Mosquito Coil Smoke Inhalation

PROPHYLACTIC EFFECT OF COENZYME Q10 ON GROSS PARAMETERS OF RAT TESTIS EXPOSED TO MOSQUITO COIL SMOKE INHALATION

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ABSTRACT

Objective: To determine the toxic effect of mosquito coil smoke inhalation on gross parameters of rat testis and explore the protective effect of Coenzyme Q10 on testicular toxicity profile.

Study Design: Laboratory-based experimental study.

Place and Duration of Study: Department of Anatomy Army Medical College, Rawalpindi in collaboration with National Institute of Health, Islamabad, from Jan 2020 to Dec 2020.

Methodology: This study was carried out among 30 male Sprague Dawley rats, distributed into three groups as 10 rats/group; group A served as control group, rats in group B were exposed to allethrin-based mosquito coil smoke 4 hours/day for 12 weeks. Rats in group C were administered Coenzyme Q10 (10 mg/kg/day) via oral gavage for 12 weeks along with mosquito coil smoke exposure. At the end of study, gross parameters of body weight gain, testicular weight and volume, and relative tissue body weight index of testis were compared among groups.

Results: Rats exposed to mosquito coil smoke alone showed significantly less weight gain (p<0.001), testicular weight (p<0.001), volume (p<0.001), and relative tissue body weight index (p<0.001) as compared to control group. Rats administered Coenzyme Q10 along with mosquito coil smoke exposure exhibited significantly higher weight gain (p<0.001), testicular weight (p<0.001), volume (p<0.001) and relative tissue body weight index (p<0.001) as compared to rats exposed to mosquito coil smoke alone.

Conclusion: Long-term inhalation of allethrin-based mosquito coil smoke caused testicular atrophy among rats, as evident by reduced testicular weight, volume and relative tissue body weight index. Whereas, prophylactic oral administration of Coenzyme Q10 among rats exposed to mosquito coil smoke prevented testicular toxicity.

Keywords: Allethrin, Coenzyme Q10, Mosquito coil, Relative tissue body weight index, Testis, Weight.

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INTRODUCTION

Mosquito-borne diseases account for the maximum number of cases, morbidity, and mortality among all vector-borne infectious diseases, worldwide. Tropical and subtropical regions of the world (Africa, Asia, and America) are the most widely affected by these mosquito-borne diseases, where approximately more than half of the human population is residing. Despite the availability of modern non-chemical methods to control mosquitoes, chemical mosquito repellents are still the most widely used especially in low socio-economic regions. Most abundantly used household chemical repellents include mosquito coils, pyrethrum-based sprays, vaporizer mats and aerosols. As per careful estimate, approximately 45-80% of deterrence can be achieved via pyrethrin-based mosquito coils within the first 4 weeks of treatment.

Mosquito repellent action of coils, vaporizer mats, and emanators includes spatial action of vaporous/airborne pyrethroid particles, as recommended by WHO. Among which mosquito coils are less desirable because of the production of smoke as side product. Despite the side effect of smoke, mosquito coils are still widely used in regions of Asia, Africa, and Australia, owing to their easy accessibility and cost-effectiveness. Pyrethroid compounds are experimentally reported to cause impaired male reproductive functions among rodents and human, either via oxidative stress or endocrinial disruption of Hypothalamic-pituitary gonadal axis. Owing to the widespread domestic use, cumulative results of long-term exposure to mosquito coil smoke can be a matter of reproductive health concern.

According to a recent review, environmental exposure is more important in causing male reproductive disorders via epigenetic mutations as compared to genetically acquired causes. As our knowledge regarding responsible environmental factors is still lacking, the effect of long-term exposure to mosquito coil smoke on the male reproductive system should be explored to allow timely prevention and treatment.

Owing to the potential antioxidant role of Coenzyme Q10 (CoQ10), its administration has shown...
promising results of improved seminal parameters in experimental trials among humans and rodents. However, there is lack of any substantial study to see the effects of CoQ10 on testis. Hence, the objective of this study was to determine the effects of mosquito coil smoke on the testicular weight toxicity of rats and explore the protective effect of Coenzyme Q10 on testicular toxicity. Testicular weight, volume and RTBWI, if taken as an indirect indicator of testicular atrophy, can be correlated with reduced fertility and sperm abnormalities in future clinical studies to have conclusive remarks on mosquito coil smoke toxicity profile.

**METHODOLOGY**

This laboratory-based experimental study was carried out at the department of Anatomy, Army Medical College, Rawalpindi/National University of Medical Sciences (NUMS) in collaboration with the National Institute of Health (NIH), Islamabad with approval from Ethics Review Committee (ERC/ID/104). The research was carried out on 30 male Sprague Dawley rats from January to December 2020. Healthy rats of average 3–4 months of age and mean weight of 250 ± 50 gm were selected by non-probability consecutive sampling. Rats with any gross abnormality were excluded from the study.

Environment, housing, and management of rats were carried out under NRC, 1996 declaration, and institutional guidelines throughout the research process. The rats were fed on standard laboratory rat chow and water ad libitum. After an acclimatization period of one week, rats were distributed into three groups including 10 rats per group as:

- **Group A**: Rats in Group A served as controls.
- **Group B**: These rats were exposed to the smoke of the mosquito coil (Allethrin-based) four hourly/day for 12 weeks.
- **Group C**: Rats in this group were given Coenzyme Q10 in a dose of 10 mg/kg/day, orally through a gavage tube 13 along with exposure to mosquito coil smoke (Allethrin-based) four hourly/day for 12 weeks.

Rats cages for mosquito coil smoke exposure were placed in transparent plastic cabins measuring 76 x 60 x 60 cm. Plastic cabins had three small openings on either side, each with a width of two cm. Rats in Group B and C were exposed via whole-body inhalation to smoke of commercially available mosquito coil with an active ingredient of d-trans Allethrin (one g/kg) for four hours (10am-2pm) every day for 12 weeks. Pure salt of Coenzyme Q10 was administered to rats in group C in dose of 10 mg/kg, between (9am-10am) every day for 12 weeks, by trained personnel orally through gavage tube.

The bodyweight of the rats was measured in grams at start and end of the experiment, with the help of digital analytical balance, sensitive up to one-tenth of a gram increment. At the end of the study, animals were euthanized by inhalant anesthetic overdose of diethyl ether. The rats were dissected in the supine position, by making a longitudinal midline incision below the rib cage to cut through skin and muscles and open the abdominal cavity. Right testes were identified, retracted through the inguinal canal into the abdominal cavity, and removed.

The volume of testes was measured in milliliter using formalin displacement method. The weight of the testis was then measured in grams using a digital analytical balance (Sartorius CP 324S), sensitive to weight increment of 0.0001 gram. Relative Tissue Body Weight Index (RTBWI) was calculated to get standardize the weight of the testis over rats of different sizes, using the following formula:

\[
\text{RTBWI} = \frac{\text{weight of testis in grams}}{\text{weight of body in grams}} \times 100
\]

The data was entered into the database using a statistical package for social sciences (SPSS-22). Quantitative variables were expressed as mean ± standard deviation. Significant difference among groups was determined by using one-way analysis of variance (ANOVA) after verification of the assumptions. Outliers in data were assessed by inspection of boxplot for values >1.5 box-lengths from the edge of the box. Normally of data was determined for each group by visual inspection of Q-Q plots and confirmed by the Shapiro-Wilk test (p >0.05). Homogeneity of variances was assessed by Levene’s Test of Homogeneity of Variance (ANOVA) after verification of the assumptions. Data with non-homogenous distribution was analyzed by Welch-ANOVA, and followed by Games-Howell post hoc test. The p-value ≤0.05 was considered significant.

**RESULTS**

Total 30 healthy male rats of average weight of 305.06 ± 3.04 grams were included in the study. Mean weight of rats in the beginning and the end of study among each group are given in table-I. One-way ANOVA was conducted to analyze the toxic effects of mosquito coil smoke and the protective effect of coen-
zyme Q10 on the body weight gain, testicular weight, volume and RTBWI among groups, after satisfying the assumptions of outliers, normality and homogeneity.

Mean weight gained by rats from start till the end of the experiment was statistically significantly different between the groups, Welch’s F (2,15.39)=595.58, p<0.001. Games-Howell post hoc analysis revealed that group C i.e., rats given CoQ10 along with exposure to mosquito coil smoke gained more weight as compared to group B i.e., the rats exposed to mosquito coil smoke alone, a statistically significant difference of 17.6gm, 95% CI (16.2 to 19 gm), p<0.001. Whereas, the group B rats exposed to mosquito coil smoke alone had lost weight till the end of the study, by an average of 3.14 ± 1.5 gm, figure.

The mean weight (Welch’s F (2,14.46)=961.1, p <0.001), volume (F (2,27) = 183.65, p<0.001) and RTBWI (Welch’s F (2,15.82) = 505.15, p<0.001) of rat testis varied significantly between study groups. Post hoc analysis revealed that weight, volume and RTBWI of rats’

Table-I: Inferential statistics of parameters among groups (n=10/group).

| Parameter                        | Group A                  | Group B                  | Group C                  | Total            | p-value |
|----------------------------------|--------------------------|--------------------------|--------------------------|------------------|---------|
| Initial Weight (gm)*             | 305.16 ± 2.65            | 304.88 ± 2.90            | 305.14 ± 3.77            | 305.06 ± 3.04    | 0.98    |
| Final Weight (gm)*               | 324.42 ± 1.86            | 301.74 ± 3.49            | 319.60 ± 3.44            | 315.25 ± 10.34   | <0.001* |
| Weight Diff. (gm)*               | 19.26 ± 2.1              | -3.14 ± 1.5              | 14.46 ± 0.76             | 10.19 ± 9.91     | <0.001* |
| Testis Weight (gm)*              | 1.74 ± 0.02              | 1.13 ± 0.02              | 1.54 ± 0.05              | 1.47 ± 0.26      | <0.001* |
| Testis Volume                    | 1.8 ± 0.09               | 1.1 ± 0.08               | 1.55 ± 0.07              | 1.48 ± 0.31      | <0.001* |
| Testicular Relative* tissue body weight index | 0.54 ± 0.01              | 0.37 ± 0.02              | 0.48 ± 0.01              | 0.46 ± 0.07      | <0.001* |

Table-II: Pairwise comparison of parameters among groups (n=10/group).

| Group Comparison         | Group-A Vs. Group-B | Group-A Vs. Group-C | Group-B Vs. Group-C |
|--------------------------|---------------------|---------------------|---------------------|
| Weight Gain*             | <0.001*             | <0.001*             | <0.001*             |
| Testis Weight*           | <0.001*             | <0.001*             | <0.001*             |
| Testis Volume            | <0.001*             | <0.001*             | <0.001*             |
| Testicular Relative* tissue body weight index* | <0.001*             | <0.001*             | <0.001*             |

Figure: Mean values of weight gain, weight, volume, and relative body weight index of testis.
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testis in group C was significantly higher as compared to group B, table-II. However, the weight, volume and RTBWI of rats' testis in group C, was significantly less as compared to the control group A, table-II.

Data given as mean ± standard deviation, group A=control group, group B=rats exposed to mosquito coil smoke, group C=rats administered CoQ10 along with mosquito coil smoke exposure, difference between rats’ weight at start and end of experiment, p-value=significant at p≤0.05 for overall F-test of One-way ANOVA, *Parameters distributed non-homogenously are depicting Welch-ANOVA statistics.

Group A=control group, group B=rats exposed to mosquito coil smoke, group C=rats administered CoQ 10 along with mosquito coil smoke exposure, difference between rats’ weight at start and end of experiment, RTBWI=Relative tissue body weight index of the testis, p-value=significant at p≤0.05 for post hoc Tuckey test of One-way ANOVA, *Parameters distributed non-homogenously are followed up by Games-Howell Test.

Group A=control group, group B=rats exposed to mosquito coil smoke, group C=rats administered CoQ 10 along with mosquito coil smoke exposure, error bars at 95% confidence interval, difference between rats’ weight at start and end of experiment, RTBWI=Relative tissue body weight index.

**DISCUSSION**

Owing to the increasing burden of male infertility worldwide, responsible and preventable environmental factors need to be explored. Among the known causes of male reproductive disorders, almost 30-80% of cases worldwide include oxidative stress. Pyrethroid chemicals, the major constituents of mosquito coils, are linked with oxidative stress in male reproductive system by experimental evidence. Therefore, it can be speculated that long-term mosquito coil smoke inhalation can be a causative factor of male reproductive functional and structural impairment. Based on the proposition and antioxidant potential of Coenzyme Q10 (CoQ10), this study was designed to determine the detrimental effects of mosquito coil smoke on the rat testes and the protective effect of CoQ10. Comparison of gross features i.e., body weight gain, testicular weight and volume, relative tissue body weight index (RTBWI), of rats' testis was done among the study groups at the end of the study.

Our study showed that the control group gained more weight as compared to those exposed to mosquito coil smoke over the duration of the study. However, the rats administered CoQ10 along with mosquito coil smoke exposure gained significantly more weight as compared to the rats exposed to the smoke alone. A similar trend was seen when other gross features were compared among groups. The control group had significantly higher mean testicular weight, volume, and RTBWI as compared to the Experimental groups. Protective effect of CoQ10 was evident by the fact that rats administered CoQ10 along with mosquito coil smoke had significantly higher testicular weight, volume, and RTBWI as compared to the rats exposed to mosquito coil smoke alone. Although, exact histopathologic changes are the more sensitive indicator of testicular damage, still decrease in testis weight and volume can be ascribed to generalized testicular degeneration as seen in comparable previous studies.

In 2013, Alalwani reported significantly decreased relative testicular weight among experimental rats as compared to the control group (p=0.014) in association with histopathological findings of testicular degeneration. Current study has compared not only the absolute testicular weight among groups, but also the relative testicular weight with respect to total body weight, hence eliminating the potential bias of different body sizes.

The pathophysiology of this gross variation can be explained by sloughing of the tubular epithelial cells caused by oxidative stress, and thus indicating gonadal toxicity by cell loss. The findings of reduced testicular weight, volume and RTBWI can also be attributed towards anti-progestogenic nature of Pyrethroids. As the 95% fluid produced in seminiferous tubules is re-absorbed across rete testis, efferent ducts, and epididymis under the influence of estrogens. Pyrethroids being able to inhibit estrogen receptors can decrease this fluid reabsorption. Which may lead to fluid accumulation and building up of backpressure along efferent ducts as well as seminiferous tubules. Longstanding pooling/backpressure may lead to damage and ultimately atrophy of germinal epithelium and interstitial cells of seminiferous tubules, as indicated by gross morphometric findings in current study. Reduced mean testicular weight, volume and RTBWI, if taken as an indirect indicator of testicular atrophy, can be correlated with reduced fertility and sperm abnormalities as seen in human cohorts and rodents exposed to long-term pyrethroids. After exposure to mosquito coil smoke to a rodent model, Madhubabu in 2012 reported oxidative distress and distorted cytoarchitecture in male reproductive tract, along with decreased sperma-
togenesis\textsuperscript{12}. Again in 2017, Madhubabu reported compromised spermatogenesis, sperm maturation and steroidogenesis in adult rats exposed to mosquito coil smoke in perinatal life\textsuperscript{22}. Compromised semen and male reproductive hormonal profile has been reported among human cohorts exposed to pyrethroids by Radwan in 2014, but their association with testicular histomorphological features still need to be explored\textsuperscript{20}.

Decreased testicular size and volume in presence of reduced Testosterone levels, can be attributed to testicular atrophy or reduced fluid production by Sertoli cells of seminiferous tubules\textsuperscript{8}. Imbalanced reproductive endocrinology upon mosquito coil smoke exposure can be also be caused by oxidative stress induced damage to the Leydig cells, as indicated experimentally among human and rodent studies\textsuperscript{8}. Bodyweight loss seen among rats exposed to mosquito coil smoke can also be related with hypothalamic-pituitary-gonadal axis imbalance\textsuperscript{23}, that could have led to impaired Testosterone production and hence testicular atrophy.

Antioxidants supplementation has been shown to improve oxidative stress-induced male reproductive disorders. The rats administered CoQ10 with mosquito coil smoke exposure had higher testicular weight, volume, and RTBWI as compared to the rats exposed to mosquito coil smoke alone. Substantial data is unavailable on the direct effect of CoQ10 on rats’ testis exposed to mosquito coil smoke to allow a healthy comparison with this study. However, the beneficial effects of CoQ10 administration on sperm parameters are supported by metaanalysis of available data by Lafuente et al\textsuperscript{10}. Spermatozoa carry a large number of mitochondria for their remarkable energy needs. In addition, the spermatozoa are at high risk of oxidative stress for carrying a high proportion of unsaturated lipids in their cell membranes and continuous exposure of free radicals being produced. For both reasons, CoQ10 plays an essential role in male reproductive physiology owing to its bioenergetic and antioxidant characteristics. This study has major limitation of not assessing the exact histopathological variation of tests and sper-matogenic count to give conclusive remarks on mosquito coil testicular toxicity. Despite the limitations, this study is unique in exploring the effects of long-term exposure to mosquito coil smoke on testicular weight toxicity while assessing the protective effects of CoQ10 at the same time.

CONCLUSION

Long-term inhalation of allethrin-based mosquito coil smoke caused testicular atrophy among rats, as evident by reduced testicular weight, volume and RTBWI. Whereas, prophylactic oral administration of CoQ10 among rats exposed to mosquito coil smoke ameliorated adverse effects on testis.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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