomorphological characteristics of testis among humans and animals. Hence, the consequences of allethrin-based mosquito coil smoke inhalation are important to be investigated. The aim of this study was to explore the potential adverse effects of prolonged inhalation of mosquito coil smoke on the testicular histomorphology and serum testosterone levels in rats.

METHODOLOGY

This experimental study was conducted at the Department of Anatomy, Army Medical College, National University of Medical Sciences (NUMS), Rawalpindi, Pakistan, from January to December 2020 in collaboration with the Institute of Health after obtaining ethical approval from the Institutional Review Board and Ethical Committee of Medical College, vide letter no: ERC/07, Dated January 2020. Inclusion criteria were male rats of 5-6 months of age. Exclusion criteria were rats with any gross abnormality. Twenty Sprague-Dawley male rats were procured from the Institute of Health. All 20 rats had a free availability of standard laboratory rat chow and water. After acclimatisation for one week, rats were divided into two groups by non-probability consecutive sampling, i.e., 10 rats per group; Group A served as the control group and Group B rats were subjected to inhalation of mosquito coil smoke (allethrin-based) for 4 hours per day for 12 weeks.[9]

Mosquito coils with an active ingredient of d-trans allethrin (1g/kg) were placed in plastic cabins having three small openings (2 cm wide) on either side.[10] Rat cages for mosquito coil smoke exposure were placed in those transparent plastic cabins measuring 76 X 60 X 60 cm. Rats in Group B were exposed *via* whole-body inhalation to mosquito coil smoke for four hours (10 am – 2 pm) every day for 12 weeks.

After 12 weeks of the experiment, blood samples were drawn from the tail vein of all rats for measurement of serum testosterone levels.[11] Two mL blood was collected from the localised tail vein at 1/3rd way along the length of the tail from the tail tip. The blood samples were centrifuged, and the sera were separated. Serum testosterone levels were measured by using the Testosterone Enzyme Immunoassay (EIA) Test Kit, by PerkinElmer Health Sciences, Inc. US 10 Division (Catalog. No. 10007).

At the end of the study, animals were euthanised by an inhalant anaesthetic overdose of diethyl ether.[12] The rats were then dissected for procurement of the testis. The abdominal cavity was approached by a midline longitudinal incision, and right testes were identified, retracted through the inguinal canal into the abdominal cavity, and removed. Epididymis and other extra-testicular tissues were removed. Testes were fixed in 10% buffered formalin (about 3X volume of tissue) and stored at 4˚C for 48 hours. A tissue sample measuring 1.5 × 1.5 cm was taken in the transverse plane from the mid of the formalin-fixed testes. This fixed tissue sample was further processed in a series of steps to obtain 5 µm thick histological sections *via* microtome. Two slides were prepared from a sample of each rat, and stained with Haematoxylin and Eosin (HE) dyes.

For microscopic observation, slides were viewed and photographed using Olympus® Digital Microscope Camera under a 40× objective lens (i.e., 400× magnification). For each animal, two slides were studied, and for each slide, three random fields were observed and photographed. All the images were analysed using ImageJ software, version 1.53c (National Institute of Health, USA), calibrated with the help of a linear stage micrometre.

For each animal, measurements of seminiferous tubule diameter (µm), germinal epithelium height (µm), and testicular capsule thickness (µm) were taken in the six fields of view and then averaged. In each field of view, only the seminiferous tubules sectioned transversely (perfectly round sections) were used for the analyses. The germinal epithelium height (µm) was obtained by measuring the distance from the basement membrane to the lumen at four random parts in each tubule.

The data were entered into the database using a Statistical Package for Social Sciences (SPSS) version 22.0. Quantitative variables were expressed as mean ± standard deviation (SD). Significant difference among groups was determined by independent-sample t-test, after looking for the assumptions. The p-value ≤0.05 was considered significant.

RESULTS

Table I shows the comparison among the two groups for histomorphological and serum parameters by Independent-samples t-test. The data were normally distributed, homogenous, and had no outliers. The mean seminiferous tubular diameter and testicular capsule thickness of experimental Group B were statistically significantly higher than the control Group A, $t(18) = 22.28$ (µm), $p < 0.001$ and, $t(18) = 10$ (µm), $p < 0.001$, respectively. Whereas, the germinal epithelium height of experimental Group B and serum testosterone levels were significantly lower as compared to the control Group A, $t(18) = 14.54$ (µm), $p < 0.001$ and $t(18) = 13.38$ (ng/ml), $p < 0.001$, respectively (Figure 1).

Table I: Comparison of histomorphological findings and serum tes tosterone levels between the experimental and control groups.

Parameter	Group A (mean ± SD)	Group B (mean ± SD)	Mean diff. (A-B)	p-value
Seminiferous tubule diameter (µm)	243.36 ± 4.38	291.02 ± 5.15	-47.65	<0.001*
Germinal epithelium height (µm)	62.41 ± 4.53	33.03 ± 4.51	29.38	<0.001*
Testicular capsule thickness (µm)	25.87 ± 2.66	39.17 ± 3.26	-13.3	<0.001*
Serum testosterone (ng/ml)	4.27 ± 0.24	2.67 ± 0.29	1.61	<0.001*

*Group A: Control Group, Group B: Rats exposed to mosquito coil smoke inhalation, SD: standard deviation, *p <0.05 significant for independent sample t-test.*

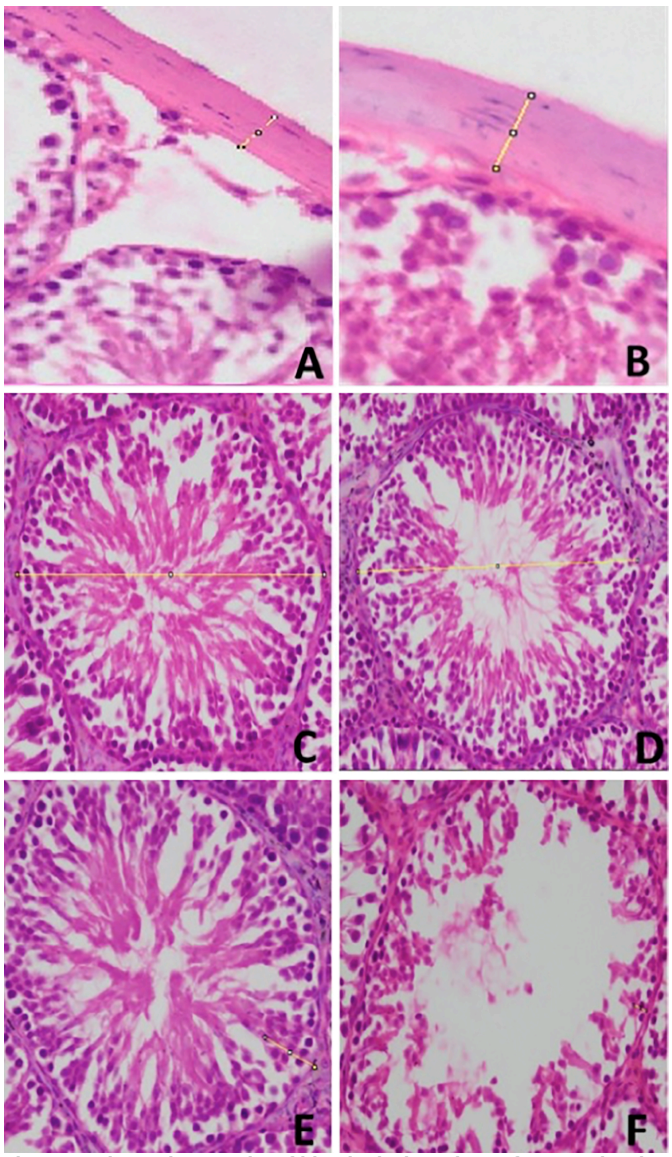

Figure 1: Photomicrographs of histological sections of testes showing the comparison of testicular capsule thickness, seminiferous tubule diameter, and germinal epithelium height in the control Group A and experimental Group B (H&E, X400). (A) Normal testicular capsule thickness in rats of control group. (B) Increased testicular capsule thickness in rats of experimental group. (C) Normal seminiferous tubule diameter in rats of control group. (D) Increased seminiferous tubule diameter in rats of experimental group. (E) Normal germinal epithelium height in rats of control group. (F) Decreased germinal epithelium height in rats of experimental group.

DISCUSSION

This study explored the effects of mosquito coil smoke on the histomorphology of rat testes. The study recorded the microscopic changes in the testicular morphology and serum testosterone levels in two groups of rats. The study aimed to establish the temporal relationship between possible harmful effects of mosquito coil smoke and testicular structural integrity while considering the testicular endocrinal role. The novelty of this study lies in its comprehensive approach to linking chronic mosquito coil smoke exposure to specific testicular changes, providing new insights into the underlying mechanisms.

The experimental group rats showed seminiferous tubule distention, reduced germinal epithelium height, thickening of the testicular capsule, and reduced serum testosterone levels as compared to the control group.

Abnormal serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, sex hormone-binding globulin, inhibin B, and calculated free testosterone have also been delineated in the Japanese male population exposed to mosquito coil smoke.[13] Various studies have proposed different mechanisms of inhibition of testosterone production upon exposure to pyrethroids. Proposed mechanisms for pyrethroids-induced hypothalamic-pituitary-gonadal (HPG) axis disruption include the involvement of steroid receptors, ion channels, and signalling molecules.[14] Pyrethrin chemicals are believed to retard testosterone binding to its receptor and serum androgen-binding globulin, thus disrupting the HPG axis.[14] By influencing endocrinal pathways and steroidogenesis, pyrethroids are considered to influence male fertility.[14] A study proposed that pyrethroid-related insecticides can reduce androgen production by altering the activity of the steroidogenic acute regulatory protein and decreasing the availability of cholesterol precursors.[15]

Synthetic pyrethroids have been associated with the downregulation of steroidogenic acute regulatory protein among pubertal male rats that impact steroidogenesis.[16]Allethrin-induced oxidative stress, cytotoxicity, and calcium-mediated apoptosis of testicular Leydig cells were reported in an experimental rodent model.[17] Testicular enzymatic activity of glucose-6-phosphate dehydrogenase (G6PD) and epididymal enzymatic functioning of sorbitol dehydrogenase were mildly affected in another rodent model exposed to allethrin-based smoke inhalation.[18]

The allethrin-induced oxidative stress likely exacerbates cellular damage through increased production of reactive oxygen species (ROS), leading to lipid peroxidation, DNA damage, and apoptosis.[18] This oxidative stress disrupts the integrity of seminiferous tubules, causing dilation and epithelial sloughing. Seminiferous tubule dilation in the experimental group of the current study can be explained by the sloughing of the epithelial cells. A similar study in 2012 on chronic mosquito smoke inhalation among rats has reported dilated seminiferous tubular lumen owing to oxidative stress.[9] Reduced mean germinal epithelium height among rats exposed to mosquito coil smoke also indicates the testicular disruption toxicity by cell loss that can lead to reduced fertility. However, further detailed analysis to identify the exact stage and nature of affected germ cell maturity could have added better information to the understanding of toxicological data. Rats' testes are normally surrounded by a compact connective tissue capsule with a single squamous epithelium on the surface. Beneath the epithelium, veins and a few arteries are visible microscopically in the white fibrous connective tissue.[19] Deep in the connective tissue of the testicular capsule, collagen Type I fibres, fibrocytes, fibroblasts, and smooth muscles are scattered.[19] The

morphometric finding of thickened testicular capsules among rats exposed to mosquito coil smoke can be attributed to the accumulation of inflammatory infiltrates in the thickened capsule in response to ongoing oxidative stress.

Few experimental studies among rodents exposed to allethrin-based mosquito coil support the histological findings of the current study. These studies have proposed oxidative stress-induced tubular dilation and loss of germinal epithelium along with morphological abnormalities of sperms.[9,17,20] As the seminiferous tubules are constituted by germinal epithelium and connective tissue, any change in their morphological characteristics can be associated with variation in sperm quality. Effects of mosquito coil smoke on male reproductive health have been variably reported among different cohorts and the quest for conclusive results is still going on.[9,15]

Morphometric variation in the testicular histology of rats in the current study can either be directly attributed to allethrin-induced oxidative stress or indirectly to the endocrinal imbalance caused by low testosterone levels. Normal sperm production is regulated by the endocrinological role of the HPG axis along with a complex interaction between neighbouring Sertoli and Leydig cells at autocrine and paracrine levels.[21] Hence, variation in serum testosterone levels could also have led to various morphological variations of reduced germinal epithelial height in the current study.

Limitations of this study include a simple evaluation of serum testosterone levels rather than measuring seminal fluid testosterone concentration. Secondly, histological characteristic of the Leydig cells were not assessed. In addition, a detailed profile of spermatogenesis by application of Johnson's criteria on histopathology and semen analysis would have delineated conclusive results on the functional integrity of the testes.

CONCLUSION

Long-term inhalation of allethrin-based mosquito coil smoke can cause testicular toxicity among rats, as shown by reduced serum testosterone, and histomorphological findings of dilated seminiferous tubules, reduced germinal epithelial height, and thickened testicular capsule.

ETHICAL APPROVAL:

Ethical approval was taken from the Ethics Committee of the Army Medical College / NUMS Rawalpindi (ERC: /ID/07) prior to the initiation of the research work.

COMPETING INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

MFA: Substantial contribution to the concept and drafting of the manuscript.
KQ: Substantial contribution to the design of the work, drafting of the manuscript, and supervision.
AZ: Interpretation of the data and drafting of the work.
SSS: Substantial contribution to interpretation of the data and drafting of the manuscript.
MRBK: Drafting of the manuscript and revision of the final version.
MI: Drafting of the histopathalogical study.
All authors approved the final version of the manuscript to be published.

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