SURABAYA INTERNATIONAL HEALTH CONFERENCE
"Empowering Community For Health Status Improvement"
Novotel Samator East Surabaya Hotel, July, 13-14 2019

The Effectiveness Extracts Of Ocimum Sanctum L Leaf On Motility And Morphology Spermatozoa Mice That Is Exposure With Cigarette Smoke

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Abstract: The incidence of infertility is still a health problem in the world including Indonesia (21.3%) throughout Asia. Fertility in men is strongly influenced by several factors, one of them is smoking behavior. Smoking can increase ROS, reduce antioxidants in semen, cause DNA damage, and morphological abnormalities of spermatozoa. Basil contains flavonoids which are rich in antioxidants, boron and zinc to stimulate of androgen hormones. This research is using 8- weeks-old Mus musculus, we exposed with smoke of 20 cigarettes/day and we administered of extract Ocimum sanctum L in dose dependency (100 mg/kg, 200mg/kg, and 300mg/kg) for 4 weeks. Then we examined the motility and morphology of spermatozoa by microscope. This research consists of 5 group. In CS-, CSBL-2 and CSBL-3 it showed that the spermatozoa motility is more progressive than CS+ and CSBL-1. The morphology of spermatozoa in CS+ and CS- found different results with a significance value 0,10 (p0.05). There is an effect of giving basil leaves to increase motility and improvement the morphology of spermatozoa that have been exposed to cigarette smoke especially in dose 300 mg/kgBB but not significant
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Keywords: .

1. Introduction

Infertility is still a health problem in the world in the Asian continent, the prevalence of infertility is quite high, including Indonesia (21.3%) (HIFERI, 2013). Mascarenhas et al., (2012) added that there were 48.5 million productive age couples who could not have children. Fertility in men is strongly influenced by several factors, one of which is due to an unhealthy lifestyle such as smoking. Cigarettes are one of the addictive substances made from processed tobacco by adding additional ingredients in the form of cloves or not. Someone who uses cigarettes can provide health for individuals and society, so smokers are known as two names namely active smokers and passive smokers. Every single cigarette burned, containing 400 kinds of chemicals is toxic, but there are three main toxic components contained in cigarette smoke, namely carbon monoxide, nicotine, and tar (Sukmaningsih, 2009). Several studies on the effects of chemicals from cigarettes indicate a disturbance in spermatogenesis. Smoking can increase free radicals (ROS) and reduce antioxidants in semen and can cause DNA damage through cellular DNA fragmentation and cause morphological abnormalities (head, neck and tail) of spermatozoa. This is evidenced by an increase in the level of 8-OhdG (marker of DNA fragmentation) by 50%

in male active smoker spermatozoa and 23% in passive smokers and lower compared to non-smokers (Sukmaningsih, 2009). Free radicals are molecules that have unpaired electrons, so they have high reactivity and tend to bind other electrons to produce new free radicals. This is what makes free radicals very dangerous (Sarma et al, 2011). Some of them are in the form of gases (CO, CO2, NO NO2 and HCN), and particular nicotine, tar, metal and phenol (Philip et al, 2015). Previous research conducted by Batubara, et al (2013), using animal models exposed to cigarette smoke for 30 days, obtained results, that the more cigarettes consumed by the subjects, the normal motility of spermatozoa decreased significantly (20.60 ± 3.64). While the percentage of morphology of abnormal spermatozoa also increased significantly (68.00 ± 1.87). Antioxidants are substances to stabilize free radicals by complementing the lack of electrons possessed by free radicals, and inhibiting the occurrence of chain reactions that can cause oxidative stress (Christijanti & Iswara, 2010). Various types of plants in Indonesia can be used as natural ingredients that can increase fertility. One of them is basil leave (Ocimum sanctum L.). According to Kuniawan (2013), empirically basil is used as an aphrodisiac because it contains arginine which can strengthen sperm resistance and prevent sterility. In addition to araginin, basil leaves also contain other secondary metabolites such as boron and zinc which function to release androgen (testosterone), and flavonoids which are antioxidants which play a role in protecting DNA and other important molecules from oxidation and damage, and can improve sperm quality increase male fertility (Gunawan & Mulyani, 2004; Sarma et al, 2011). Based on the background above, the researchers wanted to conduct further research on the effect of basil leaf extract (Ocimum sanctum L) on the morphology and motility of spermatozoa of male mice (Mus musculus) which were exposed to cigarette smoke

2. Research Methodology

2.1. Animal Intervention and Treatment This research is an experimental research with a type of true experimental laboratories design using a post-test only control group design approach. The population of this study was white male mice (Mus musculus) exposed and not exposed to cigarette smoke. The study sample was 25 which were divided into 5 groups namely: CS- (without exposure to cigarette smoke and treatment basil leaf extract), CS+ (without treatment basil leaf extract but exposed smoke of 5 cigarettes/day), CSBL-1 (exposed smoke of 5 cigarettes/day + 100mg/kg basil leaf extract), CSBL-2 (exposed smoke of 5 cigarettes/day + 200mg/kg basil leaf extract), CSBL-3 (exposed smoke of 5 cigarettes/day + 300mg/kg basil leaf extract) and were taken by simple random sampling. Inclusion criteria from this research namely, male mice, weighing 20-40 grams, age 3 months, healthy, adapted 1 week while exclusion criteria namely, animals in a condition of disability, illness (the condition of limp mice is less active, dull mice feathers, exudate discharge from eyes, mouth, anus or genital), die due to errors during treatment. The treatment of cigarette smoke is carried out after the animal experiences adaptation for one week. Every treatment of exposure smoke of cigarette is carried out in a intervention cage. After the last cigarette burned, the animal left for 10 minutes with cigarette smoke. Then the experimental animals can be released from intervention cage and given basil leaf extract for treatment. This intervention and treatment was carried out for 28 days. 2.2. Materials The materials: male mice, basil leaves (Ocimum sanctum L.), 70% ethanol, cigarettes, standard food mice, materials for preparing sperm analysis (NaCl solution, Eosin Y 1%), Aquades, NaCMC. The tool: Experimental animal cages, Digital scales, Rotavapor tools, Blenders, Glass beakers, Maceration tubes, Intragastric Sonde, Measuring pipettes, Handschon, Cotton, Filter paper, Surgical instruments, Glass jars with caps, Glass objects Glass cover, light microscope, syringe, lighters, Petri dish.

2.3. Sampling The experimental animals are anesthetized using a dose of 0.5 ml timolol and dissected using a disecting kit to take the organs of the testis and cauda epididymis. The Cauda epididymis is separated by cutting the proximal part of the corpus epididymis and the distal vas deferens. Cauda epididymis that has been separated can be placed in a petri dish containing 1 ml of 0.9% NaCl. Cauda epididymis is cut until smooth and stirred so that it is suspended with 0.9% NaCl to form a spermatozoa suspension. Furthermore, the suspension was observed using a light microscope. 2.4. Observation of Spermatozoa Motility and Morphology Observation of spermatozoa motility was using suspension of spermatozoa is dropped on the object's glass by using a dropper pipette and the lid of the glass object using a closing glass. Observe the preparation under a light microscope with a magnification of 400 times the spermatozoa movements after that categorize the results. Observation of spermatozoa motility was using suspension of spermatozoa is dropped on top of the object's glass to make smear preparations and dried in the air. The smear preparations are fixed with bunsen fire to dry and must not evaporate, then color with 1% Y eosin solution, leave it to dry. Preparations are washed with distilled water and dried. The preparation was observed under a light microscope with a magnification of 400 times to determine the morphology of 100 spermatozoa of mice. Calculate the percentage of normal and abnormal spermatozoa. 2.5. Statistical Analysis Data is recorded in Microsoft Excel 2016 and exported to SPSS Version 25 for statistical analysis. Quantitative variables are reported as amounts (%). The values are given as mean ± standard deviation (SD). The result were analyzed by Kruskal-Wallis test followed by Mann-Whitney test. The significance level used is 95% with a significance value of 5%. If the value of p (0.05) with CS- or CS+, but based on table 2 it was found that the average percentage of normal spermatozoa morphology CSBL-1, CSBL-2, and CSBL-3 increased. In CSBL-3 it has an average percentage is 75.50 ± 9.19 which is almost the same as the average CS- percentage. So there is an effect of basil leaf extract to increase the average percentage of normal spermatozoa morphology especially CSBL-3 but not significant. Table 2. Mean and Standard Deviation Value Normal Morphology of Spermatozoa Figure 1. Normal Spermatozoa Morphology The result of representation normal spermatozoa morphology in microscope consist of head, neck, and tail. Bar: decrease and increase mean percentage of normal spermatozoa morphology. CS-: Cigarettes Smoke -, CS+: Cigarettes Smoke +, CSBL-1: Cigarettes Smoke + Basil Leaf 1, CSBL-2: Cigarettes Smoke + Basil Leaf 2, CSBL-3: Cigarettes Smoke + Basil Leaf 3. No Group Mean (%) ± SD 1 Cigarettes Smoke (-) 81,25 ± 3,10 2 Cigarettes Smoke (+) 55,17 ± 10,13 3 Cigarettes Smoke + Basil Leaf 1 58,00 ± 12,73 4 Cigarettes Smoke + Basil Leaf 2 62,75 ± 19,14 5 Cigarettes Smoke + Basil Leaf 3 75,50 ± 9,19 0.00% 20.00% 40.00% 60.00% 80.00% 100.00% CS - CS + CSBL-1 CSBL-2 CSBL-3 From the results of data analysis showed that there was an increase decrease in the average percentage of abnormal spermatozoa morphology after exposed to cigarette smoke and given basil leaf extract. In Table 3 using the Kruskal-Wallis Test it showed that the average percentage of abnormal spermatozoa morphology there was no significant difference value 0.068 (p >0.05). Based on table 3 it was showed that the average percentage of abnormal spermatozoa morphology CSBL-1, CSBL-2, and CSBL-3 decreased. In C3 it has an average percentage is 24,50 ± 9,19 which is almost the same as the average CS- percentage. So there is an effect of basil leaf extract to decrease the average percentage of abnormal spermatozoa morphology especially CSBL-3 but not significant. Table 3. Mean and Standard Deviation Value Abnormal Morphology of Spermatozoa Figure 2. Abnormal

Spermatozoa Morphology The result of representation abnormal spermatozoa morphology in microscope; A. Only head, B & D. curved like the letter C, C & E. 1 Head with 2 Tails. Bar: increase and decrease mean percentage of abnormal spermatozoa morphology. CS-: Cigarettes Smoke -, CS+: Cigarettes Smoke +, CSBL-1: Cigarettes Smoke + Basil Leaf 1, CSBL-2: Cigarettes Smoke + Basil Leaf 2, CSBL-3: Cigarettes Smoke + Basil Leaf 3.

2.2. From the results of research that has been carried out shows that the ethanol extract of basil leaves is effective against increased sperm motility of mice. Ethanol extract of basil leaves is effective against the increase of motility of mice because it contains flavonoids which can neutralize the high levels of free radicals generated by cigarette smoke. These results are in accordance with previous studies conducted by Desi, et al (2018) where the flavonoid content of basil leaves can prevent a decrease in the percentage of spermatozoa motility of MSG-induced male wistar rats (Rattus norvegicus). The results of the study also showed that the ethanol extract of basil leaves was effective against the increase in the average percentage of normal spermatozoa of mice but statistically significant results were not obtained. This result is slightly different from previous studies conducted by Murod No Group Mean (%) ± SD 1 Cigarettes Smoke (-) 18,75 ± 3,10 2 Cigarettes Smoke (+) 44,83 ± 10,13 3 Cigarettes Smoke + Basil Leaf 1 42,00 ± 12,73 4 Cigarettes Smoke + Basil Leaf 2 34,75 ± 22,20 5 Cigarettes Smoke + Basil Leaf 3 24,50 ± 9,19 0.00% 10.00% 20.00% 30.00% 40.00% 50.00% 60.00% CS - CS + CSBL-1 CSBL-2 CSBL-3 (2014) which showed that the administration of basil leaves gave a significant increase in the morphology of spermatozoa. Cigarette smoke has a negative effect besides affecting the health of the respiratory system as well as disrupting the reproductive system in the form of a disruption of the process of spermatogenesis in the seminiferous tubules and influencing testosterone hormone levels. In the study of Batubara, et al (2013), showed that the increasing number of exposure to cigarette smoke was given in line with the percentage decrease in abnormal motility and morphology of spermatozoa. In the content of each clove cigarette there are 1-3 mg nicotine which is a toxoid alkaloid which can enter into the brain barrier quickly in approximately 10 seconds and circulates to all parts of the body in 15-20 minutes at the time of the last suction. Nicotine in cigarette smoke can stimulate the adrenal medulla to release catecholamines which can affect the central nervous system, so the mechanism of feedback between the hypothalamus, anterior pituitary and testicles is disrupted, as a result of the synthesis of testosterone hormone and the process of spermatogenesis. The effect of nicotine on sperm cells depends on the body's resistance to mice because each mouse has different sensitivity (Anita, 2004). Cigarette smoke can increase the production of free radicals (ROS) so much that oxidative stress occurs. Oxidative stress is an important factor in male fertility. ROS is a metabolite derived from oxygen which can modify cell function and endanger cell survival. Spermatozoa are susceptible to ROS because the plasma and cytoplasmic membranes contain large amounts of unsaturated fatty acids so that lipid peroxides can be formed (Cummins & Jequier, 1994). Cigarette smoke has free radical compounds that can increase the amount of lipid peroxidation and cause damage or decrease in membrane spermatozoa integrity (Karim, 2011). The lipid peroxide reaction will cause an increase in membrane fluidity, membrane integrity disorder and inactivation of membrane bonds with enzymes and receptors (Sukmaningsih et al, 2011). This will cause inhibition of nutritional needs and ATP in cells. If the supply of ATB is exhausted or decreases, the spermatozoa flagellum does not contract and does not move (Hafaz, 2017). The

motility of spermatozoa can also decrease due to abnormalities of the spermatozoa. The progressive progress of the spermatozoa depends on the balance of the shape of the tail. Spermatozoa with abnormal morphology will inhibit the movement and balance of the tail (Fitriani, 2009). Protective effects on cells can be done by giving antioxidants before exposure (Khaki et al, 2011). In the CS+ group, the average percentage of abnormal morphological numbers of spermatozoa increased and decreased after being given basil leaf extract. This is because exposure to cigarette smoke causes increased oxidative stress which affects the process of spermatogenesis, the quality of semen and changes in testosterone levels which cause the formation of abnormal spermatozoa morphology (Fitriani, 2009). In addition, in the study of Faranita (2009), said that high free radicals can damage the mitochondrial membrane and DNA integrity in the spermatozoa nucleus. This can induce cell apoptosis which causes changes in the morphology of spermatozoa during spermatogenesis. Basil is an herbal plant that has a high antioxidant content. Basil contains flavonoids which are antioxidants that play a role in protecting DNA and other important molecules from oxidation and damage, and can improve sperm quality so that it can increase male fertility (Gunawan & Mulyani, 2004). Flavonoids are phenolic compounds that act as free radical scavengers Flavonoids will push hydrogen ions so that they can neutralize the toxic effects of these free radicals and increase the normal motility and morphology of spermatozoa (Khaki et al, 2011; Safwan, 2016). Besides flavonoids, basil also contains arginine. Arginine is a non-essential and polar amino acid that is indispensable in protein synthesis and has an important role in the body's immune system and cellular immunity. According to Srivastava et al (2006) arginine also plays an active role in the process of spermatozoa formation. This is supported by previous research by Mahendra (2009) and Setyo Kurniawan (2013) that the arginine content in basil leaves increases the motility and concentration of spermatozoa so that it can strengthen sperm resistance and prevent sterility. The results of this study indicate that CSBL-3 has more progressive spermatozoa motility and a lower percentage of abnormal spermatozoa (24.50 ± 9.19) than CSBL-1 and CSBL-2. This is because at CSBL-3 (300 mg / kg) the antioxidant content of basil leaf extract is quite optimal in protecting against cell membrane damage, increasing motility and decreasing the abnormal morphology of spermatozoa in mice.

3. Conclusion

Conclusion Based on the results of the analysis and discussion of this study, it can be concluded that the the effectiveness extracts of ocimum sanctum l leaf on motility and morphology spermatozoa mice that is exposure with cigarette smoke there was no significant effect, but on an average percentage there was an increase in motility and a decrease in the abnormal morphological amount of spermatozoa in mice

4. Thank You Note

. I’m sincerely thank to RISTEKDIKTI for support funding this research, Nahdlatul Ulama Surabaya University for support fully our research, and Hotimah Masdan Salim, dr., Ph.D, as a supervisor who is attentive, patient, accompanies and directs the author, especially to my parents who always give support and motivate in the preparation and complete of this article.

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