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Submission date: 17-Mar-2023 02:16PM (UTC+0700)

Submission ID: 2039203765

File name: ON_THE_SPERMATOZOA_MOTILITY_OF_MALE_WISTAR.29987-67100-1-PB.pdf (437.16K)

Word count: 3786

Character count: 20332



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THE EFFECT OF COFFEE ON THE SPERMATOZOA MOTILITY OF MALE WISTAR RATS (*Rattus norvegicus*) EXPOSED TO ELECTRIC CIGARETTE SMOKE

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ABSTRACT

Background: Man infertility is usually caused by decrease of spermatozoa quality. E-cigarette smoke which contain chemicals as nicotine produce free radicals which decrease spermatozoa motility. Coffee contain caffeine and chlorogenic acid functioned as antioxidant and increase spermatozoa motility. **Aim:** To prove the effect of coffee to spermatozoa motility of male wistar rats that exposed to e-cigarette smoke. **Methods:** The samples were 28 male wistar rats (*Rattus norvegicus*), no anatomical abnormalities, 200-300 grams/BW, and 8-9 weeks old. The sample was randomly divided into 4 groups: no treatment, exposed to e-cigarette smoke, given 3ml robusta coffee solution contained of 180mg coffee powder and exposed to e-cigarette smoke, given 3ml robusta coffee solution contained of 360mg coffee powder and exposed to e-cigarette smoke. The coffees solution was given through a gastric tube. The dose of e-cigarette liquid was 3,5ml with 18mg/ml nicotine for each group. After four weeks, the rat's sperm was taken, preparation was made, and spermatozoa motility was examined through microscope. The statistical test exerted Saphiro-Wilks test and non-parametrical test from Kruskal-Wallis. **Results:** Mean value of progressive motility, non progressive motility, and immotility percentage on normal control group = 53,81%, 30,00%, 16,66%; negative control group = 0,48%, 17,14%, 82,38%; first treatment group = 10,00%, 32,85%, 57,14%; second treatment group = 11,42%, 26,19%, 62,38%. Kruskal-Wallis test showed a significant difference in progressive motility and immotility ($p=0,000$). **Conclusion:** The coffee solution could give an effect on the spermatozoa motility of male wistar rats exposed to electric cigarette smoke. Coffee can increase sperm motility percentage of wistar rats exposed by electric cigarette smoke.

Keywords: Infertility, e-cigarette, nicotine, coffee, motility

INTRODUCTION

Infertility is a form of inability on active couple sexually and without use of contraception to attain spontaneous pregnancy within period of 12 months.¹ There are 50-80 million couples of childbearing age in this world who have problem of infertility, while in Indonesia, approximately 10-15% of total population have the problem.² One-third infertility problems on couple are related to male reproductive organ. According to WHO, 30-40% of couples are difficult to have children because of infertility factor on the husband side.^{3,4} The infertility on man is caused by decrease of sperm quality. The poor quality of sperm is the main causal factor of infertility on 20% couples in England. The infertility has several risk factors, one of them is smoking. The smoking can reduce the quality of sperm, this aspect is because the cigarette contains of free radicals that will finally cause to abnormality of spermatozoa concentration, motility, and morphology.^{4,5} Based on the research data from Basic Health Research in 2018, it has indicated that

the prevalence of smoking on the population aged 15 years old and above are about 62,9% on man and 4,8% on woman.⁶

Since 2004, electric cigarette has been introduced as the substitute of tobacco cigarette.^{7,8} Along these recent years, it is found an increase of total tobacco smokers who intend to move to electric cigarette.⁹ This electric cigarette is initially a strategy to stop smoking or Nicotine Replacement Therapy (NRT). But, E-cigarettes are not currently approved by the FDA as a quit smoking aid. The FDA classifies e-cigarettes as a tobacco product and to date, no e-cigarettes have been approved as a smoking cessation aid.¹⁰

There are several types of e-cigarettes; tanks or mods, a type of rechargeable e-cigarette product designed to be used multiple times. They are modifiable devices ("mods"), allowing users to customize the substances in the device. Pod Mods, is an e-cigarette, or vaping, product with a prefilled or refillable "pod" or pod cartridge with a modifiable (mod) system. Pod Mods typically use



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(Jurnal Kedokteran Diponegoro)

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E-ISSN : 2540-8844

Volume 10, Number 3, May 2021

nicotine salts rather than the freebase nicotine used in most other e-cigarette products. Nicotine salts, which have a lower pH than free base nicotine, allow particularly high levels of nicotine to be inhaled more easily. The e-cigarette activates by exerting energy and battery to heat e-liquid and change to vapor.^{11,12} Although, e-liquid contains fewer chemicals and induce cytotoxicity or fewer side effect than the tobacco cigarette, this content permanently show oxidant reactivity by producing ROS (reactive Oxygen Species). ROS can induce the oxidative stress that leads to cell apoptosis.⁷ The increasing number of free radicals or ROS is beyond the body's antioxidant defense system which will cause to oxidative stress.^{10,13} The oxidative stress that exists in sperm plasma can be a toxic and causes to oxidative breakage of sperm.¹⁴ Moreover, ROS can cause to damage on DNA of spermatozoa and spermatozoa apoptosis, then it will occur to the reduction of spermatozoa quality.¹⁵

The excessive number of ROS must be weakened by antioxidant that aims to defend the normal cell function.¹⁵ The natural sources of antioxidant are mostly found in plant and generally the phenolic compound that spread out in whole parts of plant.¹⁶ The polyphenol compound is a kind of antioxidant which mostly found in daily food intake. For example, coffee which is categorized into one of favorite drinks in the wide world which becomes the greatest source of antioxidant among other kinds of drink.^{17,18}

The coffee is contained of many chemicals, one of them is chlorogenic acid. It is a type of polyphenol compound which becomes the strong antioxidant in coffee drink.^{18,19,20} Besides, the coffee is also contained of caffeine which is able to stimulate movement of spermatozoa. The coffee giving can be given to balance the negative impact from radicals that resulted by e-cigarette.^{19,21,22}

A number of researches have done to identify the effect that resulted from coffee giving to the quality of spermatozoa which is exposed to tobacco cigarette smoke. The research finding shows that the coffee is able to improve the spermatozoa motility on *wistar rats* which have been exposed to cigarette smoke.¹⁹ Based on this finding, it needs to a research which aims to identify the effect of coffee giving to the spermatozoa motility on male *wistar rats* which have been exposed to e-cigarette smoke.

METHODS

This was an experimental research which exerted post-test only controlled group design. This research was conducted in Biology Laboratory of Mathematics and Natural Science Faculty of State University of Semarang. The total samples of research were 28 wistar rats (*Rattus norvegicus*) which fulfilled the following criteria: male wistar rats, did not have anatomical abnormality, weight between 200-300 gram, and age of 8-9 weeks.

A week before treatment, the wistar rats were adapted in a cage and fed with standard food and drink. After the adaptation, 28 wistar rats were divided into four groups which contained of 7 wistar rats that were determined randomly.⁹

The normal control group (N) was the group with no treatment. The control group (-) was the group which only exposed to e-cigarette smoke. The first treatment group was given e-cigarette smoke and coffee solution dose of 180 mg/3 ml. The last, the second treatment group was given e-cigarette smoke and coffee solution with a dose of 360 mg/3 ml.

The solution of robusta coffee was given through a gastric tube. The exposure of e-cigarette smoke was given by putting the rats into a closed cage. The liquid of e-cigarette was then poured into a beaker glass and heated, and then put into the closed cage. This study used local brand e-liquid. The dose of e-cigarette liquid was 3,5 ml for each cage. The nicotine which contained within e-cigarette liquid was approximately 18 mg/ml. This treatment was conducted for four weeks.

After four weeks of treatment, it continued to termination which the rats would be put into a jar that has been given some cottons that contained of chloroform or ether liquid, so the rats would not be awake and dead. The process of taking rats' sperm was done by cutting vas deferens and squeezing it to secrete sperm. The sperm was dripped with NaCl 0,9% about two drops and stirred until homogeneous. A drop of sperm was dripped on the object glass and covered by deck glass. The motility of spermatozoa was examined under the light microscope with zooming of 10x10 into three visual fields. The assessment on spermatozoa motility was done qualitatively by examining the movement of spermatozoa.

The data was then analyzed by computer software, SPSS in 25.0 version. The ethical



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E-ISSN : 2540-8844

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clearance for this research was derived from Ethical Commission of Health Research of Medical Faculty of Diponegoro University Semarang.

RESULTS

The data of spermatozoa motility on Wistar rats was presented in percentage. Based on the table 1, the highest mean percentage of spermatozoa which have moved actively was the normal control group (N) in approximately 53,81% and the lowest one was the control group (-) in approximately 0,48%. For the highest mean value of spermatozoa percentage which have moved but not progressive was the 1st treatment group in approximately 32,85%, while the lowest was the control group (-) in approximately 17,14%. Next, for the highest mean value of spermatozoa percentage which did not move was the control group (-) in approximately 82,38%, while the lowest was the normal control group (N) in approximately 16,66%.

Table 1. Description of Mean Value of Spermatozoa Motility Percentage

Group	N	Progressive (%)	Non Progressive (%)	Immotility (%)
Control (N)	7	53,81±6,507	30,00±5,444	16,66±3,333
Control (-)	7	0,48±1,259	17,14±18,604	82,38±19,787
1 st treatment	7	10,00±4,304	32,85±14,065	57,14±17,259
2 nd treatment	7	11,42±1,779	26,19±8,033	62,38±8,760

The normality test which was used in this research used Saphiro-Wilks test, because the total sample were less than 50. When the significance value (p) > 0,05, the data was normally distributed, meanwhile, when the significance value (p) < 0,05, the data was not normally distributed.

Table 2. Result of Data Normality Test on Spermatozoa Motility Percentage

Group	P		
	Progressive Motility	Non Progressive Motility	Immotility
Control (N)	0,200	0,119	0,030*
Control (-)	0,000*	0,016*	0,011*
1 st treatment	0,819	0,897	0,312
2 nd treatment	0,001*	0,196	0,060

Based on the table 2, it resulted that the p value < 0,05 on progressive motility of negative control group, progressive motility of second treatment group, non-progressive motility of negative control group, immotility of normal control group, and immotility of negative control group, therefore, the result of data normality test was not normally distributed.

Further, it was continued to non-parametric test of Kruskal Wallis.

Table 3. Result of Kruskal-Wallis Test on Spermatozoa Motility Percentage

Variance Test	Sig.		
	Progressive Motility	Non Progressive Motility	Immotility
Kruskal-Wallis	0,000*	0,060	0,000*

Based on the table 3, this research showed that the significance value p > 0,05 for the non-progressive motility percentage, so there was no difference of non-progressive motility percentage among groups. Next, for the progressive motility percentage and immotility percentage, it resulted the significance value p < 0,05, so there was difference of progressive motility percentage and immotility percentage among groups.

Furthermore, the researchers conducted pairwise comparison test on spermatozoa progressive motility percentage and spermatozoa immotility percentage which was aimed to identify the significant difference between negative control and first treatment group, negative control and second treatment group, negative control and normal control group, first treatment and second treatment group, first treatment and normal control group, and second treatment and normal control group.

Table 4. Result of Pairwise Comparison Test on Spermatozoa Progressive Motility

Group A – Group B	Sig.
Negative Control – First Treatment	0,028*
Negative – Second Treatment	0,009*
Negative – Normal Control	0,000*
First Treatment – Second Treatment	0,680
First Treatment – Normal Control	0,008*
Second Treatment – Normal Control	0,026*

Description: it is significant if Sig. < 0,05



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Volume 10, Number 3, May 2021

Based on the table 4, it indicated that the significance value $p < 0,05$ for the comparison between negative control and first treatment group, negative control and second treatment group, negative control and normal control group, first treatment and normal control group, second treatment and normal control group which referred to a significant difference between the groups. Meanwhile, on the other group, it was not found significant difference.

Table 5. Result of Pairwise Comparison on Immotility

Group A - Group B	Sig.
Normal Control – First Treatment	0,017*
Normal Control – Second Treatment	0,004*
Normal Control – Negative Control	0,000*
First Treatment – Second Treatment	0,613
First Treatment – Negative Control	0,059
Second Treatment – Negative Control	0,166

Description: *Sig.< 0,05

Based on the table 5, it showed that the significance value $p < 0,05$ for the comparison between normal control and first treatment group, normal control and second treatment group, normal control and negative control group. Meanwhile, on the other group, it was not found significant difference.

DISCUSSION

E-liquids consist of a base, which is usually comprised mainly of propylene glycol (PG) and vegetable glycerin (VG), and nicotine and flavorants can be added to this PG/VG base which could affect to free radicals.^{13,23} The free radicals which resulted from e-cigarette liquid heating was able to harm DNA of sperm directly by striking purine base and pyrimidine. The free radical or ROS could initiate apoptosis in sperm which caused enzymes activity to degrade DNA sperm.²⁴ Moreover, ROS was able to affect sperm agglutination which then caused to the decrease of spermatozoa motility.²⁵

On the research finding, it found a significant difference on the progressive motility and immotility from rats group which have been exposed to e-cigarette smoke for 4 weeks and rats group which were not exposed to e-cigarette smoke. The sperm count with progressive motility on

negative control group was higher than the positive control group. The sperm count with immotility on positive control group was higher than the negative control group. This result indicated that the e-cigarette smoke which inhaled in a long time was able to cause the impairment on spermatozoa motility.

Robusta coffee has the content of polyphenol compound, specifically chlorogenic acid (CGA) that was functioned as an antioxidant and able to hamper free radicals.^{26,27} In addition, the coffee also contained caffeine which was able to hamper the action of phosphodiesterase enzyme and increase the production of cAMP, so it could stimulate the spermatozoa motility immediately.²¹

On the research finding, the researchers also found a significant difference on spermatozoa number that have progressive motility from the rats group which have been only exposed to e-cigarette smoke and rats group which have been exposed to e-cigarette smoke and given coffee solution in about 180 mg coffee powder. This significant difference was also found on the number of spermatozoa which have progressive motility from rats group which have been only exposed to e-cigarette smoke and rats group which have been exposed to e-cigarette smoke and given coffee solution in about 360 mg coffee powder. This result indicated that the coffee giving was able to increase the spermatozoa motility from the rats which have been exposed to e-cigarette smoke.

This percentage value of progressive motility on rats group which have been exposed to e-cigarette smoke and given coffee solution of 360 mg coffee powder was higher than the rats group which have been exposed to e-cigarette smoke and given solution of 180 mg coffee powder, but there was no indication of significant difference. This finding proved that the increase of coffee dose was able to improve the motility of spermatozoa.

CONCLUSION

Based on the research finding, the researchers concluded the effect of coffee giving to the spermatozoa motility on male wistar rats that have been exposed to e-cigarette smoke. The spermatozoa motility on male wistar rats that have been exposed to e-cigarette smoke and given coffee solution of 180 mg and 360 mg coffee powder was significantly higher than the spermatozoa motility



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on wistar rats who were not given coffee solution. The spermatozoa motility on wistar rats which have been exposed to e-cigarette smoke and given coffee solution of 360 mg coffee powder did not differ significantly from those given 180 mg of coffee powder.

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