THE EFFECTS OF NICOTINE EXPOSURE PER INHALATION TO THE CHANGE OF MOTILITY AND MORPHOLOGY OF THE RAT'S SPERM

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ABSTRACT

Objective:*Smoking are known to have detrimental effect on sperm. The effects are suspiciously come from nicotine as its main pharmacologic component. This study is carried out to analyze the effect of nicotine exposure per inhalation to the difference of motile sperm percentage and normal morphology sperm percentage of male wistar rats.* ***Material & Methods:*** *Thirty male rats were used for this research. They were randomly divided into 5 groups and treated with nicotine inhalation 0.5 mg/kgBW, 1 mg/kgBW, 2 mg/kgBW and 4 mg/kgBW for 30 days while the control rats were not given any treatment. At the end of experiment, sperm analysis was carried out to measure the percentage of motile sperm and percentage of sperm with normal morphology.* ***Results:*** *Percentage of motile sperm significantly decreased ($p<0.05$) in all treatment groups compared to control group. Comparison between treatment groups revealed that the percentage of motile sperm significantly decreased in 0.5 mg/kgBW vs 2 mg/kgBW, 0.5 mg/kgBW vs 4 mg/kgBW, and 1 mg/kgBW vs 4 mg/kgBW. Percentage of normal morphology sperm decreased significantly ($p<0.05$) in 1 mg/kgBW, 2 mg/kgBW, and 4 mg/kgBW compared to control groups. Comparison between treatment groups revealed that there were no significant decreased between each treatment groups.* ***Conclusion:*** *This research conclude that nicotine exposure per inhalation can deteriorate the percentage of motile sperm and the percentage of normal morphology sperm of male wistar rats.*

Keywords: *Nicotine, sperm morphology, sperm motility.*

ABSTRAK

Tujuan: *Merokok diketahui memiliki efek yang merugikan pada sperma. Efek ini diduga berasal dari nikotin yang merupakan komponen farmakologis utamanya. Penelitian ini dilakukan untuk menganalisis pengaruh paparan nikotin per inhalasi terhadap perbedaan persentase motilitas sperma dan persentase morfologi sperma normal pada tikus wistar jantan.* ***Bahan & Cara:*** *Tiga puluh tikus jantan digunakan untuk penelitian ini. Mereka secara acak dibagi menjadi 5 kelompok dan diberikan perlakuan berupa inhalasi nikotin 0.5 mg/kgBB, 1 mg/kgBB, 2 mg/kgBB dan 4 mg/kgBB selama 30 hari sementara kelompok tikus kontrol tidak diberikan perlakuan. Pada akhir percobaan, analisis sperma dilakukan untuk mengukur persentase sperma motil dan persentase sperma dengan morfologi normal.* ***Hasil:*** *Persentase sperma motil menurun secara signifikan ($p<0.05$) pada semua kelompok perlakuan dibandingkan dengan kelompok kontrol. Perbandingan antara kelompok perlakuan bahwa persentase sperma motil menurun secara signifikan dalam 0.5 mg/kgBB vs 2 mg/kgBB, 0.5 mg/kgBB vs 4 mg/kgBB, dan 1 mg/kgBB vs 4 mg/kgBB. Persentase morfologi sperma normal menurun secara signifikan ($p<0.05$) dalam 1 mg/kgBB, 2 mg/kgBB, dan 4 mg/kgBB dibandingkan dengan kelompok kontrol. Perbandingan antara kelompok perlakuan mengungkapkan bahwa tidak ada penurunan yang signifikan antar kelompok perlakuan.* ***Simpulan:*** *Penelitian ini menyimpulkan bahwa paparan nikotin per inhalasi dapat memperburuk persentase sperma motil dan persentase morfologi sperma normal tikus wistar jantan.*

Kata Kunci: *Nicotine, sperm morphology, sperm motility.*

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INTRODUCTION

Despite the massive anti-smoking campaign, cigarette smoking remains a big world health problem. Data collected by CDC shown that 36.5 million people are active smokers with 16 million people are living with smoking-related disease in America.[1] In Indonesia, Riskesdas 2013 revealed that 36.3% of the population above 15 years old are tobacco consumer, both by smoking and chewing. This data shows a rising percentage comparing to Riskesdas 2007 (34.7%).[2] Smoking are related to several health problems, such as cardiovascular disease, respiration disease and

malignancies including lung cancer, bladder cancer, cervical cancer, renal cancer, pancreatic cancer and stomach cancer. These past decades, researchers are begin to investigate the relations between smoking and fertility.[3]

Smoking are known to have deteriorating effect on sperm. Ramlau-Hansen found that smokers tend to have lower sperm volume, lower sperm count and lower motile sperm percentage compare to them who are non-smokers. The study also indicated that the relation between sperm concentration and smoking is dose-dependent.[4] The cause of the negative effect to male fertility is suspiciously coming from the substance formed when the smoke is burnt like benzopyrine, nicotine, cadmium and lead.[5-8] However, another study showed that oral administration of nicotine to male rats resulting in significantly decreasing of total sperm count and motile sperm count. Furthermore, this study also revealed that the negative effects are diminished after the nicotine administration is stopped.[9] Wong et al., measured cotinine level in plasma and found that the higher the cotinine level, the higher the abnormal morphology sperm percentage. And since cotinine is the nicotine metabolites, the theory of nicotine as the substance that affects directly to the male fertility.[10]

OBJECTIVE

The aim of this study was to analyze the effect of exposure of nicotine per inhalation (NicI) to the change of percentage of motile sperm (PMS) and percentage of normal morphology sperm (PNMS).

MATERIAL & METHODS

This was a laboratory experimental study using post test only control group design comparing PMS and PMNS after exposure of NicI between treatment-group and control-group.

The subjects of the study were 30 young male white rats (Rattus Norvegicus) Wistar strain age 2-3 months old, weight 150-250 grams obtained from Animal Lab, Faculty of Veterinary, Airlangga University, Surabaya. Management of the subject rats in this research were following International Guiding Principles for Biomedical Research Involving Animals (1985) of Council for International Organizations of Medical Sciences. The subjects were divided into 5 different groups. 1 group served as control groups, while the other 4 groups were treated with NicI. Each treatment groups were given 0.5 mg/kgBW, 1 mg/kgBW, 2 mg/kgBW and 4 mg/kgBW of NicI. The treatments were given for 30 days. After the treatment was completed, sperm were obtained from epididymal head of the left testis of rats which removed through orchidectomy.

Sperm motility examinations were performed immediately using Improved Neubauer count booster under 400x magnification microscope. Sperm morphologies were examined using Eosin/Negrosin stain, observed under 1000x magnification microscope. Normal sperms were counted from 200 observed sperms and the percentages were calculated.

Result data were analyzed using SPSS 20. If the data were normally distributed, both PMS and PMNS were tested using One Way Anova (OWA) followed with Post Hoc Test (PHT) to determine the significance between each group if the result of OWA was significant ($p<0.05$). If the data were homogen, PHT LSD was used. But if the data were not homogen, PHT Tamhanne was used. If the data were not eligible for OWA, Kruskal-Wallis test followed with Mann-Whitney test was used to determine the significance of the data. Data normality was determined using Shapiro-Wilk Test.

This research has been declared ethical by Animal Care and Use Committee (ACUC) Faculty of Veterinary, Airlangga University, Surabaya.

RESULTS

Homogeneity test of the body weight were done after randomization. The result of the test was the body weight of each group was homogen with $p>0.05$ (table 1).

Table 1. Baseline body weight characteristic of the male rats.

Characteristic	Control	Group				p value
		NicI 0.5 mg/kgBW	NicI 1 mg/kgBW	NicI 2 mg/kgBW	NicI 4 mg/kgBB	
Early body weight (gram)	150 ± 17.89	163.33 ± 13.66	163.33 ± 15.05	151.67 ± 21.37	171.67 ± 28.58	0.32

Table 2. The difference of PMS median between control and NicI treatment group.

Group	N	Median (Min-Max) (%)	Normality Test	p value
Control	6	82.50 (70–100)	0.78	0.00**
NicI 0.5 mg/kgBB	6	27.50 (5–70)	0.46	
NicI 1 mg/kgBB	6	10 (5–35)	0.10	
NicI 2 mg/kgBB	6	7.50 (0–25)	0.00*	
NicI 4 mg/kgBB	6	7.50 (0–10)	0.00*	

*: p<0.05, not normally distributed **: p<0.05, statistically significant

Table 3. Comparison of PMS after NicI between each group.

Groups	p value
Control vs NicI 0.5 mg/kgBW	0.00*
Control vs NicI 1 mg/kgBW	0.00*
Control vs NicI 2 mg/kgBW	0.00*
Control vs NicI 4 mg/kgBW	0.00*
NicI 0.5 mg/kgBW vs NicI 1 mg/kgBW	0.19
NicI 0.5 mg/kgBW vs NicI 2 mg/kgBW	0.02*
NicI 0.5 mg/kgBW vs NicI 4 mg/kgBW	0.01*
NicI 1 mg/kgBW vs NicI 2 mg/kgBW	0.10
NicI 1 mg/kgBW vs NicI 4 mg/kgBW	0.04*
NicI 2 mg/kgBW vs NicI 4 mg/kgBW	0.85

*: p<0.05, statistically significant

Table 4. The difference of PMNS mean between control and NicI treatment group.

Group	N	Mean ± SD (%)	Normality Test	p value
Control	6	90.00 ± 8.94	0.17*	0.00**
NicI 0.5 mg/kgBW	6	43.33 ± 31.41	0.31*	
NicI 1 mg/kgBW	6	23.33 ± 12.11	0.42*	
NicI 2 mg/kgBW	6	12.50 ± 10.37	0.06*	
NicI 4 mg/kgBW	6	9.17 ± 9.17	0.16*	

* : p>0.05, data normally distributed **: p<0.05, statistically significant

Tabel 5. Comparison of PMNS after NicI between each group.

Group	Mean Difference	CI 95%		p value
		Lower Border	Upper Border	
Control vs NicI 0.5 mg/kgBW	46.67	-11.56	104.89	0.13
Control vs NicI 1 mg/kgBW	66.67	44.23	89.10	0.00*
Control vs NicI 2 mg/kgBW	75.80	57.44	97.56	0.00*
Control vs NicI 4 mg/kgBW	80.83	62.17	99.50	0.00*
NicI 0.5 mg/kgBW vs NicI 1 mg/kgBW	20.00	-37.06	77.06	0.88
NicI 0.5 mg/kgBW vs NicI 2 mg/kgBW	30.83	-26.82	88.49	0.47
NicI 0.5 mg/kgBW vs NicI 4 mg/kgBW	34.17	-23.96	92.30	0.36
NicI 1 mg/kgBW vs NicI 2 mg/kgBW	10.83	-12.53	34.20	0.75
NicI 1 mg/kgBW vs NicI 4 mg/kgBW	14.17	-8.40	36.73	0.38
NicI 2 mg/kgBW vs NicI 4 mg/kgBW	3.33	-16.91	23.58	1.00

*: p<0.05, statistically significant

The data was not normally distributed. The test showed significant difference of median grouped data ($p<0.05$) as shown on table 2.

The comparison between each group test showed that all treated group had significantly lower PMS compared to the control group (Table 3). From each dose of NicI, significant difference found in 0.5 mg/kgBW vs 2 mg/kgBW, 0.5 mg/kgBW vs 4 mg/kgBW and 1 mg/kgBW vs 4 mg/kgBW as shown on table 3.

The data was normally distributed. The test showed significant difference of mean grouped data ($p<0.05$) as shown on table 4.

Based on PHT, it is known that PMNS difference is significantly decreasing in group NicI 1 mg/kgBW, NicI 2 mg/kgBW, NicI 4 mg/kgBW compared to control group (Table 5). Comparison between each dose showed descriptively decreasing of PMNS mean (Table 4) but not statistically significant as shown on table 5.

DISCUSSION

Nicotine is the main pharmacologic substance of tobacco (Nicotiana Tabacum). It is metabolized into its active form, cotinine once it enters the systemic circulation. Cotinine is known to have longer half time than nicotine.[11,12] Proven negative effects of smoking to male fertility are lower sperm count, lower motile sperm count and higher abnormal sperm count.[13-15] The mechanism responsible for the pathology is still yet unclear. Several hypothesis are developed, including increasing reactive oxygen species (ROS) that impact the sperm directly as one suspected.[16,17] Oxidative stress occurred as the consequences of the rising amount of ROS. This leads to formation of radical superoxide (O2-) and hydrogen peroxide (H2O2) which are very reactive to the plasma membrane. Once the plasma membrane broken, hydrogen peroxide enter the plasma and inhibit glycolysis process by inactivating Glyceraldehyde-3-phosphate (GAP). GAP is the intermediate substance to the formation of ATP. Since the ATP is not formed, phosphorilation of the sperm axoneme is also impaired and leads to the impairment of the motility of the sperm.[18,19]

Besides the impairment of the sperm motility, ROS also causes morphology abnormality of sperm. Suspected mechanism responsible for the abnormality is Oxidative DNA impairment. The mechanism leads to DNA condensation and decondensation failure during spermatogenesis. The result of this failure is anatomical-impaired sperm.[20-23]

The result of this research showed that NicI exposure for 30 days decreased the PMS significantly in all treatment-group compared to the control ($p<0.05$). NicI also caused significant decrease of PMS in several inter-treatment group comparison (0.5 mg/kgBW vs 2 mg/kgBW; 0.5 mg/kgBW vs 4 mg/kgBW; 1 mg/kgBW vs 4 mg/kgBW). Different results were shown on PMNS difference after NicI, where the differences begin significant in NicI 1 mg/kgBW and above compared to control group. Inter-treatment groups comparison showed insignificant differences in all group. Research by Asiyah et al (2011) showed similar result with this research. PMS and PMNS showed significant decrease after nicotine administration intraperitoneally compared to negative control. The dose given to the rats is 5 mg/kgBW.[24]

Another study conducted by Oyeyipo et al (2011) showed that orally administration of nicotine (0.5 mg/kgBW and 1 mg/kgBW) for 30 days significantly decreased the PMS, but this condition is reversible once the nicotine administration was stopped. This reversibility reflected in group 4 and 5 where the PMS is significantly increasing after the administration of nicotine is stopped for 30 days. The reduction did not occurred in PMNS, both doses did not decrease the PMNS significantly after 30 days of nicotine.[9]

This is a bit different with the result of this study that PMNS is decrease significantly in group of 1mg/kgBW, 2mg/kgBW, 4mg/kgBW compared to negative control. This can be happen because this study have different administration route and different dose of the nicotine. Inhalation administration route is pharmacokinetically known not to pass hepatic blood barrier so it has more concentration at the artery.[25,26] Inhalation route is one of the weakness of this study because we can not give individual dose on each rat. Oyeyipo et al (2014) have done advanced study about nicotine effects compared to sperm analysis and given ginger extract as an additional intervention. This 2014 study show different result with the previous one (2011) that PMNS decreased significantly ($p<0.05$). Oyeyipo et al (2014) also revealed that curved tail is dominant abnormalities at the morphology examination. The final result of the study stated that ginger (Zingiber officinale) extract could prevent decrease of rats sperm motility and morphology caused by nicotine.[27]

Yamamoto et al (1998) concluded that there was sperm motility decrease after exposure of 20 cigarettes per day (equal 8mg/kgBW) in 70 days. Yamamoto also showed that there were no differences of basal testosterone between control group and the intervention group, nevertheless testosterone response after hCG administration did not sharply increase compared to control group. This result support hypothesis where nicotine could decrease Leydig cell secretory function, although no clear mechanism about how it can affect sperm motility.[28]

Another similar study have been conducted by Oyeyemi et al in 2015. Oyeyemi et al showed decrease of sperm motility and normal morphology after 1 mg/kgBW intravenous nicotine administration for 40 days. Vitamin E administration significantly prevent nicotine negative effect to the sperm.[29]

Another study by Condorelli et al (2013) had a similar result even though it used different design and method. This was an in-vitro study of the nicotine exposure to human sperm directly. The result were PMS decrease, increase of changed chromatin density sperm percentage, and increase of Fragmented DNA sperm percentage.[30]

CONCLUSION

Investigation in this research showed that exposure of nicotine per inhalation significantly reduced the PMS and PMNS of adult male rats. However, the study cannot explain the pathology process behind the damage. Further study needed to determine the pathology process of the damage. The other limitation of the study was the dose of nicotine inhaled to systemic circulation. The doses determined in the methods were exposure dose, not the doses inside systemic circulation.

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