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THE EFFECTS OF PURPLE GRAPE JUICE (VITIS VINIFERA) ON SPERMATOZOA MOTILITY OF MALE WISTAR RATS (RATTUS NORVEGICUS) EXPOSED TO ELECTRIC CIGARETTE SMOKE

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ABSTRACT

Background: Infertility in men can be caused by decreased motility of spermatozoa. Electric cigarette smoke contains nicotine, which can produce free radicals that affect spermatozoa motility. Purple grape juice which contains antioxidants, such as flavonoid, has the potential to improve sperm motility. **Objective:** This study aims to show the effect of purple grape juice on improving sperm motility in male Wistar rats exposed to electric cigarette smoke. **Methods:** This is an experimental study with a post-test-only control group design. Sample consisted of 28 male Wistar rats divided randomly into 4 groups, C1 (healthy control), Cpos (electric cigarette smoke), P1 (electric cigarette smoke + purple grape juice 3 mL/day), and P2 (electric cigarette smoke + purple grape juice 5 mL/day). Rats were kept in cage for 1 week (adaptation process), followed by 4 weeks of treatment duration. Rats were terminated in 30th day, spermatozoa sample were extracted from the rats, then sperm motility was examined. Data were analyzed by using SPSS for Windows Software Version 26. **Results:** Progressive motility rate of C1, Cpos, P1, P2 groups consecutively: 58.33 ± 7,52; 26,66 ± 8,16; 31,66 ± 7,52; 43,33 ± 12,11. Non progressive motility rate of C1, Cpos, P1, P2 groups consecutively: 26,66 ± 10,32; 55 ± 8,36; 43,33 ± 12,11; 35 ± 12,24. Immotility rate of C1, Cpos, P1, P2 consecutively: 18,33 ± 9,83; 28,33 ± 14,71; 20 ± 6,32; 16,66 ± 5,16. Kruskal Wallis test showed a significant difference in progressive motility sperm group (P=0.002) and non-progressive motility sperm group (P=0.005). While the immotile sperm group didn't show a significantly difference between subject groups (P>0.05). **Conclusion:** Purple grape juice affects sperm motility of male wistar rats exposed to electric cigarette smoke.

Keywords: *Electric Cigarettes, Infertility, Nicotine, Purple Grape Juice, Sperm Motility*.

INTRODUCTION

According to ICMART and WHO, infertility is a disease of the reproductive system characterized by failure to achieve pregnancy for 12 months without using safety devices during sexual intercourse. Infertility is classified into 2 groups: primary if there has never been a history of pregnancy and secondary if there has been a history of pregnancy.[1] Infertility is caused by abnormalities in the sperm.[2] Low sperm concentration (oligospermia), poor motility (asthenospermia), and morphological abnormalities (teratospermia) are often the main causes of infertility.[3]

Electric cigarettes are nicotine without burning tobacco plants to keep the sensation of smoking.[4] Conventional cigarettes contain 1–1.5 mg of nicotine per stick with an average of 14-21 mg a day while electric cigarettes contain 0-16 mg of nicotine per bottle if used up to 300 puffs, with an average of 62.8 puffs per day. The average nicotine consumed in electric cigarettes is 3.36 mg per day, which is lower than conventional cigarettes.[4]

Study by Mandasari AA, et al. (2019) in Biotropic: The Journal of Tropical Biology, mice exposed to 18 mg of electric cigarette smoke for 30 days showed a decrease in the number of spermatozoa up to 5.2 million/ml. Based on the study, nicotine in electric cigarettes can cause increased production of free radicals, resulting in oxidative stress. Oxidative stress that occurs can affect abnormalities in sperm, for example, sperm motility.[5] The high content of Reactive Oxidative Stress (ROS) can damage the DNA in sperm and increase the production of superoxide anions. In addition, smoking can also cause creatinine kinase (CK) levels in sperm to decrease which results in decreased spermatozoa motility[6].

Grape juice can prevent diseases associated with oxidative stress.[7] Corte Cristiane, *et. al* (2013) in Applied Physiology, Nutrition, and Metabolism, the antioxidant effect of organic purple grape juice on exhaustive exercise, rats fed purple grape juice intake increased antioxidant levels.[8]

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Smoking using electric cigarettes containing nicotine can increase the production of Reactive Oxidative Stress (ROS) which causes oxidative stress, therefore affects sperm motility.[5,6] Purple grape juice can increase the level of antioxidants, thus increasing the motility of spermatozoa[7-9]. Since there has been no study regarding the effect of giving purple grape juice on sperm motility of male Wistar rats exposed to electric cigarette smoke, it was our interest to conduct the study.

METHODS

This research is an experimental study with a post-test-only control group design. The subjects of this study used experimental male Wistar rats (*Rattus norvegicus*) aged 8-9 weeks. The study was conducted from September to October 2021.

The study was carried out at the Laboratory of the Faculty of Mathematics and Natural Sciences, the State University of Semarang using 28 male Wistar rats divided randomly into 4 groups, healthy control (C1), positive control given electric cigarette smoke (Cpos), treatment one given electric cigarette smoke + purple grape juice 3 mL/day (P1) and treatment two given electric cigarette smoke + purple grape juice 5 mL/day (P2). Adaptation was carried out for 1 week, followed by treatment for 4 weeks. The inclusion criteria in this study were male Wistar rats, aged 8-9 weeks with a bodyweight of 150-200 grams. The exclusion criteria in this study were inactive rats and have anatomical abnormalities. Study subject which died during study periods will be considered as drop out. Then the sperm of Wistar rats were taken and the sperm motility of Wistar rats was examined.

The spermatozoa motility data obtained were analyzed statistically using the Saphiro-Wilk test. Progressive motility, non-progressive motility, and immotility were not normally distributed *(P*<0.05) so the test was followed by using the Kruskal Wallis test. com.

RESULTS

Analysis of the data obtained from the motility of spermatozoa was carried out using the Saphiro-Wilk test because the sample number was less than 50. The data distribution was not normal, so a non-parametric test was carried out using the Kruskal Wallis test.

Based on the Kruskal-Wallis test, a significance value of p <0.05 was obtained for the percentage of non-progressive motility and the percentage of progressive motility so that there were differences in the percentage of progressive motility and the percentage of non- progressive motility between groups. Next, a pairwise comparison test was performed on the percentage of progressive motility and non-progressive motility of spermatozoa to determine the significant difference between positive control and negative control groups, positive control with treatment one groups, positive control with treatment two groups, treatment one with treatment two groups, treatment one with negative control groups, and treatment two with negative control groups.

Table 1. Descriptive Analysis

Group	Progressive (%)	Non-progressive (%)	Immotile (%)
C1	58,33 ± 7,52	23,33 ± 15,05	18,33 ± 9,83
Cpos	20,00 ± 10,95	51,66 ± 13,29	28,33 ± 14,71
P1	31,66 ± 7,52	43,44 ± 12,11	25,00 ± 8,36
P2	43,33 ± 12,11	40,00 ± 10,95	15,6 ± 5,16
	P^k	0,001*	0,022*

*Significant (p < 0,005);
[k]Kruskall-Wallis

Table 2. Pairwise Comparison Test Results for Progressive Motility of Spermatozoa

Groups	Compared Group	P^M
C1	Cpos	0,000*
	P1	0,007*
	P2	0,145
Cpos	P1	0,227
	P2	0,014*
P1	P2	0,212

*Significant (p < 0,005);
[m]Mann-Whitney Test

Table 3. Pairwise Comparison Test Results for Non-Progressive Motility of Spermatozoa

Groups	Compared Group	P^M
C1	Cpos	0,002*
	P1	0,040*
	P2	0,090
Cpos	P1	0,325
	P2	0,180*
P1	P2	0,722

*Significant (p < 0,005);
[m]Mann-Whitney Test

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DISCUSSION

Electric cigarette liquid consisting of nicotine, propylene glycol, flavoring, and vegetable glycerin contains ROS.[10-13] If used continuously, it will cause an imbalance between ROS levels and antioxidants in the body, leading to oxidative stress. High levels of ROS can cause apoptosis in sperm which results in DNA destruction so that it affects the motility of spermatozoa. Oxidative stress that occurs can also affect the plasma membrane of Polyunsaturated Fatty Acid (PUFA) which is very vulnerable when oxidized by ROS so that it can cause a decrease in spermatozoa motility.[14,15]

Purple grape juice can act as an antioxidant because it contains polyphenols (flavonoids) which function as antioxidants because it can neutralize and destroy free radicals contained in electric cigarette liquid.[10-13] The activity of purple grape juice as an antioxidant can be done by inhibiting lipid peroxidation by ROS on the plasma membrane which can affect the motility of spermatozoa. In addition, the activity of polyphenols is able stop the effect of oxidative stress on PUFA preventing the decrease in sperm motility.[5,16-19]

The results of this study showed a significant difference in progressive and non-progressive motility in the group of rats exposed to electric cigarette smoke during the study and the group of rats that were not exposed to electric cigarette smoke at all. This result is in line with research conducted by Miseal Tooy, *et al.* (2016) in Journal eBiomedik, which found that there was a decrease in spermatozoa motility in rats exposed to electric cigarette smoke with a significant difference of $p=0.008$.[16] This proves that long-term exposure to electric cigarette smoke can affect the motility of spermatozoa.

The results of this study showed that there was a significant difference in the progressive motility in the group of rats exposed only to electric cigarette smoke and the group of rats exposed to electric cigarette smoke whilst administered a purple grape juice solution of 5 mL/day. A significant difference was also found in the progressive motility of the group of rats that were not exposed to cigarette smoke at all with the group of rats that were exposed to electric cigarette smoke and given a purple grape juice solution of 3 mL/day. A significant difference was also found in non-progressive motility in the group of rats that were not exposed to cigarette smoke at all with the group of rats that were exposed to electric cigarette smoke and given a purple grape juice solution of 3 mL/day.

These results are in line with research conducted by Jamalan Mostafa, *et al.* (2015) in International Journal of Fertility and Sterility, that the content of flavonoids in rutin, naringin, and kaempferol can offset the production of ROS produced by aluminum chloride ($AlCl_3$), cadmium chloride ($CdCl_2$), and lead chloride ($PbCl_4$). Naringin at a dose of 100 µM effectively increased the motility of spermatozoa with a difference of $p<0.038$ in spermatozoa exposed to aluminum chloride ($AlCl_3$).[20] Naringin, rutin, and kaempferol at a dose of 25-500 µM increased the motility of spermatozoa with a difference of $p<0.05$ for spermatozoa exposed to cadmium chloride ($CdCl_2$). Meanwhile, naringin, rutin, and kaempferol at a dose of 500 µM could significantly increase spermatozoa motility in spermatozoa exposed to lead chloride ($PbCl_4$). The results of this study prove that the content of flavonoids can effectively increase the motility of spermatozoa against exposure to metal toxicants with high levels of ROS.[20]

In the results of the study, there was a no-significant difference in the immotility between C1 and Cpos group, Cpos and P1 group, Cpos and P2 group, C1 and P1, C1 and P2 group, and P1 and P2 group. The insignificance could be caused by the high levels of ROS in the seminal plasma damaging DNA which then decreases the ATP levels so that the sperm lacks energy. In return, the sperm experience a decrease in motility. In accordance with the journal published by Nissanka Nadee in 2018 in HHS public access that high levels of ROS can cause damage in mtDNA that play a role in ATP production through susceptibility to oxidative damage caused by ROS. High levels of ROS can damage mtDNA which affects the production of ATP as the energy used by flagella in sperm for fertilization[21,22].

CONCLUSION

Based on the results of this study, it can be concluded that the spermatozoa motility of male wistar rats exposed to electric cigarette smoke was lower than that of those who were not. Purple grape juice solution (*Vitis Vinifera*) can affect spermatozoa

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motility of male wistar rats exposed to electric cigarette smoke by an effective dose of 5 mL/day. Further research activities are needed using more diverse doses of grape juice solution to find the effective dose.

ETHICAL APPROVAL

This research has received ethical clearance from the Research Ethics Commission of the Faculty of Medicine, Diponegoro University with the number 104/EC/H/FK-UNDIP/IX/2021.

CONFLICTS OF INTEREST

The author declare, there is no conflict of interest.

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