DIPONEGORO MEDICAL JOURNAL
(Jurnal Kedokteran Diponegoro)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
Volume 10, Number 5, September 2021

HISTOPATHOLOGY OF SEMINIFEROUS TUBULES OF WISTAR RATS (*RATTUS NORVEGICUS*) EXPOSED TO ELECTRIC MOSQUITO REPELLENT WITH ACTIVE INGREDIENT *D-ALLETHRIN*

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ABSTRACT

Background: An increase in mosquito-borne diseases has resulted in the use of mosquito repellents, especially electric ones in Indonesia. One of the active ingredients of electric mosquito repellent, namely *d-allethrin*, can generate free radicals resulting in oxidative stress. Oxidative stress on the testes can interfere with the spermatogenesis stage in the seminiferous tubules. **Objective:** Determine the histopathology of seminiferous tubules of Wistar rats exposed to electric mosquito repellents with the active ingredient *d-allethrin*. **Methods:** This study used a true experimental design with a post-test only control group design. A sample of 25 Wistar rats was divided into 5 groups. The control group (K) was given only standard feed. Treatment group 1 (P1), treatment group 2 (P2), treatment group 3 (P3), and treatment group 4 (P4) were exposed to electric mosquito repellents with active ingredient *d-allethrin* sequentially for 8 hours/day, 12 hours/day, 18 hours/day, and 24 hours/day. After 30 days, the rats were terminated. The testes were taken for histopathological observation with HE staining and assessed according to the Johnsen score criteria. **Results:** Based on the score, the mean value of the control group was 9.4, P1: 8.36, P2: 7.68, P3: 5.84, and P4: 5.64. The statistical tests using the One-way ANOVA showed a significant difference ($p < 0.001$). Post Hoc Bonferroni test showed significant differences ($p < 0.001$) between group K>P2, K>P3, K>P4, P1>P3, P1>P4, P2>P3, and P2>P4. Insignificant differences were found between group K>P1 ($p=0.081$), P1>P2 ($p=0.787$), and P3>P4 ($p=1$). **Conclusion:** Exposure to electric mosquito repellent containing the active ingredient *d-allethrin* caused differences in the histopathology of seminiferous tubules score of Wistar rats.

***Keywords*:** *Electrical mosquito repellent, d-allethrin, oxidative stress, seminiferous tubules, spermatogenesis*.

INTRODUCTION

An increase in mosquito-borne diseases has resulted in the use of mosquito repellents in Indonesia being frequently used. Electric mosquito repellent is now the people's choice as mosquito control in the room because it smells good, doesn't emit smoke, and is easy to use.

Allethrin is one of the active ingredients in electric mosquito repellent, which has a molecular formula $C_{19}H_{26}O_3$[1,2] Allethrin is included in the type 1 pyrethroid group, which has a risk of damaging health.[3,4] The permethrin-allethrin mixture at a concentration of 10 / 0.14 µg / ml was found in the Lucio A study to have genotoxic and cytotoxic effects on human peripheral blood lymphocytes, which were characterized by an increase in cell apoptosis and a decrease in the Nuclear Division Index (NDI).[5] Changes in the testes can occur due to *d-allethrin* compounds, namely damage to the tubular, degeneration of the epithelial cell layer, and oedema of the epididymis and testes.[6] Damage to the testes will disrupt the spermatogenesis process and affect the quality of the spermatozoa produced, which can lead to infertility problems.[7]

The purpose of this study was to determine the histopathology of seminiferous tubules of wistar rats exposed to electric mosquito repellents with the active ingredient *d-allethrin*.

METHODS

The material used in this research is an electric mosquito repellent with the active ingredient *d-allethrin*. This study used a true experimental design with a post-test only control group design. The sample size was determined based on WHO guidelines on the use of experimental animals. Each treatment group has a minimum of 5 individuals per group.

Based on the provisions, the sample used is at least 25 individuals divided into 4 treatment groups and 1 control group, using of simple random sampling, the total number of samples is 5 per group. Before research, Wistar rats were acclimatized first for 7 days with standard feed and drink. The

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treatment was carried out for 30 days. The control group (K) was given only standard feed. Treatment group 1 (P1), treatment group 2 (P2), treatment group 3 (P3), and treatment group 4 (P4) were exposed to electric mosquito repellents with active ingredient *d-allethrin* sequentially for 8 hours/day, 12 hours/day, 18 hours/day, and 24 hours/day. After 30 days the rats were terminated by using ether then performed cervical dislocation.

The testes were taken for histopathological observations with HE staining and assessed according to the Johnsen score criteria of 1-10.

Table 1. Johnsen Score Criteria[8]

SCORE	ASSESSMENT
10	There is a complete spermatogenesis process
9	The spermatogenesis process is incomplete with many late-stage spermatids
8	Spermatozoa cells <5 per tubule and some late-stage spermatid cells
7	0 spermatozoa cells, 0 late-stage spermatid cells, and many early-stage spermatid cells
6	0 spermatozoa cells, 0 late-stage spermatid cells, and some early-stage spermatid cells
5	Spermatozoa cells and 0 spermatid cells, many spermatocytes
4	Spermatozoa cells and 0 spermatid cells, some spermatocytes
3	There are only spermatogonial cells
2	There are only Sertoli cells, no germ epithelial cells
1	There is no seminiferous epithelium

Testicular preparations were observed under a microscope at 400x magnification on five fields of view. The descriptive data were the average Johnsen score of five different microscopic visual fields. The data obtained were then tested for normality using the Saphiro Wilk test, then the significance of the data was tested using the One-way ANOVA test, which was continued with the Post Hoc Bonferroni test to analyze differences between groups. The degree of significance is if $p \leq 0.05$ at the 95% confidence interval.

RESULTS

Table 2. Descriptive analysis of the average Johnsen score from microscopic image of seminiferous tubules of Wistar rats exposed to electric mosquito repellents with the active ingredient *d-allethrin*

Group	Mean ± SD	Median (mean ± SD)
K	9.44 ± 0.17	9.4 (9.2 - 9.6)
P1	8.36 ± 0.36	8.4 (8,0 - 8,8)
P2	7.68 ± 0.91	8.2 (6.2 - 8.4)
P3	5.84 ± 0.74	5.8 (4.8 - 6.8)
P4	5.64 ± 0.38	5.6 (5.2 - 6.2)

Based on table 2, it can be seen that the microscopic image of seminiferous tubules in the control group has a higher value (9.44) than the 4 treatment groups. Meanwhile, the lowest average calculation is found in treatment group 4, namely 5.64.

The normality test which used in this study used the Saphiro-Wilk test because the sample size was less than fifty. The data is normally distributed if the p-value is > 0.05.

Table 3. Normality test results (Saphiro-Wilk test)

Group	Mean ± SD	Median (mean ± SD)	Normality	Homogeneity
K	9.44 ± 0.17	9.4 (9.2 - 9.6)	0.314 *	0.063 **
P1	8.36 ± 0.36	8.4 (8,0 - 8,8)	0.377 *	
P2	7.68 ± 0.91	8.2 (6.2 - 8.4)	0.124 *	
P3	5.84 ± 0.74	5.8 (4.8 - 6.8)	0.984 *	
P4	5.64 ± 0.38	5.6 (5.2 - 6.2)	0.928 *	

Information: * Normal (p> 0.05); ** Homogeneous (p> 0.05)

Based on the data results above, the control and treatment groups have normally distributed data (p> 0.05). The homogeneity test results produced a significance value of 0.063. Seeing the value of p> 0.05, it can be concluded that the data is homogeneous. Furthermore, the parametric test was carried out with Oneway Anova because the data were normally distributed and homogeneous. The data is stated to have a significant difference if the p-value <0.05.

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Table 4. One-way ANOVA test results

Group	Mean ± SD	p
K	9.44 ± 0.17	<0.001 *
P1	8.36 ± 0.36	
P2	7.68 ± 0.91	
P3	5.84 ± 0.74	
P4	5.64 ± 0.38	

Information: * Significant (p <0.05)

Based on the One-way ANOVA test results above, the p-value was <0.05, which means that there were significant differences between the five groups. Because there are significant differences, it is necessary to conduct the Post Hoc Bonferroni follow-up test to compare the differences between groups.

Table 5. Post Hoc Bonferroni test results

Group	P1	P2	P3	P4
K	0.081	0.001 *	<0.001 *	<0.001 *
P1	-	0.787	<0.001 *	<0.001 *
P2		-	0.001 *	<0.001 *
P3			-	1,000

Information: * Significant (p <0.05)

Based on the results of the Post Hoc Bonferroni test, there were significant differences (p <0.05) between the control group (K) and treatment group 2 (P2), treatment group 3 (P3), and treatment group 4 (P4), and treatment group 1 (P1) with treatment group 3 (P3) and treatment group 4 (P4), and between treatment group 2 (P2) with treatment group 3 (P3) and treatment group 4 (P4). Meanwhile, between the control group (K) and treatment group 1 (P1), treatment group 1 (P1) with treatment group 2 (P2) and treatment group (P3) with treatment group 4 (P4) there was no significant difference (p> 0.05).

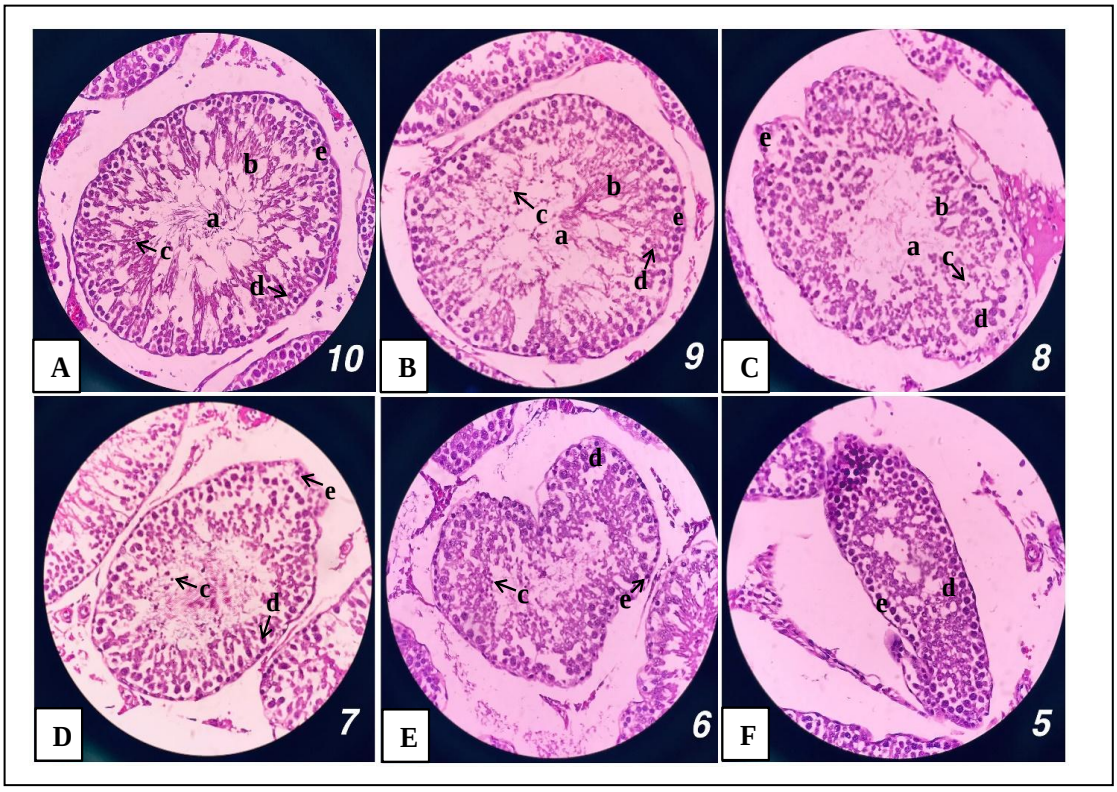

Figure 1. Microscopic appearance of seminiferous tubules in the Johnsen score criterion assessment of 400x magnification. Score 10 (A), 9 (B), 8 (C), 7 (D), 6 (E), 5 (F). Spermatozoa (a), late-stage spermatids (b), early-stage spermatids (c), spermatocytes (d), spematogonia(e).

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DISCUSSION

This study showed that the seminiferous tubule microscopic image scores of the 4 treatment groups were lower than the control group (P1, P2, P3, P4, K). Based on the score, the average value of treatment group 4 is 5.64, where this value is lower than the control group, namely 9.4, treatment group 1 is 8.36, treatment group 2 is 7.68, and treatment group 3 is 5.84. This study shows that the longer the exposure time of the mosquito repellent containing the active ingredient *d-allethrin*, the lower the Johnsen tubule seminiferus score in Wistar rats.

This study is in line with the theory of allethrin, which enters the body by inhalation for a long time, causing the liver to be unable to completely detoxify so that secondary metabolites appear which act as free radicals. Free radicals cause DNA damage and will circulate in the blood throughout the body including the testes.[2] Free radicals are produced either by cellular metabolism in the body or from external sources. In the body, free radicals are created as a by-product of ATP production when cells use oxygen to generate energy. In general, this product is in the form of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). Reactive Oxygen Species (ROS) is produced when oxygen molecules bind to other molecules. When ROS production exceeds antioxidant capacity, it directs cells to oxidative stress.[9]

Oxidative stress negatively affects the hypothalamic-pituitary-gonadal axis pathway, which results in decreased secretion of male reproductive hormones. The progressive decrease in the testosterone hormone will affect the activity of the LDH enzyme, which functions to convert NADH to NAD + as a lactate-forming material through pyruvate synthesis which will affect the metabolism of the Sertoli cells, where this will have an impact on spermatogenesis. Lactate functions as a nutrient intake for spermatozoa in the spermatogenesis process in the Sertoli cells. Inadequate nutritional intake causes the quality of the sperm produced is not good.[10–13]

Oxidative stress can also cause damage to the mitochondrial membrane and eliminate the potential function of the mitochondrial membrane, which results in membrane leakage resulting in membrane depolarization and activation of apoptotic factors, which induce cell death.

The damage that occurs is characterized by degenerative changes in the seminiferous tubules, vacuolization of the intersisium, reduction in seminiferous basal epithelial cells resulting in the release of the basement membrane, and formation of hypospermatozoa. Damage to the testes will disrupt the sperm spermatogenesis process and affect the quality of the spermatozoa produced, which can lead to infertility problems.[7, 14]

Based on the analytical analysis using the Oneway Anova test, significant results were obtained between the control group and 4 treatment groups, to determine whether the exposure time of mosquito repellent containing the active ingredient *d-allethrin* in each group had a significant effect or not, then the Post continued test was carried out. Hoc Bonferroni. The analysis found that the difference in the seminiferous tubule Johnsen scores in the control group against the P1 group did not show a significant difference. This shows that exposure to *d-allethrin* electric mosquito repellent for 8 hours/day can cause histological changes in the seminiferous tubules but not statistically significant. This insignificant difference could be due to the short duration of exposure. Anvita., Et al.[15] In their research, no significant difference was found in the microscopic image of the testicles of mice exposed to mosquito repellents with the active ingredient *d-allethrin* for 8 hours/day.

The difference in seminiferous tubular Johnsen scores showed a significant difference in the P1 group against the P3 and P4 groups, the P2 group against the P3 and P4 groups, and the control group against the P1, P2 and P3 group. This study showed that the active ingredient *d-allethrin* in electric mosquito repellent can cause differences in the microscopic image of seminiferous tubules of Wistar rats. This is in accordance with what was conveyed by Yofa Sukmawati., Et al.[16] in their research that exposure to *d-allethrin* active ingredient mosquito

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repellent can cause damage to rat testes in the form of a significant increase in the mean seminiferous tubule diameter caused by seminiferous tubule dilatation, germ cell degeneration, epithelial thinning, and stromal swelling.

The difference between the Johnsen tubule seminiferus score of the P1 group against the P2 group, the P3 group against the P4 group showed no significant difference. This insignificant difference can be due to the duration of exposure to the electric mosquito repellent used is too narrow.

CONCLUSION

Exposure to mosquito repellent with the active ingredient *d-allethrin* for 8 hours / day caused differences in the histopathology of seminiferous tubules of Wistar rats but not significantly. Exposure to mosquito repellent with active ingredient *d-allethrin* for 12 hours / day, 18 hours / day, and 24 hours / day caused significant differences in seminiferous tubular histopathology of Wistar rats. The average Johnsen score for group K (9.4) was higher than that of the P1 (8.36), P2 (7.68), P3 (5.84) and P4 (5.64) groups.

Ethical Approval

All procedures have been approved by the issuance of ethical clearance No. 77 / EC / H / FK-UNDIP / VIII / 2020 from the Health Research Ethics Commission of the Faculty of Medicine, Diponegoro University, Semarang.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

There was no specific funding provided for this article.

Author Contributions

Conceptualization, SNI; methodology, SNI; formal analysis, SNI; investigation, SNI; resources, SNI; data curation, SNI; writing—original draft preparation, SNI; writing—review and editing, DA, RRMDA, and FS; supervision, DA, RRMDA, and FS; project administration, SNI.

Acknowledgments

Authors would like to thank the parties who has helped in every process of this research.

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