



Dean Pandu Eko Sapto, Farmaditya Eka Putra,
Rr. Mahayu Dewi Ariani, Donna Hermawati

THE EFFECT OF BEETROOT JUICE (*Beta vulgaris L.*) ON THE CONCENTRATION OF SPERMATOZOA IN WISTAR RATS EXPOSED TO MOSQUITO COIL SMOKE

Dean Pandu Eko Sapto¹, Farmaditya Eka Putra³, Rr Mahayu Dewi Ariani²,
Donna Hermawati²

¹ Undergraduate Student, Faculty of Medicine, Diponegoro University, Semarang, Indonesia

² Department of Medical Biology and Biochemistry, Faculty of Medicine, Diponegoro University, Semarang, Indonesia

³ Department of Histology, Faculty of Medicine, Diponegoro University, Semarang, Indonesia

*Corresponding Author : E-mail : deanpandu@gmail.com

ABSTRACT

Background: Mosquito coils contains Allethrin that can increase the free radical effect. This process may result in the damage of the cell membranes that triggered by oxidative stress. Oxidative stress can affect spermatogenesis and decreasing the quality of spermatozoa, including the spermatozoa concentration. Beetroot contain flavonoid as an antioxidant which can counteract the free radicals in the body. **Objective:** To identify the effect of beetroot juice in the spermatozoa concentration of Wistar rats that exposed by mosquito coils. **Method:** This is experimental study with post test only control group design. The samples were 28 male Wistar rats that divided into 4 groups. The Control Group (-) was a group without treatment. The Control Group (+) was only exposed by mosquito coils. Group P1 was exposed by mosquito coils and given 8 ml of beetroot juice. P2 group was exposed by mosquito coils and given 16 ml of beetroot juice. For 56 days, on the 57th day all rats were terminated and spermatozoa concentrations were examined. **Results:** The average concentration of spermatozoa was: Group K(-)=20,00; Group K(+)=8,00; P1 group=12,40; P2 group=17,20. One Way ANOVA test found there were significant differences among the five treatment groups. Post-Hoc test found there were significant differences among K(-) with K(+), K(-) with P1, K(-) with P2, K(+) with P1, K(+) with P2 and P1 with P2. **Conclusion:** Beetroot juice can affect the spermatozoa concentration of male wistar rats exposed by mosquito coils.

Keywords: Mosquito coils, beetroot juice, spermatozoa concentration

INTRODUCTION

Infertility is the failure of a married couple in having a child after having sexually active for 1 year without using contraception.¹ Poor quality of sperm and inactive sperm are the factors that affect 50% of infertility couples.²

Spermatogenesis is a complex process in which germinativum primordial cell is relatively undifferentiated. Spermatogonia contains 46 diploid chromosomes which each contains 23 randomly given haploid set. Spermatogenesis occurs inside tubulus seminiferus in the testes.

There are several factors that affect fertility including environmental condition and lifestyle. One of the environmental influences is the usage of mosquito coil. Mosquito repellent products generally have certain additives such as *Allethrin* which can cause testicular histological change, decrease testicular weight and reduce the tubulus seminiferus diameter.³ If the testicles are disturbed, the spermatogenesis will be influenced. Free radicals may be overcome using antioxidants, one of them is the Beetroot (*Beta Vulgaris*) which contains flavonoid as a potent antioxidant to prevent free radical formation.

There is only a few further studies regarding the effect of beetroot on spermatozoa concentration.

This present study aimed at exploring the effect of beetroot on spermatozoa concentration in male Wistar rats which were exposed to mosquito coils smoke.

METHODS

Samples

This is experimental study with post test only control group design. Samples in this study were male Wistar rats (*Rattus norvegicus*) and were obtained from Laboratorium Biologi Fakultas Matematika dan Ilmu Pengetahuan Alam (FMIPA) Universitas Negeri Semarang.

The research samples were taken from randomized population. Based on WHO guideline, the minimum number of samples in each group should be 5, therefore, the minimum total sample required in this study are 20. This research used 28 male Wistar rats that divided into 4 groups. The Control Group (-) was a group without treatment. The Control Group (+) was only exposed by mosquito coils. Group P1 was exposed by mosquito coils and given 8 ml of beetroot juice. P2 group was exposed by mosquito coils and given 16 ml of beetroot juice. For 56 days, on the 57th day all rats



were terminated and spermatozoa concentrations were examined.

Data Analysis

The data obtained from four sample groups were processed using the SPSS computer program. Then, the distribution normality test was carried out with the Saphiro-Wilk test because the sample size was <50. Furthermore, the homogeneity test was carried out using the Levene test to determine whether the group had the same variant or not. The data distribution was normal therefore the parametric test was carried out with the one way Anova test and continued with LSD / Benferoni post hoc. The difference is considered significant if the p value <0.05 with the 95% confidence interval.

RESULT

The population of this study used 28 male wistar rats obtained from Laboratorium Biologi Fakultas MIPA Universitas Negeri Semarang a total of 28 were randomly selected and met the inclusion criteria. All samples in the form of 28 male wistar rats were adapted first by being fed and drinking standard for 7 days, then divided into 4 treatment groups, each group consisting of 7 rats. The treatment was carried out for 56 days.

The following content is a descriptive analysis of the spermatozoa concentration in each treatment group with units per 2 million spermatozoa cells that can be seen in the table below.

Table 1. Descriptive Analysis of Spermatozoa Concentration in each group

Group	Mean	Standart Deviation	Minimum	Maximum
Control -	20,00	1,696	18	22,5
Control +	8,00	1,767	6	10,5
Dose 8 ml (P1)	12,40	2,104	9,5	14,5
Dose 16 ml (P2)	17,20	1,037	16	18,5

Based on the table above, it can be seen that the highest mean spermatozoa concentration was found in the negative treatment group, namely 20.00, while the lowest was in the positive group treatment, namely 8.00.

The normality test used in this study used the *Shapiro-Wilk* test, because the number of samples were less than 50. The results of the test showed that the data were normally distributed, where the p value was > 0.05.

Table 2. Normality Test Results of Spermatozoa Concentration

Group	Shapiro-Wilk
	P
Control Negative	0,921
Control Positive	0,844
P1	0,566
P2	0,754

p : meaning value (meaning if p>0,05)

Furthermore, the homogeneity test was carried out using the *Levene* test to determine whether the group had the same variant or not.

Table 3. Homogeneity Test Results of Spermatozoa Concentration

Variant Test	P
Levene	0,411

p : meaning value (meaning if p>0,05)

Based on the homogeneity test above, the p value is > 0.05, which means that all groups have the same variant, so that all groups are considered homogeneous.

Table 4. ONE WAY Anova Test Results of Spermatozoa Concentration

Group	Value p
Control negative	
Control positive	
P1	<0,001
P2	

One Way Anova Test, p : meaning value, meaning if p < 0,05

According to *One Way Anova* test, the results obtained p <0.05, which means that there are significant differences between the four groups. Then, the *Post Hoc* test was carried out to determine the significant differences between each group.



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Table 5. *Post Hoc Bonferroni* Test Results of Spermatozoa Concentration

	K-	K+	P1	P2
K-	-	<0,001	<0,001	0,019
K+	<0,001	-	0,001	<0,001
P1	<0,001	0,001	-	<0,001
P2	0,019	<0,001	<0,001	-

p : meaning value, meaning if $p < 0,05$

The results of the *Post Hoc* test showed a significant difference between negative control and positive control. Negative control with P1, negative control with P1 and P2 obtained significant differences. There was a significant difference between P1 and P2.

DISCUSSION

Mosquito coils have a chemical component in the form of Transfultrin which is an endocrine disruptor. Like the active hormonal components, this substance can inhibit the proliferation of the Sertoli cells through its indirect effect on the FSH (*Follicle Stimulating Hormone*) hormone secreted by the pituitary and its direct effect at the testicular level.^{8,9}

Mosquito coils also contain Allethrin, its toxicity causes damage to the mitochondrial membrane of the Leydig cells, therefore it interferes with the steroidogenic process. This process decreasing the production of the testosterone hormone. Furthermore, as a result the spermatogenesis process disruption, sperm quality is interfered.^{10,11}

Free radicals which formed from the results of allethrin metabolism will damage the mitochondrial membrane of the Leydig cells, therefore, the ATP production for testosterone synthesis is reduced.^{12,13} As a result of spermatogenesis process disruption, the quality of the sperm produced also decreases.^{14,15} This is in accordance with previous research conducted by Qotrunnada, *et al* in 2017.¹¹

In this study, it is suggested that the smoke of mosquito repellent affects the quality of the concentration of spermatozoa in the Wistar rats. This effect can be observed from the comparison of positive controls exposed to mosquito repellent smoke with the lowest spermatozoa concentration. Based on this research, it was found that the average spermatozoa concentration in each group included negative control group (20.00), positive control (8.00), treatment 1 (12.40), treatment 2 (17.20). These results indicate that the Wistar rats which exposed to the mosquito coil

smoke had the lowest spermatozoa concentration in the positive control group (8.00). This is in accordance with the research of Purwati (2016) that mosquito coils cause oxidative stress reactions and damage the integrity of cell membranes.

The results of the *Shapiro Wilk* statistical test showed normal data distribution ($p < 0.05$), indicating that the data were normally distributed. Furthermore, the homogeneity test was carried out with the *Levene* test ($p > 0.05$), the value was 0.411, which means that all groups had the same or homogeneous variants. Followed by the *One Way Anova* test, the results obtained $p < 0.05$ so that there were significant differences in the four groups.

In the *Post Hoc* test, this study showed that the P1 group gave a more significant difference compared to K (+). Group P1 had a significant difference with groups K (+) and K (-). This shows that the Beetroot juice can increase the concentration of spermatozoa.. Group of P2 treated with a significantly higher difference than P1, but was still unable to match the spermatozoa concentration of group K (-). Group P2 has a significant difference with Group P1, K (+) and K (-). This indicates that the dosage level of the Beetroot juice can affect the rate of improvement in the resulting concentration. These results are supported by previous research by Pravitasari, *et al* in 2015 which showed that the distribution of antioxidant juice at a dose of 2 ml was more influential than the distribution of antioxidant juice at a dose of 1 ml on the histopathology of liver cells of the Wistar rats.⁷ This study also showed that mosquito coils reduced spermatozoa concentration of the Wistar rats, indicated by a significant difference between K (+) Wistar rats that were only exposed to mosquito coil smoke exposure compared to K (-) where Wistar rats were not exposed to anything and only received standard feed.

Beetroot juice distribution increases the spermatozoa concentration because it contains antioxidants such as flavonoids, betasianin and betalanin. The antioxidant compounds in beetroot juice, especially flavonoids, can protect the body from free radicals such as mosquito coils. Beetroots themselves are included in vegetables with high antioxidant content. Antioxidant compounds bind to free radical electron molecules in the body thus inhibiting the oxidation process of metabolic cells.⁵



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The limitation in this research was that the spermatozoa concentration of the Wistar rats before receiving treatment could not be checked, therefore, there was no comparison of the spermatozoa concentration of wistar rats before and after receiving treatment.

CONCLUSION

There was a significant difference in the spermatozoa concentration of the Wistar rats exposed to the smoke of mosquito coils 24 hours per day and those of the Wistar rats without exposure to the smoke of mosquito coils..

There was a significantly higher concentration of spermatozoa in Wistar rats which were given 8 ml and 16 ml Beetroot juice daily and exposed to mosquito coil smoke when compared to rats that were only exposed to mosquito coil smoke.

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