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THE EFFECT OF GIVING BIT JUICE (Beta vulgaris L.) ON SPERMATOZOA MORPHOLOGY OF WISTAR RATS THAT EXPOSED MOSQUITO COIL SMOKE

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ABSTRACT

Background : Spermatozoa have a limited amount of antioxidants that cause spermatozoa to be susceptible to oxidative stress by reactive oxygen species (ROS). Mosquito repellent contains the active ingredient Pyrethroid which is one example of external factors that cause DNA damage through oxidative stress. This results in the emergence of secondary metabolites that can act as free radicals, then these free radicals follow the circulation of blood throughout the body including the testes which will cause the process of spermatogenesis to be disrupted and will ultimately affect the quality of the spermatozoa produced. **Objective :** To find out the effect of giving beetroot juice (Beta vulgaris) on the morphology of spermatozoa of male Wistar rats that exposed to mosquito coil smoke. **Method** : This study uses an experimental study, with the type of research design is post test only control group design. The population used in this study were male Wistar rats (*Rattus novergicus*). The research sample was selected by simple random sampling. Analysis of the data used in the study is an analysis with one way anova test. **Results :** The mean morphological morphology of normal spermatozoa are: Group K (-) = 96.40; Group K (+) = 88,40; P1 group = 90.80; P2 group = 94.40. One Way ANOVA test found significant differences between the five treatment groups. Post-Hoc Test found significant differences between K (-) with K (+), K (+) with P2, P1 with P2, P1 with K (-). While in the other groups, there was no significant difference in the morphological percentage of spermatozoa. **Conclusion :** The giving of beet juice can affect sperm morphology Wistar rats that exposed to mosquito coil smoke.

Keywords : spermatozoa, bit juice, antioxidant, free radical

INTRODUCTION

Infertility is a condition where a married couple has not been able to have children despite sexual intercourse. There are two factors that influence the incidence of infertility, those are internal and external. Internal factors include hormonal disorders and oxidative stress. External factors include age, smoking habits, alcohol and drugs that could also affect fertility.[2]

Spermatozoa have a limited amount of antioxidants in accordance with a small volume of cytoplasm. This condition causes spermatozoa become vulnerable to oxidative stress caused by reactive oxygen species (ROS).

Moreover, the plasma membrane of spermatozoa that are rich in unsaturated fatty acids to maintain membrane fluidity causes spermatozoa easily bind with ROS. The mechanism causes oxidative stress as a result of membrane plasma peroxide, causing damage to spermatozoa[3].

The use of mosquito coils in Indonesia is very high along with the high incidence of dengue fever caused by mosquito bites.[4] *Pyrethroid* is one of the active ingredients contained in insect repellent can enter the body by inhalation in a long time, not only can cause disturbances in the lungs but also causes the liver unable to detoxify completely. This results in the emergence of secondary metabolites that can act as free radicals, then these free radicals follow the circulation of blood throughout the body including the testes which will

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cause damage. If the testes are damaged, spermatogenesis will be disrupted and will ultimately affect the quality of the spermatozoa produced.[5]

Antioxidants are compounds that are useful to overcome the oxidative damage caused by free radicals.[6] Red beet is a potential source of water soluble pigment, called betanin. Betanin in the betanidin form 5-O-beta-glucose is an antioxidant and active prevention of oxygen induction and oxidation by free radicals.[7]

RESEARCH METHODOLOGY

This research uses experimental, with the type of research design *post test only control group design*. The population used was male Wistar rats (Rattus norvegicus) obtained from Laboratorium Biologi Fakultas Matematika dan Ilmu Pengetahuan Alam (FMIPA) Universitas Negeri Semarang. The research sample was selected by simple random sampling to avoid bias so that all population objects have the same opportunity as the sample. The data obtained were tested for distribution normality with the Shapiro-Wilk test because of the small sample size. If the data distribution in this study is normal, perform parametric tests with the One Way Anova test. If there is data not normally distributed, then the test used is the Kruskal Wallis non parametric test followed by the Mann-Whitney post hoc test.

RESEARCH OUTCOME

The study population used 28 male Wistar rats which were used randomly and fulfilled the inclusion criteria. The entire sample of 28 male Wistar rats adapted to advance the standard of food or water for 7 days, then were divided into 4 groups, each group consisted of 7 mice. The treatment was conducted for 56 days.

Table 1. Descriptive analysis of morphology spermatozoa in each group

Group	N	Mean	Standard Deviation	Median	Minimum	Maximum
K (-)	5	96.40	1.517	97.00	94	98
K(+)	5	88.40	1.517	89.00	86	90
P1	5	90.80	2.775	90.00	87	94
P2	5	94.40	3.578	95.00	89	99

Based on the table above, it can be seen that the average percentage of spermatozoa having normal morphology shows the highest K (-) group (96.40) and the lowest K (+) group (88.40).

Table 2. Spermatozoa Concentration Normality Test Results

Group	Shapiro-Wilk
	P
K (-)	0,492
K (+)	0,492
P1	0,656
P2	0,573

p : meaningfulness value (meaningful if p>0,05)

From the results of the data above shows the normal data distribution (p> 0.05). Furthermore, the *Levene* homogeneity test using the test to determine whether the group has the same variant or not.

Table 3. Morphological Homogeneity Spermatozoa Test Results

Variant Test	P
Levene	0,464

Note : *homogent test (p>0,05)

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

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Table 4. *One-Way Anova* Morphological Spermatozoa Test Result

Group	p Value*
K (-) K (+) P1 P2	0,001*

One Way *Anova* Test, p : meaningfulness value, meaningful if p < 0,05

From the *One Way Anova* test obtained p <0.05 which means that there are significant differences between the four groups.

Table 5. *Post Hoc LSD* Morphological Spermatozoa Results Test

Group	P1	P2	Control (-)
Control (+)	0,149	0,002*	<0,001*
P1		0,037*	0,003*
P2			0,225

Note : * Significant (p < 0,05)

The resultsd from *Post Hoc* test, found significant differences between Control (+) with P2, Control (+) with Control (-), P1 with P2, P1 with Control (-).

DISCUSSION

Based on the results of this research, the giving of exposure to the mosquito coils smoke showed a difference in the morphology of the spermatozoa of wistar rats. This difference can be seen from the positive control treatments that are only exposed to mosquito coils fumes giving a normal morphology that is lower and meaningful than the negative control treatment groups who are not given exposure to mosquito coils fumes. Exposure to mosquito coil smoke given is very influential on spermatozoa due to DNA damage induced by oxidative stress.[5] Allethrin contained as basic materials of mosquito coils maker which can cause the formation of free radicals. If free radicals are not stopped it will damage the mitochondrial cell membrane. Mitochondria is ATP-producing necessary for the conversion of testosterone and Leydig cells in the process of spermatogenesis. In this case if the mitochondria are disturbed or damaged, the process of spermatogenesis will be disrupted.[8]

The results of this study indicate that exposure to mosquito coils can show normal morphological differences in spermatozoa. This can be seen from the average morphology of normal spermatozoa owned by the negative control group having the highest percentage, whereas normal spermatozoa owned by positive control had the lowest percentage compared to other groups. After being tested using the *Post-Hoc* LSD test there was a significant difference in the morphology of the spermatozoa in the negative control group compared to the positive control group (p <0.05). The results obtained support previous studies conducted by Faisal Yusuf in 2018 which stated that allethrin contained in mosquito repellent can reduce motility, number, viability and morphological abnormalities of spermatozoa.[9]

The giving of Wistar male rat beetroot exposed to mosquito coils showed normal morphological differences in spermatozoa when compared with the spermatozoa of wistar rats that were not given beets. This can be seen from the average treatment group who were exposed to mosquito coils and given beetroot with a dose of 16 ml showed a significant difference compared to positive control. This significant difference can be shown through the *Post-Hoc* LSD test, there is value of p <0.05. This shows that wistar rats given 16 ml beetroot juice were able to produce normal spermatozoa morphology. Wistar rats given daily exposure to mosquito coils smoke were higher but not equivalent to normal spermatozoa morphology of wistar rats in the

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Negative Control group. The increase was also shown by the treatment group who were exposed to mosquito coils and given beetroot at a dose of 8 ml, but had no significant difference with the positive control group. This insignificant difference was shown through the *Post-Hoc* LSD test, there was a value of p> 0.05. This might be due to the dose of beet juice given to the wistar rats which has not had the maximum effect in terms of increasing the morphological production of normal spermatozoa. The giving of beet juice improves the morphology of normal spermatozoa wistar rats that given exposure to mosquito coil smoke due to beetroot contains betacyanin pigment which has antiradical effect and high antioxidant activity.[10] In addition, based on previous research it was tested that beets contain flavonoids, saponins, sterols, and triterpenes.[11] The content of flavonoids act as an antioxidant by donating the hydrogen atoms which make free radicals become nonreactive.[12]

The limitation in this study is the morphology of spermatozoa Wistar rats could not be checked prior to treatment, so it could not be compared with morphology after treatment in the same mice.

CONCLUSION AND SUGGESTION

Conclusion

The difference in morphology of normal spermatozoa between Wistar rats that exposed to the mosquito coil smoke for 8 hours a day and Wistar rats that were not exposed was significant. There was a significant difference in the percentage morphology of normal spermatozoa between Wistar rats that received beetroot juice at a dose of 16 ml daily with exposure to the mosquito coils smoke and Wistar rats that only exposed to the mosquito coils smoke. There was no significant difference between Wistar rats that received beet juice at a dose of 16 ml daily with exposure to the mosquito coils smoke and negative control Wistar rats was not significant.

Suggestion

Need more research on the effect of different doses of beetroot juice on the morphology of spermatozoa Wistar rats. Need more research on the effect of mosquito coil smoke exposure on spermatozoa of Wistar rats with other sources of antioxidants.

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