Protective Effects of Coenzyme Q10 on Testicular Histology in Rats Exposed *via* Chronic Mosquito Coil Smoke Inhalation

Muhammad Fahad Atta[1], Khadija Qamar[2], Saba Saleem Safdar[3], Maria Iram[4], Muhammad Rizwan Bashir Kiani[2] and Muhammad Sarwar Zia[4]

[1]*Department of Anatomy, Quaid-e-Azam Medical College, Bahawalpur, Pakistan*
[2]*Department of Anatomy, Army Medical College/National University of Medical Sciences, Rawalpindi, Pakistan*
[3]*Department of Anatomy, Sialkot Medical College, Sialkot, Pakistan*
[4]*Department of Anatomy, Rawal Institute of Health Sciences, Islamabad, Pakistan*

ABSTRACT

Objective: To determine the preventive effect of coenzyme Q10 (CoQ10) on the testicular histology of rats exposed chronically to mosquito coil smoke.

Study Design: Experimental study.

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

Methodology: Thirty male Sprague Dawley rats were divided into three groups of 10 rats each. Group A was the healthy control. Group B rats were exposed to allethrin-based mosquito coil smoke for 12 weeks (4 hours/day). Group C rats received coenzyme Q10 (CoQ10, 10mg/kg/day) through oral gavage, in addition to 12 weeks of mosquito coil smoke exposure (4 hours/day). At the end of the study, testicular histology was compared among three groups including the germinal epithelium height, seminiferous tubule diameter, and testicular capsule thickness, while adjusting for the body weight variations among rats.

Results: The rats in Group B, exposed only to mosquito coil smoke showed testicular disruption, characterised by dilated seminiferous tubules ($p < 0.001$), reduced germinal epithelial height ($p < 0.001$), and thickened testicular capsule ($p < 0.007$), as compared to the control group rats. However, the germinal epithelium height ($p = 0.73$) and testicular capsule thickness ($p = 0.31$) of rats receiving CoQ10 in addition to mosquito coil smoke inhalation were not significantly different from the control group.

Conclusion: Prolonged inhalation of allethrin-based mosquito coil smoke can cause testicular disruption among rats. The oral CoQ10 administration can effectively prevent the histomorphological adverse effects on the testis among rats exposed to mosquito coil smoke.

Key Words: *Allethrin, Coenzyme Q10, Germinal epithelium, Mosquito coil, Seminiferous tubules, Testicular capsule.*

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INTRODUCTION

Reproductive health is vital for both, individuals and society. Despite variable fertility rates worldwide, developed countries grapple with economical and social imbalances due to low fertility rates.[1] Male infertility contributes to around 50% of the global infertility cases, with underreporting in low socio-economic countries. Male infertility rates are estimated to be 5-6% in North America, 9% in Australia, and 8-12% in Central and Eastern Europe.[2] Despite its significance and growing evidence of a decline in reproductive health, male infertility still remains relatively underexplored.

Correspondence to: Dr. Muhammad Rizwan Bashir Kiani, Department of Anatomy, Army Medical College/National University of Medical Sciences, Rawalpindi, Pakistan
E-mail: gakhar1811@yahoo.com
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Various physiological, environmental, and genetic factors underlie male reproductive disorders, including obstruction of male genital ducts, varicocele, idiopathic factors, cryptorchidism, immunologic issues, ejaculatory dysfunction, testicular failure, drug/radiation effects, and endocrine disruptions.[3] Many of these factors induce oxidative stress-related male reproductive dysfunctions. Globally, oxidative stress-driven epigenetic changes contribute to 30-80% of male infertility cases.[4] Therefore, it is crucial to thoroughly investigate preventable environmental causes of oxidative stress-related male infertility.

Pyrethroid compounds such as allethrin are linked to oxidative stress in the male reproductive tract, potentially impairing fertility by peroxidation of spermatozoa membrane lipids.[5] Chronic exposure to allethrin-based mosquito coil smoke might elevate toxic free radical production in the male reproductive tract, leading to reduced sperm count, motility, and fertility.[5] Infertility is prevalent among Pakistani males,[6] yet limited data exists on sperm abnormalities and potential infertility risk factors.

Coenzyme Q10 (CoQ10) is essential for adenosine triphosphate production *via* mitochondrial oxidative phosphorylation and serves as an antioxidant and membrane stabiliser.[7] Widely used therapeutically (as an adjunct in various cardiovascular, neurodegenerative, and inflammatory disorders), CoQ10 shows inconsistent effects on sperm motility, number, morphology, and seminal fluid antioxidant activity.[8] However, knowledge gaps persist regarding CoQ10's impact on testicular histomorphometry and environment-induced epigenetic modifications causing male reproductive disorders.[9] This study aimed to assess CoQ10's preventive effects on testicular histology in rats exposed chronically to mosquito coil smoke.

METHODOLOGY

This rodent experimental study was conducted from January to December 2020, at the Anatomy Department of Army Medical College/National University of Medical Sciences, Rawalpindi, Pakistan, after obtaining approval vide letter no. ERC/07, dated January 2020. The study included n = 30 male Sprague Dawley rats with an average weight of 250 ± 50 gm. Rats with any visible gross deformity were excluded from the study. The study was conducted following the institutional guidelines and rules of NRC, 199ves6 declaration.[10]

The rats were provided *ad libitum* access to standard rodent chow and water, throughout the study period. Rats were divided into three groups of 10 rats in each by non-probability consecutive sampling technique. Group A, the control group, contained healthy rats. Group B contained rats exposed to allethrin-based mosquito coil smoke for 12 weeks (4 hours/day).[11] Group C received CoQ10 (10mg/kg/day) through oral gavage,[12] in addition to 12 weeks of mosquito coil smoke exposure (4 hours/day). Group B and C rats were exposed to commercially available mosquito coil smoke with an active ingredient allethrin *via* whole-body inhalation for four hours (10 am – 2 pm) every day for 12 weeks. Exposure was achieved by placing rat cages in plastic cabins containing three 2cm-wide holes on each side.[13] Oral gavage for administering CoQ10 to Group C rats was done daily between 9 am and 10 am for 12 weeks. For this purpose, rats were grasped and firmly immobilised with their heads and bodies fixed vertically, by a trained personnel. A 5cm-curved gavage tube with a 3mm-ball tip was used for administering the calculated dose of CoQ10.[12] Gavage tube was wiped in between the animals.

Rats were weighed at the end of this study by using a digital weighing scale, sensitive up to 0.1 grams. At the end of this study, rats were euthanised by diethyl ether inhalational overdose.[14] Rats were then dissected in the supine position. The abdominal cavity was approached by a longitudinal midline incision made below the rib cage. The right testes were dissected out through the inguinal canal into the abdominal cavity. Testicular tissue was processed, and two slides were prepared from the testis of each rat. Five micrometres (5µm) thick histological sections were obtained using a microtome (Leica rm 255). For microscopic observations, tissue sections were further pro-cessed, and stained with haematoxylin and eosin dyes.

Olympus® microscope BX43 with digital camera (10 megapixels) was used for microscopic observation and photography of slides, using Cell Sens imaging software version 1.17. For each animal, two slides were studied, and for each of the slides, three random fields were observed and photographed under a 40× objective lens (i.e., 400 × magnification). All the images were analysed using ImageJ software, version 1.53c (National Institute of Health, USA). For all the measurements, the software was calibrated with the help of a linear stage micrometre which was photographed using the same system and magnification as the rest of the slides.

Using the slides, seminiferous tubular diameter (µm) was calculated by taking the average of two measurements perpendicular to one another. Multiple measurements for the thickness of the testicular capsule (µm) in each field of view were made and an average was taken. Germinal epithelium height was obtained by measuring the distance from the basement membrane to the lumen at four different parts in each tubule, equidistant from each other, and a mean value was computed. For each animal, all the measurements were taken in the six fields of view and the average was calculated to find a single final value.

The data were analysed using the Statistical Package for the Social Sciences (SPSS) version 26. Descriptive statistics of quantitative variables were computed as mean ± standard error. The significant difference in microscopic parameters among groups was assessed *via* the one-way analysis of variance (ANOVA) adjusting for body weight, after examining the assumptions. Follow-up post hoc tests were carried out to determine pairwise differences among groups when the parameter was significant for the overall F-test of one-way ANOVA (at $p \leq 0.05$).

RESULTS

The mean weight of rats with standard error at the end of the study in Groups A, B, and C were recorded as 324.42 ± 0.59, 301.74 ± 1.1, and 319.6 ± 1.09 grams, respectively. Descriptive statistics of rats' testicular histological parameters in three groups are given in Table I.

A comparison of all quantitative parameters among the three groups was carried out by one-way ANOVA while controlling for body weight. There was a statistically significant difference in seminiferous tubular diameter ($F\ 2.26 = 25.39$, $p < 0.001$, partial $\eta^2 = 0.66$), germinal epithelium height ($F\ 2.26 = 10.5$, $p < 0.001$, partial $\eta^2 = 0.45$), and testicular capsule thickness ($F\ 2.26 = 44.89$, $p = 0.007$, partial $\eta^2 = 0.32$) of the study groups (Table I). A significant difference was followed by post hoc analysis with a Bonferroni adjustment (Table II).

The mean seminiferous tubules diameter of the testis of the rats exposed to mosquito coil smoke in Group B, and simultaneously exposed to mosquito coil smoke and CoQ10 in Group C, was more as compared to the control Group A, a statistically significant difference of 14.5 µm (SE = 2.49, $p < 0.001$) and 48.06 µm (SE = 6.93, $p < 0.001$), respectively.

Table I: Histological testicular parameters among groups A, B, and C.

Parameter	Group A ($mean_{adj}$ ± SE)	Group B ($mean_{adj}$ ± SE)	Group C ($mean_{adj}$ ± SE)	p-value
Seminiferous tubule diameter (µm)	243.2 ± 3.04	291.26 ± 4.2	257.69 ± 1.93	<0.001*
Germinal epithelium height (µm)	61.47 ± 2.9	34.41 ± 3.99	58.64 ± 1.83	<0.001*
Testicular capsule thickness (µm)	25.59 ± 1.82	39.57 ± 2.51	28.1 ± 1.15	0.007*

Group A = Control group, Group B = Rats with chronic exposure to mosquito coil smoke, Group C = Rats administered CoQ10 in addition to chronic mosquito coil smoke exposure, Standard deviation, p-value = Significant at p ≤0.05 for overall F-test of one-way ANOVA adjusting for body weight at a degree of freedom = 2.26.

Table II: Comparison of histological parameters among groups A, B, and C.

Pairwise groups x	y	Mean Diff. (µm) (x-y)	p-value
Seminiferous tubules diameter (µm)			
A	B	-48.06	<0.001*
A	C	-14.5	<0.001*
B	C	33.56	<0.001*
Germinal epithelium height (µm)			
A	B	27.07	<0.001*
A	C	2.83	0.73
B	C	-24.24	<0.001*
Testicular capsule thickness (µm)			
A	B	-13.98	0.007*
A	C	-2.5	0.31
B	C	11.48	0.006*

Group A = Control Group, Group B = Rats with chronic exposure to mosquito coil smoke, Group C = Rats administered CoQ10 in addition to chronic mosquito coil smoke exposure, p-value = Significant at p ≤0.05 for Bonferroni adj post hoc test of one-way ANOVA.

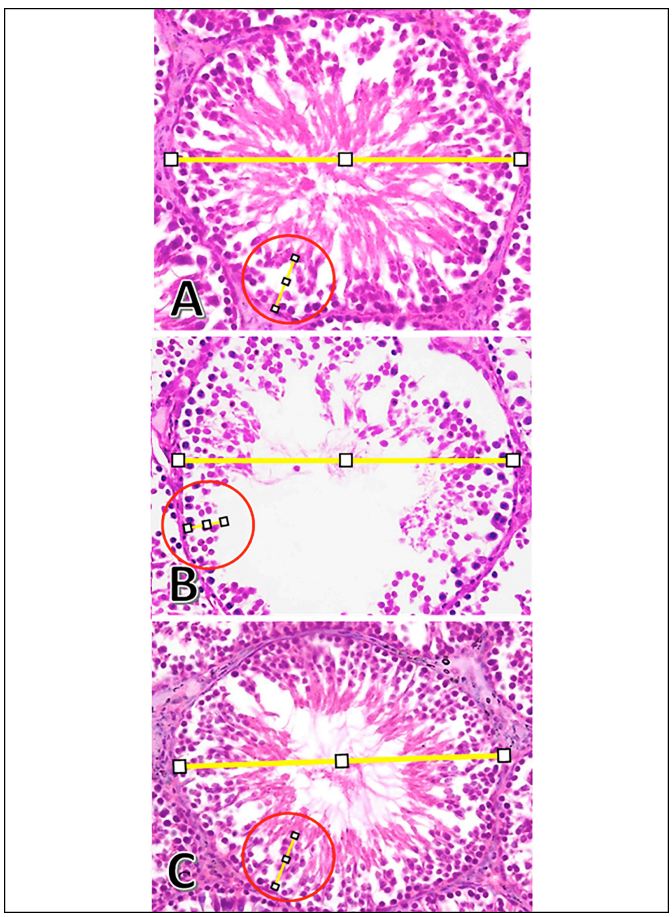

Figure 1: Photomicrograph at 400X magnification showing a comparison of testicular epithelial height and seminiferous tubule diameter among groups.
Group A = Control group, Group B = Rats with chronic exposure to mosquito coil smoke, and Group C = Rats administered CoQ10 in addition to chronic mosquito coil smoke exposure.

The mean germinal epithelium height of the testis of rats in Group C was higher as compared to Group B, with a statistically significant difference of 24.24 µm, (SE = 5.33, p <0.001), but not significantly different from control Group A, i.e., 2.83 µm (SE = 2.37, p = 0.73, Figure 1). Additionally, the mean testicular capsule thickness of rats in Group C was less as compared to Group B, with a statistically significant difference of 11.48 µm (SE = 3.35, p = 0.006), but insignificantly different from the control Group A, i.e., 2.5 µm (SE = 1.47, p = 0.31).

DISCUSSION

The primary finding of this study underscores the potential protective role of CoQ10 against testicular histological disruption induced by chronic mosquito coil smoke exposure.

This study found significant disruption of the testicular histology in rats chronically exposed to mosquito coil smoke in terms of dilated seminiferous tubules, reduced germinal epithelial height, and thickened testicular capsules. Animals simultaneously treated with CoQ10 though had shown histological changes but statistically insignificant when compared to the control group animals.

This morphometric variation of dilated seminiferous tubules among rats exposed to allethrin-based mosquito coils can be explained by the anti-progestogenic nature of pyrethroids. Being inhibitors of estrogen receptors, pyrethroids can significantly inhibit seminal fluid re-absorption across rete testis, efferent ducts, and epididymis.[15] This can cause fluid accumulation and build-up of substantial back-pressure along efferent and seminiferous tubules, as evidenced by dilated seminiferous tubules in the current study. Initially, this tubular dilatation might have been accompanied by apparently normal spermatogenesis. However, the longstanding dilatation may lead to loss of germinal epithelium accompanied by the dilated seminiferous tubules,[15] again consistent with the observation of decreased germinal epithelium height in the current study. This reduction in germinal epithelium height among the rats exposed to mosquito coil smoke can result in reduced fertility. However, further detailed analysis to identify the exact stage and nature of the affected germ cell maturity could have added better information to the understanding of the toxicological data. The morphometric finding of thickened testicular capsules among the rats exposed to mosquito coil smoke can be attributed to increased connective tissue deposition and thickened fibromuscular stroma in response to the ongoing oxidative stress. However, the exact nature and cause of the thickened testicular capsule could only be delineated with

further sophisticated staining, and microscopic and tissue composition analysis.

The histopathological findings of the current study are also supported by a few comparable rodent studies.[5,11,16] Few descriptive and cohort studies among humans have also stated the probable link between impaired male reproductive functions with long-term pyrethroid exposure (oral / inhalational / cutaneous) in terms of decreased sperm count, increased sperm abnormality,[17] sperm DNA damage,[18] translational modifications of germ cells proteins,[19] and transgenerational epigenetic changes in the spermatozoa.[19] The studies exploring the adverse impacts of inhalational exposure to pyrethroids on the male reproductive tract structural integrity are still inconclusive.

Antioxidant supplementation can help combat oxidative stress by reducing the reactive oxygen species formation or by imbibing the preformed free radicals. The current study has also shown preserved morphological characteristics of rats' testis when administered CoQ10 along with mosquito coil exposure. The standardised data are scarce on the direct effect of CoQ10 on rats' testis exposed to mosquito coil smoke to offer an effective comparison with this study. However, experimental studies are available showing improvements in seminal parameters upon the administration of CoQ10 among infertile male patients.[8,20]

Different segments of seminiferous tubules contain spermatozoa waves in different stages of the germ cell lineage cycle. The histological evaluation of seminiferous tubules may vary in different cross-sections depending upon the exact stage of the seminiferous tubule cycle and waves.[21] To address the issue multiple measurements were taken for each animal in this study and then the average value was computed. However, the exact identification of the spermatogenic stage would have allowed more standardised comparison among the groups of the current study and with the future studies. Despite this limitation, this study is distinctive in exploring the preventive effect of CoQ10 on the testicular histology of rats exposed chronically to mosquito coil smoke.

Further research should delve into comprehensive oxidative stress analysis and mechanistic studies to elucidate CoQ10's exact protective mechanisms. Longitudinal and translational investigations are crucial for validating these findings in human subjects and uncovering the precise pathways underlying CoQ10's protective effects, potentially offering novel therapeutic interventions.

CONCLUSION

Long-term inhalation of allethrin-based mosquito coil smoke can cause testicular disruption among rats, as evidenced by histopathological findings of dilated seminiferous tubules, reduced germinal epithelial height, and thickened testicular capsule. Whereas the orally administered CoQ10 among rats exposed to mosquito coil smoke can effectively prevent the adverse effects on the testicular histology.

ETHICAL APPROVAL:
Ethical approval was obtained from the Institutional Review Board and Ethical Committee of the Army Medical College, Rawalpindi, Pakistan vide letter no. ERC/07, Dated: 24-Aug-2023.

COMPETING INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
MFA, KQ, SSS, MI, MRBK, MSZ: Conception of the study, study design, data collection, draft of the manuscript, statistical analysis, data interpretation, and literature search.
All authors approved the final version of the manuscript to be published.

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