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THE INFLUENCE OF BEETROOT (*Beta vulgaris*) ON SPERMATOZOA VITALITY OF WISTAR RATS EXPOSED TO MOSQUITO COILS

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ABSTRACT

Background: Spermatozoa vitality is one of the sperm analysis test. Application mosquito coil repellent generates free radical enhancement. Free radical contribute to external factor that influence the quality of spermatozoa vitality. Beetroot (*Beta vulgaris*) contain a considerably high amount of antioxidant. It reduce free radical in the body and improve quality of spermatozoa vitality. **Aim:** To find out and analyze the effect of beetroot (*Beta vulgaris*) on the spermatozoa vitality of male wistar rats exposed to mosquito coils. **Methods:** This research uses experimental methods, with a post-test only control group design. The research sample was selected using a simple random sampling technique 28 male wistar rats met the inclusion and exclusion criteria, then divided into 4 treatment groups K (-), K (+), P1 and P2. Data obtained after 56 days of treatment with 7 days of adaptation before treatment, the data will be tested for normality with Shapiro-Wilk then proceed one way ANOVA if the data distribution is normal. **Results:** Shapiro-Wilk test showed no significant differences between groups K (-), K (+), P1 and P2 ($p > 0.05$). One way ANOVA test on sperm vitality analysis showed a significant difference between groups. Post-Hoc test showed sperm vitality on K (+) there was significant difference compared to K (-) ($p < 0.001$), K (+) no significant difference compared to P1 ($p = 0.244$) and K (+) there was significant difference compared to P2 ($p = 0.001$). **Conclusion:** Giving beetroot juice with the right dose has a significant effect on the spermatozoa vitality of male wistar rats exposed to the smoke of mosquito coils.

Keywords: *Spermatozoa, Beetroot, Sperm Vitality, Free Radical, Antioxidant*

PREFACE

Analysis of a person's infertility can be done by semen analysis using indicators of vitality, morphology, motility, and spermatozoa concentration. Spermatozoa vitality is an assessment of sperm quality by looking at the ratio of live and dead spermatozoa, an indication of a dead spermatozoa that is by staining with *eosin nigrosine*. Sperm vitality examination must be done in condition of straight forward sperm motility below 40%.¹A person's sperm vitality can be influenced by external and internal factors, external factors that can affect sperm vitality are temperature, food, lifestyle, pollution and excess work.²Mosquito coils have several active substances that can interfere with sperm vitality, inhalation of this *pyrethroid*

group causes histological changes, decreased testicular weight and reduced diameter of the seminiferous tubules. The toxicity of active ingredients of mosquito coils is determined by the duration of use, frequency of use and concentration of active substances in one room. The use of high frequency and duration will increase the toxicity of active ingredients of mosquito coils, the higher the level of toxicity of mosquito coils will lead to the emergence of secondary effects in the form of free radicals.³Free radicals are fragile substances that are highly reactive and can affect the process of spermatogenesis.⁴Free radical products namely oxygen specimens (ROS) can produce fat peroxidation effects.⁵Fat peroxidation affects the permeability of the sperm membrane, the sperm membrane



itself consists of the composition of the phospholipids making it easier to reduce sperm vitality.⁶

The results of these free radical side effects can be treated with antioxidants, they are free radicals antidote by donating electrons to free radicals.⁷ Micro intake as well as macronutrients must be enough intake containing high antioxidants, with the consumption of beetroot one of the highest content is antioxidant compounds 1.98 mmol / 100g. Beetroot has several substances like vitamin C, betalain, betacyanin (840-900 mg/kg) and betanin (300-600 mg/kg). The content of betanin in beetroot can reduce the free radicals so the secondary effects of free radicals from burning mosquito coils can be neutralized.^{8,9}

RESEARCH METHOD

Sample and Treatment

This study is a true experimental study with a post-test only control group design research design, with 28 male wistar rats as the studied samples. Tested variables were given for 56 days. The samples were divided into 4 experimental groups (Table 1), each group had 6 samples meeting these criteria :

1. Inclusion criteria : (1) Male wistar rat, (2) Age 6 week, (3) Weight 100-150 gram, (4) Has no anatomical anomaly, (5) Male wistar (*Rattus norvegicus*) which is exposed burning mosquito coils.
2. Exclusion criteria: Rat dies during adaptation and variable testing.

Table 1. Experimental group

Group	Tested Variable
P1	Wistar rats smoked with mosquito coil and given 8 ml beetroot
P2	Wistar rats smoked with mosquito coil and given 16 ml beetroot
K (-)	Wistar rats which not treated
K (+)	Wistar rats smoked with mosquito coil only

The population used was male wistar rats (*Rattus norvegicus*) obtained from Laboratorium Biologi Fakultas Matematika dan Ilmu Pengetahuan Alam (FMIPA) Universitas Negeri Semarang. All wistar rats were acclimatized in the cage and standard feeding for 1 week. The rats were then given the tested variables under grouping for 56 days. Sperm vitality was measured after variable testing, sperm vitality was tested with *eosin nigrosine* for staining and measured by 2 people.

STUDY RESULTS

Data obtained from the examination of sperm vitality are ordinal data in units of percent (%). Analysis of sperm vitality by making semi-permanent preparations, analysis can be done by the method of observe the painted or not painted as an indication of living or dead sperm. Data analysis shows sperm vitality can be seen in table 2.

Table 2. Vitality Spermatozoa

NO.	Group	Mean \pm SD Sperm Vitality(%)
1.	K (-)	62,80 \pm 8,32
2.	K (+)	38,80 \pm 9,73
3.	P 1	44,80 \pm 5,72
4.	P 2	59,60 \pm 7,02



Table 3. Shapiro-Wilk normality test results in sperm vitality

Group	Mean ± SD(%)	Median (min – max)(%)	P
Control (+)	38,80 ± 9,73	38 (29 – 54)	0,609*
Dose 8 ml	44,80 ± 5,72	48 (36 – 49)	0,108*
Dose 16 ml	59,60 ± 7,02	58 (50 – 69)	0,879*
Control (-)	62,80 ± 8,32	62 (54 – 74)	0,715*

Note: * Normal ($p > 0.05$)

The results of sperm vitality analysis were tested for normality with Shapiro-Wilk that the data is evenly distributed. The quality of spermatozoa

vitality of wistar rats was obtained sequentially from the lowest to the highest consecutive K (+), P1, P2 and K (-).

Tabel 4. One Way ANOVA sperm vitality test results

Group	Mean ± SD(%)	p [§]	p [‡]
Control (+)	38,80 ± 9,73	<0,001*	0,793**
Dose 8 ml	44,80 ± 5,72		
Dose 16 ml	59,60 ± 7,02		
Control (-)	62,80 ± 8,32		

Note: * Significant ($p < 0.05$); ** Homogeneous ($p > 0.05$)

One Way ANOVA test results indicate that the value of $p < 0.001$ ($p < 0.05$) which can be interpreted that there are

significant differences in the quality of sperm vitality.

Tabel 5. Post Hoc LSD test results in sperm vitality

Group	Control (-)	Dose 8 ml	Dose 16 ml	Control (+)
Control (+)	<0,001*	0,244	0,001*	-
Dose 8 ml	0,002*	-	0,009*	0,244
Dose 16 ml	0,528	0,009*	-	0,001*
Control (-)	-	0,002*	0,528	<0,001*

Keterangan: * Signifikan ($p < 0,05$)

Based on the Post Hoc Test, it was found that there were significant differences between the K (+) and K (-) groups ($p < 0.001$) meaning that the mosquito smoke produced a significant difference in the quality of the spermatozoa vitality of male wistar rats. No significant difference emerged between groups K (+) with P1 ($p = 0.244$), while a significant difference between K (+) and P2 ($p = 0.001$) which meant that the 16 mL beet juice dose

decreased the effect of mosquito smoke more than the dose 8 mL.

DISCUSSION

Control vitality analysis (+) in this study showed that the average sperm vitality presentation was $38.80\% \pm 9.73$, which means that it was below WHO 58% normal sperm vitality standard. In the control group (-) had an average of $62.80\% \pm 8.32$, so when compared to K (+) with K (-) there were significant differences in the vitality of



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spermatozoa. In previous studies the use of electric mosquito repellent also caused a decrease in sperm quality. The effect of transflutrin and allethrin mosquito repellent fumes is proven to be an external factor (air pollution) that influences spermatogenesis of sperm.³

The effect of mosquito coil smoke inhaled by wistar rats causes an increase in free radicals and fat peroxidation. ROS is a free radical product that causes an unbalanced exchange of electrons in the body of the wistar rat. Electron ROS displacement results in the failure of homeostasis to stimulate growth, survival, and cell signaling depending on how much ROS is produced. The higher ROS or free radicals in wistar rats will cause oxidative stress, programmed apoptosis and cell necrosis. In addition to smoke free radicals, mosquito coils also cause fat peroxidation. The composition of spermatozoa contain fat in the wall membrane, so that if there is fat peroxidation, the integrity of the spermatozoa membrane will be deformed and not intact. The peroxidation of allethrin fat in wistar mice attacks the expression of StaR mRNA, a protein product produced by this gene that is essential for the transport of cholesterol into cells. Significant reduction in StAR mRNA expression occurs in rat testes to reduce cholesterol transport into steroidogenic cells. Important enzymes in the steroidogenic pathway 3β -hydroxysteroid dehydrogenase (3β -HSD) and 17β -hydroxysteroid dehydrogenase (17β -HSD) decrease and the activity of this enzyme also decreases, so if peroxidation occurs, the integrity of the sperm membrane becomes brittle and deformed to reduce sperm quality. The active ingredient allethrin significantly decreases testosterone in wistar rats so that spermatogenesis decreases and the quality defects.^{10,11}

In the analysis of the sperm vitality of the P1 group with an average of $44.80\% \pm 5.72$ given 8 ml beet juice every day showed no significant difference when compared with K (+) ($p = 0.244$). With the increase of free radicals and fat peroxidation in wistar rats, free radical antidotes are needed, beet juice has a significant antioxidant content. Previous studies have found that the effective dose depends on the level of free radicals obtained. Previous studies get free radicals from cigarette smoke, compared to mosquito coils smoke, the free radicals obtained will be higher mosquito coils smoke. At a dose of 8 mL of beetroot every day, previous studies have found significant differences.¹²

In the spermatozoa vitality analysis treatment group 2 had an average of $59.60\% \pm 7.02$ giving a significant difference when compared with the K (+) group ($p = 0.001$). The antioxidant ability of 16 mL beetroot juice per day can provide a good potential therapeutic effect so that it can maintain the quality of spermatozoa when compared with P2 with K (-) the difference is not significant ($p = 0.528$) and it is also proven if P2 compared with P1 produces significant differences ($p = 0.009$). Previous studies also showed that a 16 mL beet dose gave a significant difference. The high level of free radicals in the body must be proportional to antioxidants to counteract the results of free radicals, in the treatment of 2 doses of 16 mL every day provides a potential dose so that shows a meaningful difference. These results indicate the right dose of antioxidants will provide a free radical deterrence effect in the body that is well aimed with meaningful results between P2 versus K (+).

Determination of free radicals with antioxidants by donating electrons to prevent free radicals from reacting. The benefits of beetroot can be tested for bioavailability, bioavailability test in vivo that is



after consumption of an active compound then absorbed through the digestive tract is immediately available in the blood circulation in sufficient quantities to be utilized by cells. In other studies, betalain bioavailability shows that it is perfectly absorbed by humans. In further studies two betalain metabolites (betanin and betanidin) have been shown to reduce linoleic damage caused by cytochrome C oxidase and lipid membrane oxidation induced by metmyoglobin-H₂O₂-activated and free iron (AA-Fe).¹³

CONCLUSION AND SUGGESTIONS

Conclusion

1. There is a significant difference in the spermatozoa vitality of wistar rats exposed to the smoke of mosquito coils compared with those not exposed to the smoke of mosquito coils.
2. There is no significant difference in the spermatozoa vitality of wistar rats exposed to the smoke of mosquito coils and administration of beet juice (*Beta vulgaris*) at a dose of 8 mL.
3. There is a significant difference in the spermatozoa vitality of wistar rats exposed to the smoke of burning mosquito coils and administration of beet juice (*Beta vulgaris*) at a dose of 16 mL.

Suggestions

1. Need another research by monitoring the spermatozoa serial vitality of wistar rats to determine the effect of beet juice (*Beta vulgaris*) appropriately.
2. It is necessary to prove other studies the effect of mosquito repellent smoke on the vitality of spermatozoa by using different active mosquito coils.
3. It is necessary to further prove the bioavailability of beet juice (*Beta vulgaris*) in the process of maintaining the vitality of spermatozoa.

4. Further research needs to be done about the toxicology test of beet juice.
5. Further research on beet juice needs to be done in humans.

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