

RESEARCH ARTICLE

The Effect of Coffee on Spermatozoa Motility and Morphology of BALB/c Mice Exposed to Electric Mosquito Repellent

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ABSTRACT

Introduction: Electric mosquito repellent which contains *d-allethrin* producing free radicals, affects the quality of sperm motility and morphology. Antioxidants contained in coffee can counteract the free radicals in the body. **Objective:** To evaluate the effect of coffee on sperm motility and morphology of BALB/c mice exposed to electric mosquito repellent.

Methods: In this experimental study was conducted with post-test only control group design, 24 BALB/c mice randomized into 4 groups. The negative control group was a group without treatment. The positive control group was exposed to electric mosquito repellent. Group P1 and P2 were exposed to electric mosquito and given the coffee solutions with the doses respectively 52 mg/0.5 mL and 78 mg/0.5 mL. The treatment was given for 35 days. On the 36th day, all mice were terminated and their sperm motility and morphology were examined. The data were then analyzed with the non-parametric test of Kruskal-Wallis. Differences were considered significant when the value of $p < 0.05$ with confidence interval 95%.

Results: The result of pairwise comparison test progressive sperm motility shows that there was a significant difference between positive control group with P2 group ($p = 0.006$), positive control group with negative control group ($p < 0.001$), and P1 group with negative control group ($p = 0.004$). The result of Kruskal-Wallis test for sperm morphology shows that there was no significant difference between those four groups ($p = 0.885$).

Conclusion: Coffee can significantly increase the sperm motility but didn't significantly increase the normal sperm morphology percentage of BALB/c mice exposed by electric mosquito repellent.

Keywords: *d-allethrin*; coffee; sperm motility; sperm morphology

ABSTRAK

Pendahuluan: Obat nyamuk elektrik berbahan aktif *d-allethrin* menghasilkan radikal bebas yang memengaruhi kualitas motilitas dan morfologi spermatozoa. Antioksidan yang terkandung dalam kopi dapat menangkal radikal bebas di dalam tubuh. **Tujuan:** Mengetahui pengaruh pemberian kopi terhadap motilitas dan morfologi spermatozoa mencit BALB/c yang dipapar obat nyamuk elektrik.

Metode: Pada penelitian eksperimental dengan desain *post-test only control group* ini, 24 ekor BALB/c jantan dibagi menjadi 4 kelompok. Kelompok kontrol negatif adalah kelompok tanpa perlakuan. Kelompok kontrol positif adalah kelompok yang hanya diberi paparan obat nyamuk elektrik. Kelompok P1 dan P2 dipapar nyamuk elektrik dan diberi larutan kopi dengan dosis masing-masing 52 mg / 0.5 mL dan 78 mg / 0.5 mL. Perlakuan selama 35 hari, pada hari ke-36 semua mencit diterminasi dan diperiksa motilitas dan morfologi spermatozoanya. Data dianalisis dengan uji non parametrik *Kruskal-Wallis*. Perbedaan dianggap bermakna apabila nilai $p < 0.05$ dengan 95% interval kepercayaan.

Hasil: Hasil uji *pairwise comparison* untuk motilitas progresif spermatozoa menunjukkan perbedaan yang bermakna antara kelompok kontrol positif dengan P2 ($p = 0.006$), kelompok kontrol positif dengan kontrol negatif ($p < 0.001$), dan kelompok P1 dengan kontrol negatif ($p = 0.004$). Hasil uji *Kruskal-Wallis* untuk morfologi spermatozoa menunjukkan tidak terdapat perbedaan yang bermakna diantara keempat kelompok ($p = 0.885$).

Kesimpulan: Pemberian kopi meningkatkan motilitas secara signifikan, tetapi tidak meningkatkan persentase morfologi normal secara signifikan pada spermatozoa mencit BALB/c yang dipapar obat nyamuk elektrik.

Kata kunci: *d-allethrin*; kopi; motilitas spermatozoa; morfologi spermatozoa

INTRODUCTION

Infertility is a reproductive system disorder defined by the inability to achieve pregnancy for a couple that actively performs sexual activity without contraceptive for minimum 12 months (World Health

Organization, 2010). Infertility is expected to occur to 15% couples globally, or about 48.5 million couples. Infertility can occur to men and women. However, there is a stigma among the society that infertility occurs to women, while 30-40% factors of infertility is from men

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(Mulyantoro, 2018).

Male fertility disorder can be divided into pre-testicular disorder, testicular disorder, and post-testicular disorder. Pre-testicular disorder is commonly related to hormonal disorder which influences spermatogenesis process, such as decreasing production of follicle stimulating hormone (FSH) and luteinizing hormone (LH) which causes hypogonadism. Testicular disorder occurs in seminiferous tubules, such as testis getting infected or trauma. Meanwhile, post-testicular disorder is various disorders occurring after spermatozoa gets out of seminiferous tubules, such as spermatozoa viability and motility disorders (Luhulima, 2012).

Mosquito repellent is repellent used to kill mosquito. Mosquito repellent can easily be found in Indonesia and there are some types of it, such as mosquito repellent coil, mosquito repellent lotion, mosquito repellent spray, and electronic mosquito repellent. One of the active substances of electronic mosquito repellent is d-allethrin which is a derivative substance of pyrethroid. The reactions caused by this substance exposure are cough, dizziness, headache, nausea, vomiting, paresthesia, and skin irritation (Saillefait et al., 2015). If d-allethrin enters into the body through inhalation for a long period, besides causing lung disorders, it will also cause the liver not able to perform complete detoxification. This causes emergence of secondary metabolites acting as free radicals. Excessive free radicals can decrease the level of LH, FSH, and testosterone that play important roles in the reproductive system (Atere & Osadolor, 2010). It can also cause tissue damage, such as in mitochondrial cell membrane. In this case, mitochondrial cells produce ATP which is needed for conversion of testosterone in leydig cells in spermatogenesis process. If mitochondria get impaired or damaged, spermatogenesis process will be impaired. Researches show that d-allethrin may cause declining amount, motility, viability of spermatozoa, and abnormal spermatozoa morphology (Christijanti et al., 2010).

Robusta coffee is a drink favored by many people because of its distinctive taste and properties when consumed according to the recommended doses. Many people drink a cup of coffee in the morning or in the afternoon, and some also drink more than one cup daily. Coffee contains caffeine which can increase the performance of psychomotor, making the body to keep awake and increasing energy, caffeine also increases the production of cyclic adenosine mono phosphate (cAMP) that will stimulate spermatozoa movement (Nabavi et al., 2013). In addition, coffee also contains abundant antioxidants which are able to prevent free

radicals in the body, including chlorogenic acid, ferulic acid, and caffeic acid (Yashin et al., 2013).

In this research, the researcher is to observe the effect of coffee on spermatozoa motility and morphology of BALB/c exposed to electronic mosquito repellent.

METHODS

This experimental research employed a post-test only control group design. In this research, 24 male BALB/c mice (*Mus musculus*), 6-8 weeks old and with body weight of 20-40 gram, were randomly divided into four groups, each consisting of 6 mice. The negative control group received no treatment. The positive control group was only exposed to electronic mosquito repellent. Group P1 and P2 was exposed to electronic mosquito repellent and given with coffee solution at dose 52 mg/0.5 mL, and 78 mg/0.5 mL respectively. The mice were exposed to electronic mosquito repellent for 8 hours daily. The treatment was given for 35 days according to group division. Food and drink for the mice were given ad libitum to all groups.

Dose and Preparation of Coffee Solution

Doses 52 mg/0.5 mL and 78 mg/0.5 mL were resulted from 2 and 3 cups of Robusta coffee for human in a cup of coffee containing 200 ml air and 10 g coffee powder. The conversion dose for mice equal to 20 g coffee powder was 0.0026×20 g, which was 0.052 g or equal to 52 mg. Meanwhile, the conversion dose for mice for 30 g coffee powder was 0.0026×30 g, which was 0.0078 gram or equal to 78 mg. If the volume of coffee solution given was 0.5 ml, making coffee solution at doses 52 mg and 78 mg can be calculated using the comparison and by dissolving respectively 26 g and 39 g coffee into 250 ml boiling water. Robusta coffee solution was filtered and left until room temperature and given to the mice using nasogastric tube. On day 36, all of the mice were terminated, and their sperm was taken from vas deferens and examined for sperm motility and morphology.

Exposure to Electronic Mosquito Repellent

Mosquito repellent exposure was given for 8 hours/day from 08.00-16.00 by inserting groups exposed with mosquito repellent into a room of 100x75x50 cm with small ventilation within which electronic mosquito repellent had been deployed.

Sperm Examination

On day 36, all mice were terminated and their sperm was taken from vas deferens. The collected sperm was dripped with NaCl 0.9% for 0.5 ml and stirred until

Table 1. Description of Mean Value±SD Percentage of Normal Spermatozoa Motility and Morphology

Variables	Groups				P value*
	NC-G N: 6 (X±SD)	PC-G N: 6 (X±SD)	C52-G N: 6 N (X±SD)	C78-G N: 6 (X±SD)	
Progressive (%)	48.33±6.581	1.11±2.719	8.89±12.589	33.88±14.819	0.003
Non-progressive (%)	32.77±7.123	24.44±12.588	27.22±16.112	31.66±11.689	
Immotility (%)	18.89±7.201	74.44±13.111	63.89±23.798	34.44±24.826	
Normal Sperm Morphology (%)	92.17±3.251	91.00±2.366	92.17±2.858	92.17±3.545	0.885

Note: * Kurskall Wallis

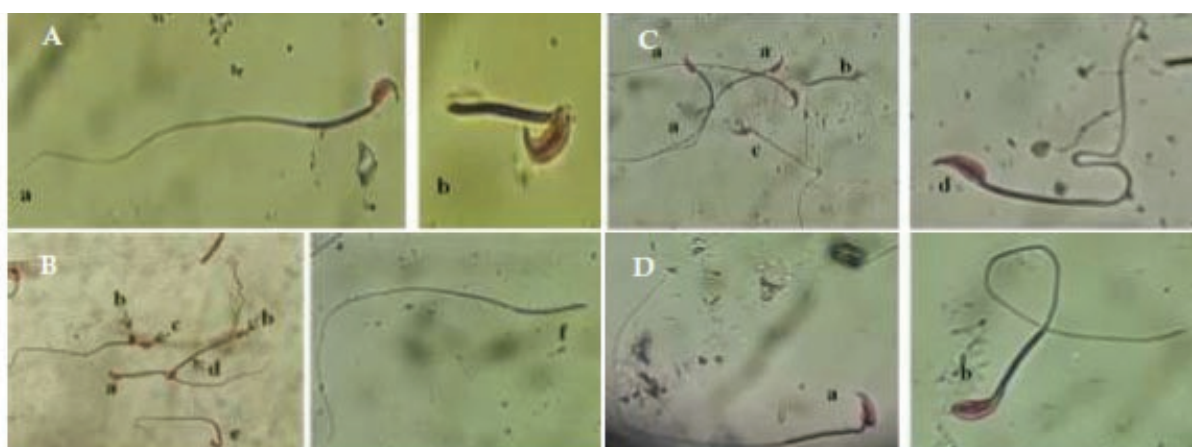


Figure 1. Normal and abnormal sperm Morphology in each group; A. NC-G; B. PC-G; C52-G; D. C78-G. There was no significant difference in number of normal sperm morphology amongst groups

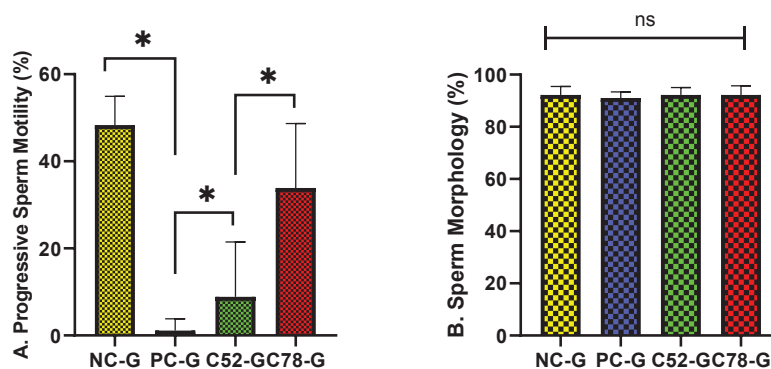


Figure 2. A. Percentage of mean of Progressive Sperm Motility; and B. Morphology in each group; * Pair wise comparrison: p<0.05; Kruskal-Wallis. ns: not significant

they were homogenous.

For sperm motility, the sperm was put on object glass, covered with deck glass. The preparation was observed under microscope at 400x magnification. The progressive sperm motility (spermatozoa motioned and moved), non-progressive sperm motility (spermatozoa

motioned but did not move), and immotility (spermatozoa unable to move) were considered pursuant to WHO 2010 standards.

For sperm morphological examination, preparation was made by dyeing eosin. The preparation was examined using microscope at 400x magnification

and calculated for percentage of normal spermatozoa morphology according to WHO 2010 standard.

Statistical Analysis

The data were analyzed using Kruskal-Wallis's non-parametric test. The difference was considered significant if p value < 0.05 with confidence interval 95%.

Ethical Clearance

The research had been under ethical clearance of the Health Research Ethical Commission (KEPK) Faculty of Medicine, Diponegoro University under No. 42/EC/H/FK-UNDIP/VI/2020 and No. 47/EC/H/FK-UNDIP/VI/2020.

RESULTS

The data of normal spermatozoa motility and morphology of BALB/c mice are in the form of percentage. The analysis on the data of mean percentage of mice's spermatozoa motility and morphology can be observed in Table 1.

The normality test employed in this research was Saphiro-Wilk test, since the number of samples was less than 50. The p value of < 0.05 showed abnormal data distribution. A Kruskal-Wallis's non-parametric test was then conducted.

The results stated that there is a significant difference in the percentage of progressive spermatozoa motility among groups ($p < 0.05$). On the other hand, no significant difference on spermatozoa morphology among groups, $p > 0.05$ (figure 1, 2B).

A pair wise comparison test applied to validate which group differ significantly. Results showed the percentage of progressive spermatozoa motility in PC-G is lower than that of NC-G, $P < 0.05$. While the percentage of progressive spermatozoa motility in C58-G and C78-G were significantly higher than that of PC-G ($p < 0.05$). The percentage of progressive spermatozoa motility in C78-G is found statistically higher than C58-G, $p < 0.05$ (figure 2A).

DISCUSSION

Electronic mosquito repellent with active agent d-allethrin can induce toxicity through oxidative stress by producing free radicals, thus antioxidant is required to prevent it. Roasted coffee has complex compounds of more than 1000 bioactive components, with antioxidant's therapeutic, anti-inflammatory and anti-cancer effects (Poole et al., 2017). Antioxidant substances in coffee include, for example, chlorogenic acid, ferulic acid, and caffeic acid (Yashin et al., 2013).

The research results in a significant decrease in the percentage of progressive spermatozoa motility and increase in spermatozoa immotility of groups of mice exposed to electronic mosquito repellent for 8 hours daily for 35 days compared to the group of mice not exposed to electronic mosquito repellent. This proves that electronic mosquito repellent containing d-allethrin inhaled for a long period may impair spermatozoa motility because of the free radicals formed and damage mitochondrial membrane. The previous research conducted by (Christijanti et al., 2010) also states that administering allethrin causes a decrease in spermatozoa motility.

The research results in a significant increase in progressive spermatozoa motility and decrease in spermatozoa immotility of groups of mice exposed to mosquito repellent and given with coffee solution with 78 mg coffee powder compared to mice exposed to electronic mosquito repellent. This proves that administering coffee may increase spermatozoa motility because of its antioxidant content and conforms to the previous research conducted by (Dja'afara et al., 2015) that administering coffee may increase the spermatozoa motility of male wistar rat exposed to cigarette smoke.

According to the research result, exposure to electronic mosquito repellent with active agent d-allethrin decreases the quality of mouse's spermatozoa morphology but statistically insignificant. The result of previous research conducted by Christijanti et al. (2010) also explains that spermatozoa morphological disorder is found in white mouse exposed to allethrin contained in electronic mosquito repellent (Christijanti et al., 2010). The insignificant decrease in the quality of mouse's spermatozoa morphology in this research may be caused by non-sufficient duration of exposure to electronic mosquito repellent for it to cause significant damage to spermatozoa morphology.

Administered with coffee, mice exposed to electronic mosquito repellent have higher percentage of normal spermatozoa morphology than the positive control group, but the difference is insignificant. Meanwhile, the result of research conducted by (Dja'afara et al., 2015) shows that administering coffee may increase the quality of male wistar rat's spermatozoa morphology exposed to cigarette smoke. The research conducted by (Wulandari et al., 2019) also shows that infusing Robusta coffee seed may increase the quality of infertile model mice's spermatozoa morphology. The increase in the quality of normal spermatozoa morphology in this research is insignificant, which may be caused by insufficient duration of coffee solution

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administration in order to increase the quality of mice's spermatozoa morphology significantly.

CONCLUSION

Coffee administration can increase the spermatozoa motility of BALB/c mice exposed to electronic mosquito repellent significantly at dose 78 mg/0.5ml but does not significantly increase the percentage of normal spermatozoa morphology of BALB/c mice exposed to electronic mosquito repellent.

CONFLICT OF INTEREST

There is no conflict of interest in this research.

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